
'15
10TH
YES MEETING
YOUNG EUROPEAN SCIENTIST MEETING
17 TO 20 SEPTEMBER 2015
FACULTY OF MEDICINE OF PORTO
YES GUIDE

YES MEETING

YOUNG EUROPEAN SCIENTIST MEETING

10TH EDITION ANNIVERSARY

17TH-20TH SEPTEMBER 2015

Porto - Portugal

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YES Meeting
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Message from the Organizing Committee

Dear colleagues,

Welcome to the 10th edition of
YES – Young European Scientists - Meeting!

It is with great pleasure that we present you this year's programme, with remarkable scientific sessions, hold by the greatest scientists, more practice with great diversity of workshops occurring in 3 periods during the conference.

The pre-congress day will count, not only with soft skills workshops in the morning hold by science authorities but also the **Leyden Academy** program on ageing and vitality, which will occur during the afternoon. This day is a great opportunity for you to develop and become a better doctor and scientist, with better and wider range of skills.

Concerning the scientific programme specifically, this year YES Meeting counts with the presence of renowned scientists such as **Venkatraman Ramakrishnan, the 2009 Chemical Nobel Prize winner, Frits Rosendaal, 2002 Spinoza Prize winner and Mario Raviglione, Director of the Global Programme of Tuberculosis of World Health Organization**. Other areas of interest that will play an important role in this edition are: Neurosciences – epilepsy, deep brain stimulation –, HIV, Microbioma, Surgery – obstetrics and gynecology, etc.

Our social programme has also suffered some changes. In this edition you will have the opportunity to visit the Futebol Clube do Porto Museum as well as a guided tour through the Stadium; visit the World of Discoveries Museum where you'll learn a bit more about the Portuguese navigators' odyssey throughout the world; take a Douro River tour in Rabelo's boats and discover the Port Wine Cellars or just enjoy a long tour along the river; enjoy a Sightseeing Bus Tour around the magnificent historical city of Porto or visit the Serralves Foundation and wondering around its beautiful gardens.

The workshops were also renewed, in this year's programme you will enjoy an even more interactive experience thanks to some new sessions and a greater focus on a hands-on approach.

Once again, the YES Meeting Organizing Committee is very proud to be able to give to all the young scientists a chance to present their own work in an international conference. You should not miss the Parallel Oral Sessions and the Plenary Session. The quality of all the presentations from your colleagues will not disappoint you!

We hope you enjoy this meeting and use this opportunity to make new friends and develop your network with the fellow young scientists in the congress!

Now it's up to you make this an unforgettable scientific experience!

Our best regards,
The 10th YES Meeting Organizing Committee

Programme

Outline

Pre-Course - 17th September

9.00 am	Opening Session
9.30 am	Soft Skills Session 1
11.00 am	Soft Skills Session 2
2.30 pm	Ageing: regenerating the future of medicine and care?
3.30 pm	Workshop 1
4.30 pm	Coffee-Break
5.00 pm	Workshop 2
6.00 pm	Workshop 3

18th September

8.45 am — Opening Session - Internal Medicine

- *Fecal transplants: From anecdote to modern medicine*
  Ilan Youngster – Children's Hospital, Harvard University, USA
- *From knife to seeking answers to life - a surgeon's journey to medical biotechnology*
  Manson Fok – Faculty of Health Sciences, Macau University of Science & Technology

10.30 am — Coffee-break – "Oncology & Molecular Biology" Poster Presentations

11.30 am — Infectious Diseases Session

- *TB 2015: burden, challenges, response*
  Mario Raviglione – Director of WHO Global TB Programme
- *HIV: a moving target*
  Fernando Garcês Ferreira – The Scripps Research Institute, USA

12.30 — Lunch

2.00 pm — Parallel Oral Sessions

3.30 pm — Social Programme

7.00 pm — Epilepsy Session

- *Neurobiology of cognitive deficits in epilepsy*
  Heinz Beck – University of Bönn, Germany
- *Clinical challenges in Epilepsy*
  Christian E. Elger – University of Bönn, Germany

19th September

9.00 am — **Oncology and Molecular Biology Session**

- *Strategies against hard to treat cancers: Bringing surgery and therapies inside cancer cells*
  Dmitri Lapotko – Rice University, USA
- *Oncolytic virus for the treatment of cancer*
  Magnus Essand – Uppsala University, Sweden

10.00 am — **Coffee-break** – "Internal Medicine", "Physiology & Immunology", "Surgery" and "Public Health & Medical Informatics" Poster Presentations

11.00 am — **Gynecologic-Obstetric Surgery Session**

- *The transvaginal laparoscopy: a challenging future?*
  Stephan Gordts – Leuven Institute for Fertility and Embryology, Belgium
- *Uterine Transplantation: From animal research to Clinical application*
  Randa Akouri – Sahlgrenska University Hospital, Sweden

12.00 — **Lunch**

1.30 pm — **Spinoza Award Session**

- *Clots that kill: genes and lifestyle*
  Frits Rosendaal – Leiden University, Netherlands

3.00 pm — **Workshops 1**

4.30 pm — **Coffee-break**

5.00 pm — **Workshops 2**

8.30 pm — **Gala Dinner**

20th September

9.30 am — **Speed Meeting with Scientists** – an informal talk with our speakers

11.00 am — **Nobel Prize Session**

- *The ribosome and how antibiotics block it*
  Venkatraman Ramakrishnan – MRC Laboratory of Molecular Biology, Cambridge, UK

12.00 — **Lunch**

1.30 pm — **Plenary Session**

3.00 pm — **Workshops 3**

4.30 pm — **Coffee-Break**

5.30 pm — **Neurosciences Session**

- *Mind-controlled transgene expression by a wireless-powered optogenetic designer cell implant*
  Marc Folcher – ETH Zürich, Switzerland
- *Current Issues and Future Perspectives of DBS: from Electrons to Photons*
  Alim-Louis Benabid – Université Joseph Fourier, France

6.30 pm — **Award and Closing Ceremony**

7.30 pm — **See'YES next year**

Extended Programme

THURSDAY, 17TH SEPTEMBER | PRE-COURSE

Opening Session – 9.00am – Auditorium CIM (Centre of Medical Investigation)
9.00am – Ana Vaz, Vice-President of the 10th YES Meeting Organizing Committee
9.05am – Ana Luísa Cunha, Pre-Congress Creator
9.15am – TUFEMED *(Tuna Feminina de Medicina do Porto)* Performance

Soft Skills Sessions – 9.30am and 11.00am
Scientific Integrity – Laura Ribeiro
Physical examination: a communication skill needing new emphasis – Elizabete Loureiro
Team Building – Joaquim Cunha
Time Management – Isabel Lourinho
How to Write a Successful CV – Maria Amélia Ferreira
Leadership – Ana Jorge
How to Write a Scientific Paper – Amândio Sousa
Project Management for Clinical and Health Researchers – António Soares | Altamiro Pereira
What's your big idea? Medical Entrepreneurship challenge – Tiago Taveira Gomes
Non-technical skills in crisis resource managment – Carla Sá Couto | Luís Monteiro
Public Speaking – Manuel Sobrinho Simões

Afternoon - Leyden Academy
Ageing: regenerating the future of medicine and care? – 2.30pm – Auditorium CIM
David van Bodegom – Leyden Academy on Vitality and Ageing

Workshops – 3.30pm, 5.00pm, 6.00pm
Saving medicine? Non-communicable diseases and the promise of robotics – Jolanda Lindenberg – Pharmacology and Therapeutics Amphitheatre (CIM)
Environmental change for healthy ageing – Lex van Delden – Room 3 (CIM)
Evolutionary thinking about ageing – David van Bodegom – Room 4 (CIM)
Coffee-Break – 4.30pm – CIM Hall

FRIDAY, 18TH SEPTEMBER

Opening Session – 8.45am – Auditorium CIM
8.45am – Marisa Martins, President of 10th YES Meeting Organizing Committee
8.50am – Amélia Ferreira, Director of Faculty of Medicine
9.00am – Diana Rodrigues, President of Students Association
9.05am – Henrique Almeida, Director of the Integrated Master in Medicine Course
9.10am – Miguel Guimarães, Head of North Section of College of Physicians
9.15am – Francisco Rocha Gonçalves, President of the Scientific Committee of the 10th YES Meeting
9.20am – Sebastião Feyo de Azevedo, Dean of Porto University

Internal Medicine Session – 9.30am – Auditorium CIM
- *Fecal transplants: From anecdote to modern medicine*
  Ilan Youngster – Children's Hospital, Harvard University, USA
- *From knife to seeking answers to life - a surgeon's journey to medical biotechnology*
  Manson Fok – Faculty of Health Sciences, Macau University of Science & Technology

Chairperson: Miguel Mascarenhas Saraiva

Coffee Breaks – **"Oncology & Molecular Biology" Poster Presentations** – 10.30am – CIM Hall

Pavle Banović, Pedro Medeiros, Wout Devlies, Henrique Duarte, Maria Salazar, Katarzyna Tyrak, Maria Leticia Carraro, Mário Fontes, Gertjan A.C. Rasschaert, Joana Moreira, Ana Rita Moreira, Vanessa Botelho, Gabriela Moreira, Agostinho Lemos, Rita Rocha, Fatime Hawchar, Pedro C. Nunes, Mafalda Carrington, Nuno Almeida, Sara Petronilho, Sara Veiga, Mariana Ribeiro, Sara Isabel Fernandes, Jelena Spasic

Infectious Diseases Session – 11.30am – Auditorium CIM
- *TB 2015: burden, challenges, response*
  Mario Raviglione – Director of WHO Global TB Programme
- *HIV: a moving target*
  Fernando Garcês Ferreira – The Scripps Research Institute, USA

Chairperson: Maria Lurdes Santos

Lunch – 12.30 – Students' Hall

Parallel Oral Sessions – 2.00pm

INTERNAL MEDICINE – Nascente Amphitheatre
• PS2: Gabrijela Dukic - Obesity as a risk factor for occurrence of hypertensive disorders of pregnancy
• PS31: Zala Novak - Ivabradine for heart rate lowering in patients undergoing coronary computed tomographic angiography

• PS76: Iolanda Jelea - Fatigue correlates in systemic lupus erythematosus patients – a single center cohort of 96 patients
• PS84: Patrycja Pieczka - The prevalence of insulin resistance and its impact on mortality in different types of Pulmonary Arterial Hypertention.
• PS104: Yeganeh Pasebani - assessing the left atrial pressure in patients with severe mitral stenosis by echocardiography and catheterization.

NEUROSCIENCES – Poente Amphitheatre
• PS34: Amy Hung - The Effects of Systemic Infection on the Neuropathology in Alzheimer's disease
• PS51: Catarina Carmo - Altered mitochondrial dynamics caused by sirtuin 3 overexpression in a cellular model of Huntington's disease
• PS72: Inês Cruz - Xanthone amino derivatives with potential dual mode of action for Alzheimer's Disease
• PS94: Spas Kerimov - Tackling the neuroinflammation - implementation of selective COX-2 inhibitors in combined therapy of epilepsy
• PS136: Lucas Lopes - Incretin drugs have anti-inflammatory effects at the spinal cord of diabetic rats
• PS143: Małgorzata Kozioł - Differences in morphometric parameters of ruptured and unruptured intracranial aneurysms.

ONCOLOGY & MOLECULAR BIOLOGY – Novo A Amphitheatre
• PS17: Verónica Alheia Cabreira – Performance of Lynch Syndrome Predictive Models in quantifying the likelihood of MLH1 mutation in a Portuguese clinical genetic setting
• PS80: Hugo Fernandes-Silva – The role of Retinoic Acid signaling in chick lung branching
• PS81: Ana Filipa Sobral – Cell cycle arrest and apoptosis activation in breast cancer MCF-7aro cells induced by new aromatase inhibitors
• PS109: Francisca Soares da Silva – Hes5: a novel player in the specification of cardiac fate
• PS113: Vasco Sampaio Pinto – An Integrative Model Of Neonatal Cardiac Injury Response
• PS131: Gonçalo Brites – Evaluation of the cytotoxic response of photodynamic therapy in combination with doxorubicin, cisplatin, methotrexate and ifosfamide in osteosarcoma cell line

PHYSIOLOGY & IMMUNOLOGY – Norte Amphitheatre
• PS73: Ana Guerra – Angiogenic Effects of Structurally-Diverse Xanthones
• PS78: Catarina Santos Ribeiro – Hypertrophic Cardiomyopathy and CRISPR: a genomic sewing kit
• PS122: Rui Ribeiro – Restoring the heartbeat: Tracking cardiomyocyte proliferation
• PS132: Sílvia Rodrigues – Dissecting cardiogenic mechanisms activated upon neonatal cardiac injury
• PS138: Renáta Gáspár – Doxorubicin-induced cytotoxicity in primary cardiomyocyte cultures
• PS161: André Leite Moreira – Acute myocardial stretch reduces cardiac stiffness through titin phosphorylation by PKG

PUBLIC HEALTH & MEDICAL INFORMATICS – Pharmacology and Therapeutics Amphitheatre (CIM)
• PS11: Kriti Tripathi – Relevance of Postprandial Glucose to Body Mass Index and Waist Circumference in young North and South Indians
• PS37: Manuel Gonçalves Pinho – Burden of ophthalmic procedures in public hospitals of Portugal
• PS46: Aleksandra Zdravković – Medical students attitude towards histology knowledge and its application in pre-clinical and clinical medicine
• PS52: Kughan – HIV surveillance in russia-positives and negatives and their impacts on epidemiology of HIV infection
• PS128: Joanna Radzimowska – Etiological and epidemiological analysis of superficial fungal infections in southern Poland, including their age and gender-related prevalence- a 5-year study.
• PS162: João Vasco Santos – Clinical and epidemiological burden of Hidroadenitis Suppurativa in Portugal: 1,391 patients from 2000 to 2010

SURGERY – Novo B Amphitheatre
• PS25: Blaz Kacijan – Kinematic analysis of the knee joint with anterior cruciate ligament injury or knee osteoarthrosis using 3-D laser triangulation
• PS64: Anna Kot – Endovascular treatment for cerebral arteriovenous malformation: predictors of multiple stage treatment.
• PS69: Alicja Lachowska – Time is (not?) a great healer - day of surgery affects length of stay.
• PS88: Jiang Bochao – Predictors of Survival in Ampullary Carcinomas
• PS93: Anna Łaskawska – Patients' views on the Enhanced Recovery After Surgery Programme.
• PS118: Daromir Godula – Influence of time period on the number of admissions to ER and patients' outcomes

Social Programme – 3.30pm
Sightseeing Tour – Historical Porto
World Of Discoveries – Interactive Museum And Thematic Park
Douro River By Rabelo Boats and Porto Wine Cellars
Douro River By Blue Boats
Serralves Foundation and Park
FC Porto Stadium and Museum

Epilepsy Session – 7.00pm – Auditorium CIM
• *Neurobiology of cognitive deficits in epilepsy*
Heinz Beck – University of Bönn, Germany
• *Clinical challenges in Epilepsy*
Christian E. Elger – University of Bönn, Germany
Chairperson: Patrício Soares da Silva

SATURDAY, 19TH SEPTEMBER

Oncology & Molecular Biology Session – 9.00am – Auditorium CIM
• *Strategies against hard to treat cancers: Bringing surgery and therapies inside cancer cells*
Dmitri Lapotko – Rice University, USA
• *Oncolytic virus for the treatment of cancer*
Magnus Essand – Uppsala University, Sweden
Chairperson: Margarida Damasceno

Coffee-Break – 10.30am – CIM Hall

"Internal Medicine" Poster Presentations

Konstantin Dotsenko, Szymon Ciuk, Lynn Debels, Tetyana Yaroshyk, Karolina Jaworska, Justyna Maciejiczek, Michał Moździerz

"Physiology & Immunology" Poster Presentations

Sava Kovacevic, Ricardo Cardoso, Borodai Inna, Skorbach Olena, Diana Tarawneh, Mário Sousa Pimenta, Ernesto Ruivo, Emilija Djuric, Carina Brito, Salah Izat Shokry

"Public Health & Medical Informatics" Poster Presentations

Aleksandra Bjegovic, João Ramos, Divya Goil, Magdalena Czerżyńska, Dynnik Oleksandra, Leila Azimi, Marjan Rashidan, Burak Goren, Daniel S. Joffe, Iana Fedorenko

"Surgery" Poster Presentations

Edit Vigh, Fereshteh Yazdanpanah, Piotr Kwiatkowski, Aleksandra C. Wolny, Monika Wenio, Mariana Matias

Gynecologic-Obstetric Surgery Session – 11.30am – Auditorium CIM

- *The transvaginal laparoscopy: a challenging future?*
  Stephan Gordts – Leuven Institute for Fertility and Embryology, Belgium
- *Uterine Transplantation: From animal research to Clinical application*
  Randa Akouri – Sahlgrenska University Hospital, Sweden

Chairperson: Nuno Montenegro

Lunch – 12.00 – Students' Hall

Spinoza Award Session – 1.30pm – Auditorium CIM

- *Clots that kill: genes and lifestyle*
  Frits Rosendaal – Leiden University, Netherlands

Chairperson: Armando Mansilha

Workshops 1 – 3.00pm – See pages 12/13 for more information

Coffee-Break – 4.30pm – CIM Hall

Workshops 2 – 5.00pm – See pages 12/13 for more information

Gala Dinner – 8.30pm – Fundação Dr. António Cupertino Miranda

SUNDAY, 20TH SEPTEMBER

Speed Meeting with Scientists – 9.30am – CIM Hall | An informal talk with our speakers

Nobel Prize Session – 11.00am – Auditorium CIM

- *The ribosome and how antibiotics block it*
  Venkatraman Ramakrishnan – MRC Laboratory of Molecular Biology, Cambridge, UK

Chairperson: Cláudio Sunkel

Lunch – 12.00 – Students' Hall

Plenary Session – 1.30pm – Aula Magna, 3rd Floor

• INTERNAL MEDICINE | PS24: Bianca Barbuta – Anatomical variants associated with atrial fibrillation

• NEUROSCIENCES | PS68: Joe Wild – Microglial Behaviour in Response to Systemic Infection in the White Matter of the Alzheimer's Afflicted Brain

• ONCOLOGY AND MOLECULAR BIOLOGY | PS82: Lia Costa – Effects of Synthetic cannabinoids in placenta: WIN 55,212 induces a mitochondrial-dependent cell death mechanism in trophoblast cells

• PHYSIOLOGY AND IMMUNOLOGY | PS10: Wu Yilong – Using cell penetrating peptide (CPP) as a novel strategy to deliver siRNA into lung cells

• PUBLIC HEALTH AND MEDICAL INFORMATICS | PS158: Ahmed Nabil Shaaban – Predictors of HIV Pre Exposure Prophylaxis Among Men Who Have Sex With Men in Portugal

• SURGERY | PS125: Mariusz Ligocki – Hepatic resection for metastatic melanoma: the role of surgical treatment.

Workshops 3 – 3.00pm – See pages 12/13 for more information

Coffee-Break – 4.30pm – CIM Hall

Neurosciences Session – 5.30pm – Aula Magna, 3rd Floor

- *Mind-controlled transgene expression by a wireless-powered optogenetic designer cell implant*
  Marc Folcher – ETH Zürich, Switzerland
- *Current Issues and Future Perspectives of DBS: from Electrons to Photons*
  Alim-Louis Benabid – Université Joseph Fourier, France

Chairperson: Rui Vaz

Award and Closing Ceremony – 6.30pm – Aula Magna, 3rd Floor

See'YES next year – 7.30pm – Aula Magna, 3rd Floor

Workshops Locations

SATURDAY

WORKSHOP	LOCATION
Accelerate Critical Thinking & Clinical Decision-Making for Acute and Chronic Care	Health Information and Decision Sciences Room L4
Arthroscopy – Hands-On Approach	Pharmacology Classroom 1
Basic Obstetric Ultrasound: A Hands-On Experience	Obstetrics and Gynecology Department
Basic Urological procedures: Hands-on Approach	Urology Department
Breath in, Breath out	Health Information and Decision Sciences Room L2
Cervical Arthrodesis: a Hands-on Approach	Biochemistry Classroom 1
Clinical Cases in Surgery	Auditorium CIM
Diabetic Foot: A 21th Century Problem	Outpatient Clinic
Dizziness	General Therapeutics and Clinical Pharmacology amphitheater
Do you have the guts to handle it?	Gastroenterology Department
Dress code: Ebola and heamorrhagic fevers	Room 2 – CIM
Good practises when communicating and supporting a victim of domestic violence or sexual assault in the emergency department	Room 3 – CIM
Handling, Physical Restrant, Blood Sampling and Parental Administration in Lab Animals	Vivarium
Histologic staining: Hands on!	Molecular structure of the cell (MSC) laboratory
Inside your Brain	Library of Neurocritical Patients Department
Interventional Radiology	Anatomic Theater
Invasive Hemodynamic Evaluation	Physiology Department; 4th floor
Kids: how to make them happy?	Room 4 - CIM
Laparoscopic Techniques	Health Information and Decision Sciences Classroom L5
Lumbar Arthrodesis: a Hands-on Approach	Biochemistry Classroom 2
Medical Emergency - INEM	Health Information and Decision Sciences Room L3

WORKSHOP	LOCATION
Minor Surgery	Library of the Physiology Department; 4th floor
Neonatal Reanimation	Physiology Classroom 1
Respiratory clinical challenges: technical solutions	Physiology Theory/practice (TP) classroom
Targeting the Brain	Pharmacology Classroom 2
The Forefront of Cardiac Auscultation	Health Information and Decision Sciences Room L1
Training basic obstetric skills in a delivery simulator	Biomedical Simulation Center; 7th floor
U, the ICU team	Biomedical Simulation Center

SUNDAY

WORKSHOP	LOCATION
Amphetamines: a common drug in the medical world	Preventive medicine library
Breath in, Breath out	Health Information and Decision Sciences Room (L1/L2)
Clinical Thinking in Heamatology	Physiology Theory/practice room
Curious Cases in Cardiology	Pharmacology classroom 2
Histologic staining: Hands on!	Molecular structure of the cell (MSC) laboratory
Hypnosis on parturition in pregnant women	Room 2 CIM
In the Pursuit of Happiness	General Therapeutics and Clinical Pharmacology amphitheater
Kids: how to make them happy?	Room 4 CIM
Kinesio Taping	Auditorium CIM
Nuclear Medicine: where all the shots are hot	Health Information and Decision Sciences Room L5
Osteopathy and Pregnancy	Physiology practice classroom
Postmodern approaches to couple relationships and human sexuality. Clinical implications.	Health Information and Decision Sciences Room L6
Splints & Casts	Biochemistry classroom 2
The Forefront of Cardiac Auscultation	Health Information and Decision Sciences Room (L1/L2)
Walking in Tightrope	Room 3 CIM

Admission

Participants are requested to wear their credentials at all times during the congress. Admission to the meeting rooms, social program events, lunches and coffee-breaks is limited to those wearing the YES credentials.

YES Meeting Staff

You can identify YES Organizing Committee members and YES Task Force members by their red coloured credentials. May you have any question or problem, please feel free to contact us any time.

Internet Access / Wireless LAN

Free internet stations are available in the YES Meeting exhibition area.

Mobile phones

Mobile phones must always be switched off inside lecture halls.

Smoking policy

Smoking in the Meeting area will not be allowed. You should go outside to do so.

Coffee-breaks and lunches

From September 19th to the 21st coffee-breaks and lunches will be served free of charge to participants wearing YES credentials.

Lost and Found

If you loose anything, please ask the Information Desk about it. If you find anything in the YES Meeting premises please deliver it at the Information Desk.

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ORMATION

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Marisa Martins
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Prizes & Awards

Prizes will be announced during in a ceremony held in the afternoon of the 20th September. Each prize will be given for each of the Themes. The prizes are as follows:

Best Plenary Session Award – 1000€

Professor Ernesto Morais Best Oral Presentations Award (per area) – 250€

Best Poster Presentations Award (per area)– 150€

Quality Assessment Sheets and Attendance Certificates

After the congress you will receive a quality assessment sheet by email. After you fill it and submit it you will receive your certificate of attendance by email. If you attended workshops or presented your work, this will be stated in your certificate.

We would like to thank all our Official Sponsors

and everyone who made this congress possible!

ABSTRACT BOOK

PLENARY SESSION

PS 24

Bianca Barbuta

Plenary Session Plenary Session

ANATOMICAL VARIANTS ASSOCIATED WITH ATRIAL FIBRILLATION

1-Barbuta B., 2-Rusu A.C., 3-Chistol R.O., 1-Furnica C.

1-"Gr.T. Popa" University of Medicine and Pharmacy, Iasi, Romania, 2-University of Medicine and Pharmacy, Targu Mures, Romania, 3-Institute of Cardiovascular Diseases, Iasi, Romania

AIM

The aim of the study is to evaluate by multidetector computed tomography (MDCT) the prevalence, location and size of LA (left atrial) diverticula and accessory appendages, the prevalence of PVs (pulmonary veins) anatomical variants and LAA (left atrial appendage) shapes in patients with atrial fibrillation (AF) compared to patients in sinus rhythm (SR).

INTRODUCTION

The left atrial appendage (LAA) is a complex morpho-anatomical structure distinct from the rest of the left atrium from an embryological, anatomical and pathophysiological point of view. Atrial fibrillation (AF) is a well-known supraventricular arrhythmia, but its exact relation to the LAA shape, left atrium (LA) accessory appendages, LA diverticula and anatomical variants of pulmonary veins (PVs) still remains unclear. In up to 94% of patients with AF, ectopic foci with electrical activity triggering AF have been found in myocardial muscle extensions located at the junction of PVs with the LA (1). Specialized conduction tissue with pacemaker activity remains at the specified level after the looping process of the heart tube during development (2). Due to its high spatial and temporal resolution, multidetector computed tomography (MDCT) is the non-invasive imaging method of choice for evaluation of PVs and LA prior to radiofrequency catheter ablation (RFCA) in AF. Anatomical variants of LA, LAA shape and PVs with unknown electrical impact are frequently identified when performing cardiac MDCT. The aims of the current study were to evaluate by means of MDCT the morphology of the LAA; to determine the prevalence, location, and size of LA diverticula and accessory appendages and to analyse PVs anatomical variants in patients with AF compared to patients in sinus rhythm (SR) in order to determine if the incidence of particular aspects of these structures is higher in patients with AF.

METHODS

Images obtained by MDCT in a group of 100 patients with <1 year paroxysmal or persistent AF prior to radiofrequency catheter ablation and in a group of 100 patients in SR with unconfirmed or insignificant (<50%) coronary arteries stenoses were compared.

RESULTS

The prevalence, mean width and length of left atrial diverticula (22%, 8.8 mm, 7.5 mm in AF group; 19%, 8.1 mm, 6.5 mm in SR group) and left atrial accessory appendages (6%, 7.42 mm, 9.38 mm in AF group; 5%, 7.34 mm, 9.94 mm in SR group) were not significantly different between the two groups. Left atrial appendages had a complex shape and pulmonary veins presented with several drainage variants but no significant differences concerning the prevalence of each left atrial appendage shape or pulmonary vein drainage variant were registered between the groups.

CONCLUSION

The prevalence, location and size of left atrial diverticula and accessory appendages, the prevalence of pulmonary veins anatomical variants and left atrial appendage shapes did not differ between patients with atrial fibrillation compared to patients in sinus rythm, results that plead against a relation between the analyzed structures and the genesis of atrial fibrillation.

1.Lazoura O, Reddy T, Shriharan M, Lindsay A, Nicol E, Rubens M, et al. Prevalence of left atrial anatomical abnormalities in patients with recurrent atrial fibrillation compared with patients in sinus rhythm using multi-slice CT. J Cardiovasc Comput Tomogr 2012; 6: 268-73. 2.Natale A, Raviele A, Arentz T, Calkins H, Chen SA, Haïssaguerre M, et al. Venice Chart international consensus document on atrial fibrillation ablation. J Cardiovasc Electrophysiol 2007; 18: 560-80.

PS 68

Joe Wild

Plenary Session Plenary Session

MICROGLIAL BEHAVIOUR IN RESPONSE TO SYSTEMIC INFECTION IN THE WHITE MATTER OF THE ALZHEIMER'S AFFLICTED BRAIN

Joe Wild, Sonja Rakic, Clive Holmes, Hugh Perry, James Nicoll, Delphine Boche
University of Southampton

AIM

Our aim was to assess how the phenotype of microglia changes with systemic infection in AD

INTRODUCTION

Alzheimer's Disease (AD) is characterised by aggregation of amyloid and tau proteins on a background of on-going inflammatory processes, orchestrated by microglia. Little is known about the effect of infection on these processes in AD. Expanding on this knowledge has the potential to reveal clues about the pathophysiology of AD

METHODS

Immunohistochemistry of cortical brain slices from four groups was performed – those whom had died with AD with (AD+) or without systemic infection (AD-), and controls whom had died with (Ctrl+) or without systemic infection (Ctrl-). Slides were stained with Iba1 (marker of microglial motility) CD64 (IgG mediated immune response) and CD68 (phagocytosis). Thirty fields of white matter at magnification 20x were taken and quantified using ImageJ 1.41 to obtain protein load (percentage area examined).

RESULTS

Iba1 displayed no significant difference between any of the groups (P=0.063). CD64 was significantly increased in the control group (Ctrl- vs Ctrl+; P<0.001) and between controls and AD cases without systemic infection (P=0.016) (P=<0.001). CD68 showed a significant increase between Ctrl and AD cases in the absence of infection (P<0.001) and a significant decrease in the AD cohort (AD- vs AD+; P=0.007). Correlation with the microglial data obtained in the grey matter showed significant associations for (i) Iba1 in all groups; (ii) for CD64i n Ctrl-, Ctrl+ and AD+ groups but not in the AD- cohort; and for (iii) CD68 in the AD cohots (AD- and AD+) but not in the Ctrl groups.

CONCLUSION

Our findings suggest that microglial response to infection seems to be impaired in AD. A complex picture of microglial behaviour has emerged with evidence for both distinct and unified microglial populations between the grey and white matter.

1. developed by Wayne Rasband, NIH, USA

PS 82

Lia Costa

Plenary Session Plenary Session

EFFECTS OF SYNTHETIC CANNABINOIDS IN PLACENTA: WIN-55,212 INDUCES A MITOCHONDRIAL-DEPENDENT CELL DEATH MECHANISM IN TROPHOBLAST CELLS

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AIM

Synthetic cannabinoids are psychoactive chemicals contained in abuse products marked as “spice” or “herbal incense”. Their impact in reproductive health namely during placentation is unknown. In this work, we intent to study the effects of the synthetic cannabinoid WIN 55, 212 in BeWo cells, a model of trophoblast cells.

INTRODUCTION

Originally synthetized for research purposes, synthetic cannabinoids are the most commonly identified psychoactive chemicals contained in abuse products. These compounds act in a similar way to phytocannabinoids, such as Δ9-tetrahydrocannabinol (THC), the major psychoactive substance of Cannabis sativa, whose effects are achieved through the activation of the specific cannabinoid receptors CB1 and CB2. These receptors are also activated by endogenous cannabinoid-like compounds called endocannabinoids (eCBs). The eCB levels need to be tightly regulated for a proper decidualization and implantation. During placentation, cytotrophoblasts, the specialized placental epithelial cells, suffer proliferation, differentiation and apoptosis, ensuring a normal placental development. Recently, we have shown that eCBs induce apoptosis and impair the differentiation into syncytiotrophoblasts [1]. The exogenous administration of cannabinoids may interfere with this delicate balance of trophoblast turnover. In this study we analyzed the effects of the synthetic cannabinoid WIN 55, 212.

METHODS

BeWo cells were cultured in DMEM/F12 medium with 1% FBS. After 12 h of adhesion, cells were treated with WIN 55, 212 for 24 h at different concentrations (0,1-20 μM). The impact of WIN treatment versus control was assessed by parameters related to cell death. We performed MTT assay to evaluate cell viability, and measured lactate dehydrogenase (LDH) released as a biomarker for cytolysis. Mitochondrial membrane potential (Δψm) and ROS/RNS species generation were evaluated by fluorimetry assays. Morphological changes were observed by phase contrast microscopy, Höechst and Giemsa staining.

RESULTS

MTT assay results indicated that WIN-55,212 is able to decrease cell viability above 5 μM, reflected in the decrease of NAD(P)H-dependent cellular oxidoreductase mitochondrial enzymes activity. The treatment also resulted in a marked decrease in Δψm although no changes in ROS/RNS generation were observed. These findings were supported by phase contrast and by Höechst and Giemsa staining morphological analysis. The highest concentrations caused LDH release suggesting a necrotic process of cell death.

CONCLUSION

To our knowledge, this is the first study on the effects of synthetic cannabinoids in trophoblast cells. Here we evidenced that WIN-55,212 is potentially harmful to placenta and thus to embryonic development and successful pregnancy, reinforcing the importance of cannabinoid signaling in placenta.

[1] Costa, M.A., et al. 2-Arachidonoylglycerol impairs human cytotrophoblast cells syncytialization: influence of endocannabinoid signalling in placental development. Molecular and Cellular Endocrinology, 399, 386-94 (2015)

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PS 10

Wu Yilong

Plenary Session Plenary Session

USING CELL PENETRATING PEPTIDE (CPP) AS A NOVEL STRATEGY TO DELIVER SIRNA INTO LUNG CELLS

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AIM

This study aims to investigate cellular uptake of novel fluorophore-labelled CPPs that localize to the lungs of mice, to demonstrate siRNA-triggered target gene knockdown, as well as to evaluate and to optimize CPP-siRNA conjugates delivery in vitro and in vivo.

INTRODUCTION

Cell penetrating peptides (CPP) are short peptides that have the ability to cross plasma membrane via various mechanisms. They are used as delivery tools for macromolecules and charged molecules. SiRNA are commonly used for gene knockdown in laboratory. However, the major challenge for siRNA in clinical uses is its delivery efficiency and specificity in vivo. In our studies, we described a novel CPP named 33Q, which is derived from gliadin proteins. Since 33Q is resistant to digestive enzymes and specific for lung cells in vivo, it is proposed to be a potential strategy for siRNA delivery into lung cells.

METHODS

33Q labeled with FAM or conjugated with siRNA at N-terminus was incubated with human lung cell lines, as well as PBMC (Peripheral blood mononuclear cells) from subjects. FACS (Fluorescence-activated cell sorting) analysis was performed to quantify the amount of CPPs within cells. CPPs entry was confirmed by confocal microscopy. Dual-luciferase assay was performed to investigate siRNA delivery. CyNA414 labeled 33Q was orally administrated into 8 week old mice. Images of harvested organs were acquired 48 hours after administration. Independent two-sample t-test was performed by SPSS 17.0 with significance level 0.05.

RESULTS

The florescence mean value of mammalian lung epithelial cells is increased by 203.3% ($p=0.022$) after 24 hours incubation with FAM labeled 33Q. The FACS analysis suggests that 53.7% ($p=0.002$) of the cell population has detectable uptake of 33Q. In addition, 18.9% ($p=0.012$) lymphocyte population and 4.0% ($p=0.064$) monocytes population have significant fluorescent signal. Furthermore, endosomes containing FAM labeled CPP can be visualized by confocal microscopy. Interestingly, fluorescence in cytosol is increased by 196.3% ($p=0.006$) according to image measurement. On the other hand, dual-luciferase assay results shows a 72.3% ($p<0.001$) luciferase knockdown effect after 24 hours incubation with siRNA conjugated 33Q. In vivo imaging of organs indicates that fluorescent signal from CyNA414 labeled 33Q localized in the lung tissue 48 hours after administration.

CONCLUSION

In conclusion, 33Q exhibits a unique combination of cell penetration, organ specificity and protease resistance properties. The results highlight its potential as a promising siRNA delivery agent in clinical for the treatment of lung related diseases.

1. Junghans C, Schroff M, Koenig-Merediz SA, et al. Form Follows Function: The Design of Minimalistic Immunogenically Defined Gene Expression (MIDGE®) Constructs. Plasmids for Therapy and Vaccination. 2007:139-146. 2. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. Science. 2002:2275-2279. 3. Köberle C, Kaufmann SHE, Patzel V. Selecting effective siRNAs based on guide RNA structure. Nature Protocols. 2006:1832-1839. 4. Manceur A, Wu A, and Audet J. Flow cytometric screening of cell-penetrating peptides for their uptake into embryonic and adult stem cells. Annual Biochemistry. 2007: 51-59.

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PS 158

Ahmed Nabil Shaaban

Plenary Session Plenary Session

PREDICTORS OF HIV PRE EXPOSURE PROPHYLAXIS AMONG MEN WHO HAVE SEX WITH MEN IN PORTUGAL

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AIM

We aimed to estimate the proportion of MSM eligible for PrEP participating in the European MSM Internet Survey (EMIS) among: 1) Portuguese-born living in Portugal, 2) non-Portuguese-born living in Portugal, and 3) Portuguese-born living abroad, and to identify predictors of eligibility

INTRODUCTION

HIV pre-exposure prophylaxis (PrEP) comprises the use of antiretroviral medications by HIV negative individuals to decrease infection risk. Men who have sex with men (MSM) have higher rates of HIV infection. Among migrants MSM, certain types of cultures, stigma, and barriers to seek medical advice, may increase the risk of acquiring the infection.

METHODS

Data were obtained through EMIS, a cross sectional study that took place from June to August 2010. An anonymous questionnaire was completed online (38 European countries using 25 languages) by 184 469 participants, of which 5187 lived in Portugal (80.7% Portuguese-born and 17.4% migrants) and 375 Portuguese-born lived abroad. Migrants were defined as men who were born in other country than their current country of residence. Information on HIV status, socio-demographic and behavioral characteristics was collected. Eligibility for PrEP was estimated according to the guidelines of the Centers for Disease Control and Prevention. Proportions were compared using the Chi-squared test and Odds Ratios (OR) with 95% confidence interval (95% CI) were computed using logistic regression.

RESULTS

Similar proportions of MSM eligible for PrEP were found among migrants living in Portugal (46.3%), Portuguese-born living in Portugal (44.4%) and Portuguese-born living abroad (45.1%) (p=0.621). In migrants living in Portugal, participants from Latin America and Caribbean region origin were more likely to be eligible for PrEP in comparison to Portuguese-born (OR=1.27; 95%CI 1.04-1.58; p=0.023). Among migrants living in Portugal those Migrants who reported having sex abroad with a man who was not from the country of residence in the previous 12 month were more likely to be eligible (OR=1.72; 95%CI 1.22-2.41; p= 0.002). Migrant MSM who visited a social commercial venue in their country of residence were more eligible for PrEP in comparison to those who never sex venues (OR= 2.14; 95% CI 1.36-3.38; p=0.001). In Portuguese living abroad (table6), individuals whose level of education were middle were more eligible for PrEP (OR= 1.74; 95% CI 1.06-2.86 P=0.029). In the same category, 62.2% of participants who received HIV results in the last 12 month were eligible for PrEP. Participants who received HIV test results in the last 12 months, after exclusion of HIV positive cases, were more eligible for PrEP in comparison to participates who didn't receive HIV results (OR= 1.84; 95%CI 1.17-2.90; p= 0.007). Portuguese-born living in Portugal (table4), and as the other 2 groups, being diagnosed of any STI in the last 12-month increase the odd of being eligible for PrEP. Individuals who had ever undergone post-exposure prophylaxis were more likely to be eligible for PrEP (OR=2.26; 95% CI1.30-3.93; p=0.004). In the same category, participates who identified themselves as homosexuals were more like to be eligible for PrEP in comparison to individuals who identified them selves as bisexuals or other, (OR= 1.46; 95%CI 1.26-1.69; p= <0.001). In addition, having HIV test result in the last 12 months, having sex abroad with a man who was not from country of residence or Consumption of any drugs typically associated with sex and parties in the last 12 month increase the odd of being eligible for PrEP.

CONCLUSION

No differences were found for PrEP eligibility between the three groups, but the predictors for PrEP were different among them

PS 125

Mariusz Ligocki

Plenary Session Plenary Session

HEPATIC RESECTION FOR METASTATIC MELANOMA: THE ROLE OF SURGICAL TREATMENT.

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AIM
The aim of this study was to assess survival and prognostic factors of stage-IV melanoma patients who underwent surgical resection of hepatic metastases.

INTRODUCTION
Melanoma liver metastasis has a very poor prognosis, with median survival shorter than 6 months when unresected. Liver resection for colorectal liver metastases (CLM) has become a gold standard, but value of surgery in patients with melanoma liver metastasis still remains unclear.

METHODS
Clinical data of 38 patients with cutaneous (n=14; 36.8%) and ocular (n=24; 63.2%) melanoma metastasis limited to the liver who underwent hepatic resection in between 1999-2014 was reviewed retrospectively. The long-term survival was estimated with the Kaplan-Meier method and compared with the survival after hepatic resection of CLM evaluated in the same department (n=1,029). Potential prognostic factors including resection margin and interval between the diagnosis of the primary and liver metastasis were evaluated.

RESULTS
Overall survival rates after liver resection at 1,3,5 years were 64.9%, 36.6%, 25.3% for patients with metastatic melanoma and 88.0%, 61.5%, 47.6% for patients with CLM, respectively ($p<0.05$). Improved post-resection survival was observed in patients with microscopically clear resection margins. The median and 1,3,5 years survival of patient after R0 resection (n=19, 50%) was 49.5 months (ranges 7.01- 146.1) and 88.9%, 57.9%, 43.5% compared to the group of patients received R2 resection (n=19, 50%) 13.5 months (ranges 0.7-62.6) and 40.5%, 13.9%, 6.9%, respectively ($p=0.0001$). The second independent positive prognostic factor was time between primary tumour treatment and hepatic secondaries resection longer than 48 months ($p=0.024$).

CONCLUSION
Obtained clinical efficacy of liver resection in patients with metastatic melanoma is not satisfying when compared to metastatic colorectal cancer. Due to the limited treatment options of metastatic liver melanoma, the aggressive surgical approach should be considered when complete resection is feasible. Patients with long interval between primary and liver metastasis melanoma may also benefit from liver resection.

PARALLEL ORAL SESSIONS

OBESITY AS A RISK FACTOR FOR OCCURRENCE OF HYPERTENSIVE DISORDERS OF PREGNANCY

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AIM

Aim of our research was to determine whether high BMI in pregnancy is a risk factor for hypertensive disorders of pregnancy.

INTRODUCTION

Hypertensive disorders affects 5-10% of all pregnancies and they are associated with high rates of morbidity and mortality of pregnant women and the fetus. Obesity is one of the risk factors for hyperetensive disordes of pregnancy. Obesity is a chronic disease and WHO's global health observatory datas shows that in 2008, more than 1.4 billion adults were overweight and more than half a billion were obese. Recent studies shows that the systemic and local inflammatory responses of the body in pregnancy are increased in women who were obese before conception, which is manifested primarily through an increase of CRP level in serum, and this can be cause of hypertensive disorders.

METHODS

This is a retrospective research that was conducted by exploring the medical records in Clinical center of Vojvodina.The study included 69 pregnant women. Criteria for inclusion of patients were pregnant women older than 18 years, with singleton pregnancies. Exclusion criteria were: genetic abnormalities of the fetus, immunological, renal, hematologic, inflammatory and malignant diseases of pregnant women. The study group included 29 women with a BMI higher than 25, and the control group included 40 women with a BMI lower than 25. Study group was divided in subgroups: H1 overweight - 18 women, H2 obese class I - 9 women and H3 subgroup obese class II - 2 women. Blood samples from pregnant women were collected for analysis. Complete blood count was determined by automated haematology analyser, as well as CRP values, by nephelometric method. The amount of uric acid was measured by enzymatic colorimetric method with uricase and peroxidas. The determination of serum urea and creatinine has been done by U.V. Kinetic Method. Hypertensive disorders were confirmed by according standards criterias. All of the statistical tests were performed using SPSS software, version 13.

RESULTS

The prevalence of pregnant women with hypertensive disorders in the study group was statistically significantly higher compared to the control group (44,8% vs 7,5%, p=0,000287). The risk of hypertensive disorders typically increases with increase of body mass index. The levels of CRP, creatinine and urea are statistically significantly higher in the study group ($p=0.0252$, $p<0.0001$, $p=0.0016$). The duration of pregnancy was significantly shorter in the study group, APGAR score (1st and 5th minute) was statistically significantly lower in the study group. There is a higher prevalence of caesarean sections in the study (44.8%) compared to the control group (20%).

CONCLUSION

The results of this study suggest that overweight and obesity appear to be important risk factors for occurens of hypertensive disorders in pregnancy.

PS 31

Zala Novak

Parallel Oral Session Parallel Oral Session

IVABRADINE FOR HEART RATE LOWERING IN PATIENTS UNDERGOING CORONARY COMPUTED TOMOGRAPHIC ANGIOGRAPHY

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AIM

The aim of our study was to determine the efficacy and safety of ivabradine on heart rate reduction prior to coronary computed tomographic angiography (CCTA).

INTRODUCTION

CCTA is a non-invasive method for the diagnosis of coronary artery disease. A reduced heart rate (60 beats per minute or less) during the scan is essential for the optimal image quality, diagnostic accuracy and low radiation exposure. Currently beta blockers are routinely used to achive appropriate heart rate prior to CCTA. Ivabradine is a novel heart rate lowering drug that could be a suitable alternative.

METHODS

Patients referred for CCTA because of anginal symptoms were prospectively randomised to either ivabradine (2,5 – 7,5 mg twice per day) for seven days prior to CCTA or standard medical treatment. The patients' heart rate was measured at eight to fifteen days prior to CCTA and on the day of CCTA.

RESULTS

A total of 101 patients (50 on ivabradine and 51 controls) were included in the study. There were no significant differences in age, sex, risk factors and medical therapy between the two groups. The heart rate of patients on the day of CCTA was 61,1 ± 7,0 (mean ± standard deviation) with ivabradine and 74,7 ± 9,7 beats per minute in the controls (paired Student t test, $p<0,001$). The heart rate was reduced by 15,4 ± 8,6 with ivabradine, whereas it increased by 1,6 ± 11,2 beats per minute in the control group ($p<0,001$). The rate of patients who reached a heart rate of 60 beats per minute or less was 48 % with ivabradine and 8 % in controls ($\chi 2$ test, $p<0,001$). Additional heart rate reduction with beta blocker prior to CCTA was needed in 40 % of patients on ivabradine and in 86 % of controls ($p<0,001$). Mild adverse effects of ivabradine were detected in 7 (14 %) patients.

CONCLUSION

Our findings suggest that ivabradine is a safe and effective drug in reducing heart rate prior to CCTA. Larger proportion of patients achieve recommended acquisition heart rate when pre-treated with ivabradine. Patients pre-treated with ivabradine are also less likely to need additional heart rate lowering with beta blockers prior to CCTA in comparison to patients not receiving ivabradine.

PS 76

Iolanda Jelea

Parallel Oral Session Parallel Oral Session

FATIGUE CORRELATES IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS – A SINGLE CENTER COHORT OF 96 PATIENTS

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AIM

In our study we investigated which of SLE's characteristics could be indicative of a patient's level of tiredness and therefore offer a more thorough understanding of the clinical approach.

INTRODUCTION

Fatigue is one of the most frequent symptoms in systemic lupus erythematosus (SLE) patients (1), affecting a patient's physical as well as emotional wellbeing (2). Although subjective in nature, the Functional Assessment of Chronic Illness Therapy (FACIT) Fatigue Scale is used to standardize a patient's perception of their degree of fatigue and was also validated in SLE patients (3).

METHODS

We prospectively, successively included patients fulfilling the 2012 Systemic Lupus Collaborating Clinics (SLICC) SLE's diagnosis criteria. Disease activity was assessed using Systemic Lupus Activity Measure (SLAM) score and the organ damage by the SLICC Damage Index. Fatigue was determined by FACIT Fatigue Scale; according to this the cohort was divided in two groups (group A and B with low, respectively high fatigue degree).

RESULTS

A total of 96 patients with a mean (±SD) age of 44.5 (± 13.4) years, of which 83 (86.5%) of feminine sex, were included. We found significant differences between group A and B for active articular involvement, alopecia, SLAM score, ESR and creatinine (14/51 vs. 17/14, p=0.001; 18/47 vs. 21/10, p<0.001; 3(0;22) vs. 6(1;24), p<0.001; 14(2;88) vs. 26(4;90), p=0.016; respectively 0.70(0.36; 3.82) vs. 0.76(0.59; 2.69), p=0.033; chi-squared, respectively Mann-Whitney test). Moreover, in univariate analysis by Spearman test, the fatigue degree was significantly associated with ESR (r=0.241; p=0.018), serum creatinine (r=0.253; p=0.014), SLAM score (r=0.462; p<0 .001), and SLICC Damage Index (r=0.333; p=0.001). The analysis by ROC curve has shown that the best predictors for fatigue were SLAM score, SLICC damage index, ESR and fibrinogen [AUC (95%CI): 0.742(0.639; 0.844), 0.618(0.495; 0.741), 0.653(0.530; 0.775), respectively 0.654(0.485; 0.822)]. When logistic regression was applied, only the articular involvement and alopecia were identified as independent predictors for fatigue [p, OR (95%CI): 0.03, 3.27(1.12; 9.53); respectively 0.02, 3.40(1.14; 10.15)] In a model including all factors identified as possible predictors in univariate analysis.

CONCLUSION

Clinically active alopecia and/ or articular involvement are carrying a significant impact on the degree of fatigue in SLE patients and so, the treatment should also focus on these aspects in order to ameliorate the quality of life of SLE patients.

1 - Tench CM, McCarthy J, McCurdie I, White PD, D'Cruz DP. Fatigue in systemic lupus erythematosus: a randomized controlled trial of exercise. Rheumatology (Oxford). 2003 Sep;42(9):1050-4. 2 - Pettersson S, Boström C, Eriksson K, Svenungsson E, Gunnarsson I, Henriksson EW. Lifestyle habits and fatigue among people with systemic lupus erythematosus and matched population controls. Lupus. 2015 Feb 18. pii: 0961203315572716. 3 - Kosinski M, Gajria K, Fernandes AW, Cella D. Qualitative validation of the FACIT-fatigue scale in systemic lupus erythematosus. Lupus. 2013 Apr;22(5):422-30.

PS 84

Patrycja Pieczka

Parallel Oral Session Parallel Oral Session

THE PREVALENCE OF INSULIN RESISTANCE AND ITS IMPACT ON MORTALITY IN DIFFERENT TYPES OF PULMONARY ARTERIAL HYPERTENTION.

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Student Scientific Group on Pulmonary Vascular Diseases and Thromboembolic Diseases

AIM

To assess the prevalence of IR and its impact on survival in three groups of patients with PAH of different patomechanisms: idiopathic pulmonary arterial hypertension with positive acute vasoreactivity test (vIPAH), IPAH with negative acute vasoreactivity test (nvIPAH) and Eisenmenger's syndrome (ES).

INTRODUCTION

Pulmonary arterial hypertention (PAH) is a progressive disease caused by dysfunction of pulmonary arteriols leading to elevated pulmonary artery pressure, right heart failure and death if the diseases remains untreated. Idiopatic pulmonary arterial hypertention (IPAH) and Eisenmenger's syndrome (ES) are the most common types of PAH. Insulin resistance (IR) has been shown to be more prevalent in patients with PAH than in general population. It also predicts mortality in this heterogeneous group of patients.

METHODS

We included 108 non-diabetic patients with PAH including 45 patients with nvIPAH, 11 with vIPAH and 53 with ES. Fasting blood samples were taken twice from each patient to measure serum glucoes, triglyceride and high density lipoprotein cholesterol levels and the mean value was taken for further analysis. TG to HDL cholesterol ratio was used as a marker of insulin sensitivity/resistance and IR was defined as TG to HDL ratio >3.0. Weight and height of every patient was measured to calculate the body mass index (BMI) as weight [kg]/ height [m]2.

RESULTS

We included 108 non-diabetic patients with PAH including 45 patients with nvIPAH, 11 with vIPAH and 53 with ES. Fasting blood samples were taken twice from each patient to measure serum glucoes, triglyceride and high density lipoprotein cholesterol levels and the mean value was taken for further analysis. TG to HDL cholesterol ratio was used as a marker of insulin sensitivity/resistance and IR was defined as TG to HDL ratio >3.0. Weight and height of every patient was measured to calculate the body mass index (BMI) as weight [kg]/ height [m]2.Kaplan-Meier survival analysis proved that IR predicts mortality in patients with nvIPAH (p=0.04) but not in patients with ES (p=0.13).

CONCLUSION

IR occurs more frequently in patients with nvIPAH than in patients with vIPAH or ES. IR is not related to BMI among patients dying because of PAH. IR is associated with increased mortality only in patients with IPAH

PS 104

Yeganeh Pasebani

ASSESSING THE LEFT ATRIAL PRESSURE IN PATIENTS WITH SEVERE MITRAL STENOSIS BY ECHOCARDIOGRAPHY AND CATHETERIZATION.

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Iran University of Medical Sciences

AIM
left atrial pressure in patients with severe mitral stenosis by echocardiography and catheterization is assessed.

INTRODUCTION
Background: Left atrial pressure in patients with severe mitral stenosis not also is a marker of severity of stenosis, but also reprehensive of some complications in these patients. It can be estimated by invasive methods such as transseptal puncture during mitral valvuplasty, and also with non-invasive methods such as transthoracic echocardiography.

METHODS
Methods: 102 patients with significant mitral stenosis who were candidate for mitral valvuplasty were evaluated in this study. Left atrial pressure was measured by transthoracic echocardiography using pulsed Doppler and tissue doppler profile and then directly by trasseptal puncture during valvuplasty.

RESULTS
Results: all of the 102 patients were evaluated (13 men, 89 women). The mean age of the patients was 43.7±12.3. Left atrial pressure by echocardiography was measured by echocardiography was 40.9±12.5 and catheterization 22.8±5.6 mmHg with p value< 0.01. Conventional calculation of left atrial pressure was less reliable compared with a new formula used in this study for calculation of left atrial pressure by E velocity.

CONCLUSION
Conclusion: Our study did not denote that transthoracic measurement of the left atrial pressure can be used as a feasible and reproducible method to assess this parameter in patients with mitral stenosis.

PS 34

Amy Hung

Parallel Oral Session Parallel Oral Session

THE EFFECTS OF SYSTEMIC INFECTION ON THE NEUROPATHOLOGY IN ALZHEIMER'S DISEASE

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AIM

This study will test the hypothesis that systemic infection exerts a change in the neuropathology of Alzheimer's disease (AD).

INTRODUCTION

AD is a progressive neurodegenerative disease, characterised pathologically by abnormal aggregation of amyloid-beta (Aβ) peptides as extracellular plaques and deposition of hyperphosphorylated tau as intraneuronal neurofibrillary tangles (N FTs) in the brain. Evidence from clinical studies indicated that systemic infection, mediated by systemic inflammation and cytokines, contributes to cognitive decline in AD patients. However, little is known whether systemic infection enhances the AD pathology and aggravates neuroinflammation, and hence exacerbates the disease process.

METHODS

Post-mortem neocortex of control (ctrl) and AD patients who died with (ctr n=16; AD n=40) or without (ctrl n=24; AD n=28) systemic infection was provided by BRAIN UK and the SWDBB. Aβ and phospho-tau were assessed by immunohistochemistry; specific monoclonal antibodies 4G8 and AT8 were used, respectively. Quantifying the staining includes capturing 30 fields of each slide, using the ImageJ software and statistical analysis performed by SPSS. Qualitative analysis involves identifying the presence and/or number of specific structures: diffuse and core plaques and vasculature for Aβ and neuropil threads, NFTS and dystrophic neurites for phospho-tau.

RESULTS

Quantitative and qualitative analysis of AD pathologies demonstrated that systemic infection had no significant effects on AD neuropathology; but disease effect was identified. Additionally, these results were correlated with previously collated data on microglial phenotype in AD in the same cohort; microglial markers: Iba1, CD64 and CD68 were assessed. Conversely, an association was found between systemic infection with microglial and AD pathology, notably with phospho-tau.

CONCLUSION

An association between systemic infection, microglial and AD pathology was exhibited, which may contribute to acceleration in AD. However, the exact mechanism remains unclear. As a result, the null hypothesis stating systemic infection exerts a change in the neuropathology in AD can neither be accepted nor rejected with certainty. Besides AD neuropathology, other areas such as neuroinflammation are being explored to enable a better understanding of the role of systemic infection in AD disease progression. Greater insight of the concept can have readily applicable clinical implications.

Many thanks to Dr Boche and Dr Rakic for their support throughout the project. Additional thanks to BRAIN UK and SWDBB for providing the materials and Alzheimer Research UK for funding the work.

PS 51

Catarina Carmo

Parallel Oral Session Parallel Oral Session

ALTERED MITOCHONDRIAL DYNAMICS CAUSED BY SIRTUIN 3 OVEREXPRESSION IN A CELLULAR MODEL OF HUNTINGTON'S DISEASE

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AIM

In this study we aimed to evaluate the effect of sirtuin 3 (SIRT3), the main mitochondrial deacetylase, on mitochondrial dynamics (fission/fusion balance and mitophagy).

INTRODUCTION

Huntington's disease (HD) is an inherited autosomal dominant and progressive neurodegenerative disorder. One of its main characteristics is the specific degeneration of GABAergic medium spiny neurons in the striatum and later, neurons in the cerebral cortex. The HTT gene, which encodes for the huntingtin protein, is affected in HD due to an unstable expansion of CAG trinucleotide repeats in the exon 1. Recently, altered mitochondrial dynamics has been implicated in the pathogenesis of several neurodegenerative disorders, including HD [1]. Sirtuins, NAD+-dependent lysine deacetylases, have emerged as important cellular targets that can interfere with mitochondrial biogenesis, fission/fusion, motility and mitophagy [2].

METHODS

We used striatal cells derived from HD knock-in mice (STHdhQ111/Q111) versus wild-type cells (STHdhQ7/Q7). Proteins involved in mitochondrial dynamics were analyzed by western blotting and immunocytochemistry.

RESULTS

STHdhQ111/Q111 cells presented a significant increase in endogenous mitochondrial SIRT3 levels and activity in relation to control cells. Untransfected mutant cells also exhibited an overall decrease in the levels of mitochondrial fusion proteins (Mfn2, Opa1) and an increase in fission-related Fis1. Drp1 (also fission-related) was preferentially accumulated in the mitochondrial fraction of STHdhQ111/Q111 cells, but its protein levels were significantly decreased in mutant cells. SIRT3 overexpression (OE) appears to decrease Drp1 accumulation in mitochondria and protein levels of Fis1 in STHdhQ7/Q7 and STHdhQ111/Q111 cells, with no differences in the levels of fusion proteins. Parkin, a marker of mitophagy, was also assessed. Untransfected mutant cells exhibited lower parkin levels. Although no significant differences in parkin were found after SIRT3 OE, increased parkin phosphorylation at activating Ser65 was detected in STHdhQ111/Q111-SIRT3 cells. Additionally, increased LC3-II/I ratio (a marker of autophagosome formation), was observed in STHdhQ111/Q111 cells, with an additional significant increase in STHdhQ111/Q111 -SIRT3 cells.

CONCLUSION

Enhanced levels of SIRT3 seem to reduce mitochondrial fission, partially restoring the balance between fission and fusion. Mitophagy might also be activated after SIRT3 OE, although it remains uncertain if the process is completed.

[1] BBA, 2012, 1822:101–10. [2] BBA, 2013, 1832:1345-59.

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Inês Cruz

Parallel Oral Session Parallel Oral Session

XANTHONE AMINO DERIVATIVES WITH POTENTIAL DUAL MODE OF ACTION FOR ALZHEIMER'S DISEASE

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AIM

This work aims to obtain new AChE inhibitors with antioxidant activity based on the xanthonic scaffold.

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent form of dementia. It is known that the malfunctions of different, but interconnected, biochemical complex pathways are related to the pathogenesis of AD. Inhibition of acetylcholinesterase (AChE) is one of the most accepted therapy strategies for AD. Consequently, several AChE inhibitors have been approved for commercial use. Nevertheless, due to the lack of selectivity of AChE inhibitors, AD-patients suffer from side-effects, suggesting that there is a considerable need for development of new clinical tools [1]. The multifactorial nature of AD requires new therapeutic strategies. Among the multipotent approaches, the association between cholinesterase inhibition and antioxidant activity has been considered as an attractive strategy [1]. Considering that concept, this work aims to obtain new AChE inhibitors with antioxidant activity based on the xanthonic scaffold. Here in, we describe the synthesis of 1,8 – dihydroxi-3-methoxyxanthone (X1) and its amino derivative (X2) and the evaluation of their antioxidant and AChE inhibitory activities.

METHODS

The synthetic approach used for the synthesis of 1,8-dihydroxy-3-methoxyxanthone (X1) involves the reaction of 1,3,8-trihydroxyxanthone with methyl iodide in the presence of K2CO3 in anhydrous acetone at reflux (60ºC). The amino derivative of X1 was synthesized by a Mannich type reaction. These compounds were purified by chromatographic techniques and their structure elucidation was established by 1H and 13C NMR. The antioxidant activity was determined using different spectrophotometric assays that evaluate the ability of compounds to scavenge free radicals and to chelate iron and copper ions [2][3][4]. The AChE inhibitory capacity was evaluated using the Ellman's microplate assay with modification [5].

RESULTS

From this study, the derivative X2 emerged as dual agent with antioxidant and AChE inhibitory activities.

CONCLUSION

In conclusion, this amino derivative can be considered a hit compound for the design and synthesis of other xanthones with the referred dual activity.

[1] Cavalli, A.; Bolognesi, M. L.: Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. (2008), Multi-target-directed Ligands to Combat Neurodegenerative Diseases, Journal of Medicinal Chemistry, 51 (3), 347-372. [2]Fukumoto, L. and Mazza, G. (2000). Assessing antioxidant and prooxidant activities of phenolic compounds, Journal of Agricultural and Food Chemistry 48(8), 3597-3604. [3]Dinis, T. C.; Madeira, V. M.; Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, Archives of biochemistry and biophysics 315(1), 161-169. [4]Brown, J.; Khodr, H.; Hider, R. and Rice-Evans, C. (1998), Structural dependence of flavonoid interactions with Cu2+ ions: implications for their antioxidant properties, Biochem. j 330, 1173-1178. [5] Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, Biochemical pharmacology, 7(2), 88-95.

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PS 94

Spas Kerimov

TACKLING THE NEUROINFLAMMATION - IMPLEMENTATION OF SELECTIVE COX-2 INHIBITORS IN COMBINED THERAPY OF EPILEPSY

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AIM

The purpose of this study was to assess the effect of single and combined application of two anti-inflammatory drugs one of which is selective COX-2 inhibitor on pilocarpine-induced epileptogenic activity and markers of brain neuroinflammation. Giving impetus and highlight an important approach to neuroprotection and anti-epiletogenic therapy.

INTRODUCTION

Neuroinflammation provoked by prolonged seizures is emerging as key factor in the epilpeptogenesis. We hypothesized that not only, but multiple proinflammatory pathways are activated after initial epileptogenic event. It is already known that antibiotics, like doxycycline (DOXY) possess not only antibacterial, but also immunomodulating effects. On the other hand, it was found that COX-2 regulates cell membrane excitability and COX-2 inhibitors, like celecoxib (CELE) possess anticonvulsive effects.

METHODS

We selected anti-inflammatory drugs directed against COX-2 - celecoxib, and an antagonist of microglial activation - doxycycline and pretreated the rats, subject of pilocarpine model of epilepsy. Male immature Wistar rats were allocated into 5 groups of 6 animals each (n=6): Negative control group, animals were given saline, 0,9%; Positive control group, animals were administered with pilocarpine hydrochloride; Experimental 1 group animals received DOXY; Experimental 2 group animals received CELE; Experimental 3 group animals received combination of DOXY and CELE. Drugs were administrated via gavage for 7 days and pilocarpine was injected 30 min after last administration in each experimental group. During the observation period of 2h., after pilocarpine administration, the Racine´s scale was used to score the degree of seizures. We also assessed the number of the so called “wet dog shakes” (WDS), which are specific characteristics of experimental seizures in mammals. 24h. later animals were sacrificed and plasma and brains were collected to measure the levels of TNF-alpha and IL-1β by sandwich-ELISA.

RESULTS

None of those anti-inflammatory drugs were effective in attenuating epileptogenic activity and levels of TNF-alfa and IL-1β when administered individually. When combination of these drugs were administered, the combined targeting of COX-2 and microglia resulted in attenuation of increased levels of proinflammatory cytokines, evaluated by ELISA, scores on Racine´s scale and number of WDS. Evaluating our results, a 3D video was created with Molecular Flipboard and Sony Vegas to visualise our hypothesis of multiple proinflammatory pathways that are activated after initial epileptogenic event and their response to combine treatment. Source of the molecules structure was the Protein Data Bank (PDB).

CONCLUSION

Administration of drug combination targeting multiple inflammatory signaling pathways for a limited duration after an inicial insult like SE, may provide an important approach to neuroprotection and anti-epiletogenic therapy. Clinacal importance of our results is that only a specific combination therapy, targeting several proinflammatory pathways provided discernible effect in halting the seizures and the progression of epilepsy.

PS 136

Lucas Lopes

INCRETIN DRUGS HAVE ANTI-INFLAMMATORY EFFECTS AT THE SPINAL CORD OF DIABETIC RATS

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AIM

This study aimed at evaluating the expression of inflammatory mediators and factors involved in cytokines production in type 1 diabetic rats treated with central-acting incretin drugs.

INTRODUCTION

Diabetes is known to affect the central nervous system, namely the spinal cord. Increasing attention has been given to diabetes-induced neuroinflammation as a possible mechanism underlying spinal cord dysfunction. Indeed, microglia hyperactivation and increased expression of pro-inflammatory cytokines were reported in the spinal cord of type 1 diabetic rats. Drugs with anti-inflammatory properties acting in the central nervous system may then be useful to prevent/treat the central effects of diabetes. Incretin drugs are known to elicit glucose-independent anti-inflammatory effects in several organs, namely in the brain. Importantly, these drugs were shown to cross the brain-blood barrier (BBB) and GLP1 receptors were shown to be expressed by spinal microglial cells. We then hypothesized that treatment with incretin drugs may abrogate the exacerbated inflammatory responses detected in the spinal cord of type 1 diabetic rats.

METHODS

Diabetes was induced by intraperitoneal injection of streptozotocin (STZ), whereas control rats (CTR) received vehicle solution only. One week after diabetes induction, STZ rats initiated a treatment with liraglutide (1g/kg subcutaneously) or saxagliptin (10 mg/kg orally) or were maintained untreated for 10 weeks. The incretin drugs used were selected based on their demonstrated ability to cross the BBB. Body weights and blood glucose concentration were evaluated before, at 4 and 10 weeks after diabetes induction. Rats were then decapitated and the L4-L5 spinal segments were removed. The expression of interleukin 1β (IL1β), tumor necrosis factor alpha (TNFα), transforming growth factor β1 (TGFβ1), nuclear factor kappa B (NF-κB) and phosphorilated forms of extracellular signal-regulated kinases (pERKs) was quantified by western blot. The results were compared by One Way ANOVA, followed by Tukey post-hoc test for multiple comparisons. Statistical significance was settled as $p<0.05$.

RESULTS

The STZ rats presented increased glycaemia and lower body weights at 4 and 10 weeks post-injection when compared with CTR rats, which was not altered by treatments. The expression of IL1β and TNFα were significantly increased in untreated STZ rats when compared with CTR, which was accompanied by a significant increase in the expression of pERKs. The STZ rats treated with incretin drugs presented IL1β levels significantly lower than untreated-STZ rats. Although significantly lower than in untreated STZ rats, the expression of pERKs in the spinal cord of incretin-treated rats was still increased when compared with CTR rats. The levels of TNFα were unchanged by treatments and the expression of TGFβ1 and NF-κB was identical in all groups.

CONCLUSION

This study suggests that incretin drugs may reduce the inflammatory insult of diabetes upon the spinal cord, by diminishing the activation of ERKs pathway and preventing the expression of IL1β.

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Małgorzata Kozioł

Parallel Oral Session Parallel Oral Session

DIFFERENCES IN MORPHOMETRIC PARAMETERS OF RUPTURED AND UNRUPTURED INTRACRANIAL ANEURYSMS.

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AIM

The aim of this study was to analyze the morphometric factors differentiating ruptured and unruptured IAs.

INTRODUCTION

The morphometric parameters of intracranial aneurysm (IA) and patent arteries which are associated with aneurysm rupture are poorly described. Recent studies suggest that certain morphometric features and changes in aneurysm morphology are related with their inevitable rupture.

METHODS

The study consisted of 51 patients (aged: 50 to 69) diagnosed between August 2014 and February 2015 who underwent CT angiography. All patients were separated into two groups on the basis of the rupture of IAs: Maximum Intensity Projections, Multiplanar Reconstructions and Volume Rendering Reconstructions were used in order to examine morphological parameters. The calculations were accomplished by using Statistica v. 10.0 software.

RESULTS

We analyzed 51 aneurysms in 51 patients. Forty of them (78.4%) were detected in anterior cerebral circulation, 31 of them (60.8%) were unruptured. Notable diversities between ruptured and unruptured IAs were discoered for: aneurysm dome depth (7.5±2.77 mm vs. 4.66±2,63 mm; $p<0.01$), neck size (4.11±3.35 mm vs. 2.47±0.66 mm; $p=0.01$) and patent artery diameter (2.13±0.34 mm vs. 1.7±0.61 mm; $p<0.01$).

CONCLUSION

Aneurysm dome depth, neck size and patent artery diameter are essentially related with aneurysm IA rupture.

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Verónica Alheia Cabreira

Parallel Oral Session Parallel Oral Session

PERFORMANCE OF LYNCH SYNDROME PREDICTIVE MODELS IN QUANTIFYING THE LIKELIHOOD OF MLH1 MUTATION IN A PORTUGUESE CLINICAL GENETIC SETTING

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AIM

This study aims to evaluate the performance of two predictive models for germline mutation in MLH1 gene in a sample of individuals at risk for Lynch syndrome.

INTRODUCTION

In Portugal, colorectal cancer (CRC) is the second most common diagnosed cancer. Lynch syndrome (LS) is the most common cause of inherited CRC. The primary defect in LS is a germline mutation in one of the four mismatch repair genes (MLH1, MSH2, MSH6 and PMS2). Early LS diagnosis and appropriate CRC surveillance improves mortality caused by LS-associated CRC and other LS-associated tumors. The gold-standard to the diagnosis of LS remains the finding of a pathogenic mutation in one of the MMR genes. All high-risk patients whose tumors show loss-of-function of MMR protein(s) when analysed for microsatellite instability (MSI) and/or by immunohistochemistry (IHC) are submitted to germline DNA mutation testing. However 15% of the cases identified by MSI or IHC are sporadic, due to hypermethylation of the MLH1 gene promotor. Recently, quantitative predictive models including PREMM1,2,6 and MMRpro were developed to facilitate the decision-making process and identify individuals who should actually be referred for molecular investigation. This is especially important in countries with limited economic resources.

METHODS

This study includes 60 members of high risk families for LS, all of which have been referred to genetic diagnosis at Genetics Department of the Portuguese Oncology Institute, Porto. Data including the demographic, clinical and tumor characteristics were retrospectively reviewed for each individual. The results from MSI and/or IHC analysis and the germline mutation test of the index proband tumor were obtained and incorporared into the analysis. Family pedigrees were traced at least to second-degree relatives, with the Cancer Gene 6.0 version software. Each individual's risk of mutation was calculated using PREMM(1,2,6) and MMRpro models. Testing characteristics were calculated for each of the models. To evaluate the sensitivity and specificity of the models, receiver operating characteristic curves were constructed.

RESULTS

We analysed 60 individuals of which 29 (48%) were MLH1 mutation carriers. Areas under the receiver operator curves were 0,80 (95% CI 0,68-0,91) and 0,89 (95% CI 0,80-0,98) for PREMM1,2,6 and MMRpro respectively. Considering a threshold of 5%, the models sensitivity varied between 0.69 (PREMM1,2,6) and 0.97 (MMRpro) and specificity ranged from 0.32 (PREMM1,2,6) to 0.48 (MMRpro). While similar in overall performance, the tests have unique characteristics and MMRpro performed better according to the aim of the study. With MMRpro, at this threshold, there was one missing-case (a man with a CRC diagnosis at the age of 64 without familiar history) and 48% of the performed germline tests could be avoided.

CONCLUSION

These results suggest that the MMRpro model can be used as a screening tool to correctly predict whose individuals should be screened for MLH1 mutation by germline DNA mutation testing. These model may be a feasible alternative to the algorithm followed in Portugal which is not cost-effective and only identifies a subgroup of the mutation carriers. This strategy could simplify the decision-making process and facilitate the implementation of screening programs to high-risk for LS individuals.

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Hugo Fernandes-Silva

Parallel Oral Session Parallel Oral Session

THE ROLE OF RETINOIC ACID SIGNALING IN CHICK LUNG BRANCHING

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AIM

The aim of this work was to characterize, for the first time, the expression pattern of several retinoic acid signaling members, such as raldh2, raldh3, cyp26a1, rarα and rarβ at early stages of chick lung development, by in situ hybridization. Moreover, the effect of exogenous retinoic acid supplementation in early stages of chick lung branching was assessed using in vitro lung explant culture.

INTRODUCTION

Retinoic acid (RA), a derivative of vitamin A, is an essential key player during vertebrate embryonic development which has been associated with several processes such as proliferation, differentiation and morphogenesis of numerous organs, namely the lung. Retinoic acid is obtained from retinol through a series of intracellular oxidative reactions catalyzed by Retinaldehyde Dehydrogenase (RALDH) and its levels are regulated by CYP26A1 (cytochrome P450 family). Once in the nucleus, it interacts with transcription factors of retinoic acid nuclear receptors (RAR), that recognize specific DNA promoter sequences (retinoic acid response elements, RARE) and therefore regulate gene expression. Regarding the fetal lung, RA is recognized as an important molecular player in mammalian pulmonary development. This complex process depends on epithelial-mesenchymal interactions governed by several growth and transcription factors, and seems to be conserved among species. However, little is known about the role of RA in avian lung development.

METHODS

In order to characterize the expression pattern of the retinoic acid signaling members an in situ hybridization was performed. Moreover to study the effect of exogenous retinoic acid, chick lung explants were supplemented with three doses of RA. Explants were assessed for cyp26a1 expression to confirm pathway activation. Furthermore, morphometric/branching analysis (the ratio of the total number of peripheral airway buds between day2 and day0) was determined.

RESULTS

In general, the expression of retinoic acid signaling members raldh2, raldh3, cyp26a1, rarα and rarβ at early stages of chick lung development is similar to their mammalian counterparts. Moreover the morphometric analysis from exogenous retinoic acid supplementation revealed an increase in lung branching in a RA dose-dependent manner and an increase in cyp26a1 expression, assessed by in situ hybridization, confirming that the observed phenotype is due to retinoic acid action.

CONCLUSION

This study demonstrates that retinoic acid signaling is crucial for precise chick lung branching. Moreover, it supports the avian lung as a good model for branching studies due to its similarity to early mammalian pulmonary development, and suggests that despite the differences in the adult lung the molecular mechanisms underlying developmental processes are preserved.

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Ana Filipa Sobral

Parallel Oral Session Parallel Oral Session

CELL CYCLE ARREST AND APOPTOSIS ACTIVATION IN BREAST CANCER MCF-7ARO CELLS INDUCED BY NEW AROMATASE INHIBITORS

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AIM

Bone loss and acquired resistance to aromatase inhibitors (AIs) remain important clinical problems in estrogen receptor (ER)-positive breast cancer treatment and efforts to identify new molecules to overcome this challenge are needed. In this work, newly synthesized steroidal aromatase inhibitors - 49, 50, 51 and 52 - were investigated for their anti-tumor effects in a cell model of breast cancer.

INTRODUCTION

Aromatase inhibitors (AIs) are a class of anti-tumor drugs that block aromatase, the enzyme responsible for estrogens synthesis. Once hormone-dependent (ER+) breast tumors require estrogens to progress, its suppression avoids tumor growth. Three AIs are clinically approved for postmenopausal patients, however, the drawbacks associated motivate the search for new compounds with lower side effects. Our group have been synthesizing and studying different series of steroidal compounds in order to obtain efficacious AIs for potential clinical use [1]. In the present work we characterize four new compounds (49, 50, 51 and 52) in terms of aromatase inhibition and clarify the subjacent anti-tumor mechanisms on ER+ breast cancer cells that overexpress aromatase (MCF-7aro).

METHODS

Aromatase assays were performed in MCF-7aro cells, to study the potential anti-aromatase activity of new compounds, followed by a Western blot analysis to characterize the effects in aromatase expression. Antiproliferative effects were investigated by cell cycle analysis. Cell death mechanisms were explored by measuring reactive oxygen species (ROS) release, mitochondrial transmembrane potential ($\Delta\Psi m$) and caspase-7 activity.

RESULTS

All compounds had the ability to inhibit aromatase activity in more than 70% and, in the case of AI 49, inhibition was accompanied by a reduction in aromatase expression. Moreover, all AIs were able to induce cell cycle arrest: G2/M phase for AIs 49 and 50 and G0/G1 phase for AIs 51 and 52. Also, an increase in caspase-7 activity, along with $\Delta\Psi m$ alterations, was found for all the compounds. However, no alterations in ROS release were observed.

CONCLUSION

All compounds proved to be potent AIs in MCF-7aro cells. AI 49 decreased aromatase expression, suggesting a mechanism of enzyme degradation, similar to steroidal AI Exemestane. The AIs induced apoptosis, possibly via mitochondrial pathway, but ROS independent. Moreover, the retention of cell cycle in different phases indicates the involvement of alternative molecular mechanisms. In conclusion, we obtained four new promising potent AIs with anti-tumor properties in MCF-7aro cells, which are mainly associated to antiproliferative effects and cell death mechanisms.

[1] Amaral, C., et al. (2013) J Steroid Biochem Mol Biol, 135, p. 51-9.

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PS 109

Francisca Soares da Silva

Parallel Oral Session Parallel Oral Session

HES5: A NOVEL PLAYER IN THE SPECIFICATION OF CARDIAC FATE

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AIM

By using the murine embryonic stem cells (mESCs) in vitro system we aim to dissect the mechanisms trough which Hes5 (a novel player we recently identified in cardiac specification) mediates early heart formation.

INTRODUCTION

Heart formation involves a complex crosstalk between various signaling pathways in a temporal and context–dependent manner. The molecular events taking place from pre-gastrulation up to formation of cardiomyocytes are recapitulated in vitro by differentiating embryonic stem cells . Following previous findings demonstrating the role of Notch in specifying a cardiac fate from mesodermal progenitors[1], our laboratory identified a novel function for Hes5, as a downstream effector of Notch1, in the onset of cardiogenesis. Loss and gain of function studies unveiled that Hes5 instructs mESC-derived mesodermal progenitors to commit preferentially towards cardiac over hematopoietic lineages in a confined transient temporal window, in part by promoting the upregulation of Isl1 levels.

METHODS

mESCs with a Doxycycline (Dox)-inducible system for Hes5 overexpression were differentiated into Day (D) 3.75 mesodermal progenitors and Hes5 expression was induced for different time pulses. Contracting cell colonies were counted on D10 of differentiation and gene and protein expression of cardiac maturation markers was assessed.

RESULTS

Hes5 activation in D3.75 mesodermal progenitors at a confined time window results in an enhancement of cardiac specification, however, when activated for periods longer than 120h an impairment in cardiac maturation occurs, as reflected by reduced contracting foci and downregulation of mRNA levels and protein expression of cardiac structural markers vs control. Moreover, Isl1, a cardiac progenitor marker, is upregulated in such conditions. Furthermore, Hes5 endogenous levels transiently upregulate from D4-D6 of in vitro differentiation, correlating with the temporal window we have identified for enhanced cardiac differentiation induced by transient exogenous Hes5 overexpression.

CONCLUSION

Owing to that Isl1 downregulation in cardiac progenitors is required to allow cardiac differentiation [2] we suggest that the continuous promotion of high Isl1 levels blocks the progression in the cardiomyocytic differentiation program. These data show that after cardiac induction Hes5 withdrawal is required to allow cardiac maturation. In conclusion, we herein demonstrate for the first time a role for Hes5 in the specification of cardiac fate. Future studies aim at evaluating Hes5 expression in nascent mesodermal cells during embryo development.

1 Chen, V. C. et al (2008) Nat Biotechnol ; 2 Cai, C. L. et al. (2003) Dev Cell.

PS 113

Vasco Sampaio Pinto

Parallel Oral Session Parallel Oral Session

AN INTEGRATIVE MODEL OF NEONATAL CARDIAC INJURY RESPONSE

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AIM

This work aims to determine (i) the type of response, i.e. regeneration/repair, initiated upon neonatal apex-resection and (ii) the functional impact of this severe injury in a post-birth mammalian heart.

INTRODUCTION

Cardiovascular diseases lead the ranking of lethal causalities worldwide mainly due to limited regenerative capacity of the adult heart. Recently, the existence of a developmental window for mammalian heart regeneration restricted to a short period after birth was demonstrated using the mouse as a model-system. Hearts of 1 day old mice (P1) after surgical apex resection progressively re-established the amputated region with negligible fibrosis. Contrarily, when the same procedure was performed at P7, fibrosis worsened and hearts failed to regenerate [1]. This work has revolutionized the cardiovascular field and has been fully acknowledged by over 300 citations. However, in 2014, controversy was settled when an independent laboratory, while attempting to establish the neonatal apex resection model, reported absence of signals for cardiac regeneration [2]. Overall, the concept of "regeneration" has become frail and unclear in the neonate as the injury outcome is dependent on small variations in the surgical procedure and the formation of a fibrotic scar has since been reported by independent laboratories.

METHODS

Murine hearts (P1.5) subjected to apex-resection were harvested at different time-points following surgery for histological (Masson's Trichrome and Hematoxilin & Eosin) and non-invasive echocardiography. The cellular/extracellular tissue dynamics, triggered upon injury, were evaluated by immunohistochemistry and flow cytometry. Special attention was given to cardiac stroma (e.g. cardiac fibroblasts), an often neglected cell population. The proliferative activity of cells was established and correlated to cellular morphometric parameters.

RESULTS

We demonstrate that neonatal heart response to severe mechanical injury involves the recruitment of inflammatory cells, fibroblasts activation, ECM production and neovascularization. Moreover, our results point towards a partial re-establishment of the removed tissue structure, via proliferation of resident cardiomyocytes. Despite the formation of scar tissue (inner core of the injured area) and incomplete histological restoration, resected hearts remained functionally competent at 21 days post-injury.

CONCLUSION

We hereby propose a model illustrative for the biological events following neonatal apex resection. Overall, our data provides credibility for the use of the neonatal cardiac apex resection model to study the activation of regenerative mechanisms, despite that no complete regeneration is observed.

[1] Porrello, E.R., et al. Science. 2011 Feb 25;331(6020):1078-80; [2]. Andersen, D.C., et al. Stem Cell Reports. 2014 Apr 3;2(4):406-13

PS 131

Gonçalo Brites

Parallel Oral Session Parallel Oral Session

EVALUATION OF THE CYTOTOXIC RESPONSE OF PHOTODYNAMIC THERAPY IN COMBINATION WITH DOXORUBICIN, CISPLATIN, METHOTREXATE AND IFOSFAMIDE IN OSTEOSARCOMA CELL LINE

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AIM

Evaluate the effect of chemotherapy alone and in combination with photodynamic therapy in osteosarcoma cells

INTRODUCTION

Osteogenic Sarcoma (OS) is a malignant tumor that arises from primitive mesenchymal cells and is characterized pathologically by spindle cells and formation of osteoids (unmineralized bones) or bones. Doxorubicin, cisplatin, methotrexate and ifosfamide are chemotherapeutic agents used in OS treatment. Combined treatments may target different key signal transduction pathways and may be more efficient in destroying cancer cells and in eluding the cellular resistance mechanisms. Photodynamic therapy (PDT) is a non-mutagenic therapeutic modality for treating cancer. Several studies reported that combination treatment of chemotherapy and PDT may overcome tumor drug resistance and increase anticancer activity and became a therapeutic approach in cases that surgery is not possible

METHODS

MNNG-HOS (osteosarcoma cell line) was cultured in DMEM supplemented with 10% fetal bovine serum (FBS). For each experiment, cells were plate in 48 multiwells in a concentration of 50000 cells/mL. After 24, 48 and 72 hours, cells were incubated with several concentrations of doxorubicin, methotrexate, cisplatin (0,001-500 μM) and ifosfamide (0,01-150μM). In order to determine the IC50, the cell proliferation was evaluate through MTT assay after 24, 48 and 72 hours of incubation. In order to evaluate the effect of photodynamic therapy in combination with chemotherapy at 72 hours, we incubated the cells with selected concentrations of doxorubicin, of methotrexate and of cisplatin. After 24 hours we administered the photosensitizer (5,15-bis(2-bromo-3-hydroxyfenil)porphyrin), previously synthetized by us and irradiated the cells with a flux of 7,5mW/cm2. 48 hours later we proceeded to MTT assay.

RESULTS

In osteosarcoma cell line, 24 hours after the treatment with doxorubicin, the results showed a decrease of cell proliferation with increasing doses of doxorubicin. The results are similar with 48 and 72 hours. The IC50 obtained for doxorubicin was 31,62nM. When incubated with cisplatin the cell proliferation decrease with low IC50 values (29,51μL). The in vitro studies performed for methotrexate and ifosfamide showed that the IC50 is superior to the higher concentration tested. Regarding the combination studies, our preliminary results showed that 72 hours after doxorubicin/PDT the cell proliferation decreased more than PDT alone.

CONCLUSION

Our study suggests that some chemotherapy agents, alone, have an anti-proliferative effect on osteosarcoma cell line. Analyzing the preliminary results of the combination with PDT the outcome is promising and deserved to be further explored.

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Ana Guerra

Parallel Oral Session Parallel Oral Session

ANGIOGENIC EFFECTS OF STRUCTURALLY-DIVERSE XANTHONES

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AIM

The aim of this work was to investigate if several synthesized xanthones with prenyl (XP13), epoxy (12EPOXI), glycosyl (XG and M) and sulfate (XGS and MS) substituents are able to modulate angiogenesis.

INTRODUCTION

In the last years, natural prenylated and glycosylated flavonoids have been emerging as promising anti-angiogenic agents. Nevertheless, different results were obtained concerning hydrophobic and hydrophilic derivatives. We have been investigating xanthones with antitumor and antiplatelet properties.

METHODS

Endothelial cells (HUVEC) were incubated with 0-500μM of XG, XGS, M, MS, XP13 and 12EPOXI for 24h. Cell's viability was assessed by MTS. Apoptosis and necrosis were evaluated measuring caspase-3 and lactate dehydrogenase by spectrophotometry. Proliferation was quantified by BrdU incorporation. Migration capacity was measured by wound-healing assay. Angiogenic properties were confirmed by quantification of tubular structures formed on matrigel. Variables are expressed as mean ± SEM and were evaluated by T-test and Mann-Whitney test. Results were considered significant when $p<0.05$.

RESULTS

HUVEC exposure to 500μM of XG, XGS, M and MS decreased viability by 10%, and increased apoptosis by 28-36%. 100μM of 12EPOXI and XP13 decreased viability (30% and 70%) and increased apoptosis (76% and 43%) and necrosis (58% for XP13). XG, XGS, M and MS decreased HUVEC proliferation (30-44%, for 500μM) and migration (25-42%, for 100μM and 200μM). HUVEC exposure to 100μM XP13 and 12EPOXI decreased proliferation (69% and 35%) and migration (37% and 29%). All compounds tested decreased formation of tubular-like structures on matrigel by 31-60% for 100-200 μM, being 12EPOXI the most potent, inhibiting the formation of these structures by 70%.

CONCLUSION

All tested compounds seem to manifest anti-angiogenic properties, affecting several steps of the angiogenic process. XP13 and 12EPOXI are more toxic for HUVEC at higher concentrations but seem to decrease tubular-like structures formed on matrigel more efficiently. The anti-angiogenic properties of these xanthones deserve to be better studied as they may be interesting regarding therapeutic targets in pathologies where angiogenesis is deregulated.

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PS 78

Catarina Santos Ribeiro

Parallel Oral Session Parallel Oral Session

HYPERTROPHIC CARDIOMYOPATHY AND CRISPR: A GENOMIC SEWING KIT

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AIM

This work aims to establish a proof-of-principle for a novel, genome editing-based therapy for HCM at cellular level, taking advantage of the CRISPR/Cas system.

INTRODUCTION

Hypertrophic Cardiomyopathy (HCM) is the most common hereditary disease of the heart and the main cause of sudden cardiac death in young athletes. HCM-causing mutations affect genes that encode for proteins of the sarcomere, the functional unit of the cardiomyocytes. There are currently over 1400 HCM-associated mutations in 11 genes. TNNT2 (Troponin T gene) accounts for 10-15% of all HCM cases. At present there is no effective treatment for this disease. Clustered regularly interspaced short palindromic repeats (CRISPR) system and CRISPR associated proteins (Cas) are an emerging genome editing tool that is highly specific and easy to design. This microbial nuclease system uses a programmable RNA-guided DNA endonuclease to create double strand breaks (DSBs). Being an RNA-guided system, it is easily designed by complementarity to the target DNA sequence: a 20 nucleotide sequence adjacent to a protospacer adjacent motif with the form 5′-NGG give this system its specificity.

METHODS

In order to target different sequences within the murine TNNT2 exon 10, different guide RNAs (gRNAs) were designed, using the CRISPR Design Tool. The gRNAs were then cloned into px459 CRISPR/Cas9 backbone cloning construct, containing a wild-type Cas 9. HL-1 and E14 tg2a cells were transfected with those vectors and the cleavage efficiency of each gRNA:Cas9 complex was evaluated by checking the presence of indels (insertions/deletions upon DSB and DNA repair) into the TNNT2 target region. A donor plasmid with an exogenous wild type therapeutic cDNA (Exon 10 – Exon 18), was also constructed and co-transfected with the CRISPR plasmid into E14 tg2a cells.

RESULTS

Good levels of transfection were achieved in both HL-1 and E14 tg2a cells. However, cleavage efficiency in E14 tg2a cells was considerably higher. Two gRNAs showed a high efficiency: G1 and G8. The remaining gRNAs did not seem to induce DSB, making it impossible to assess their efficiency. We were also able to replace the cleavage region by our therapeutic cDNA, as a way of correcting all the mutations downstream from TNNT2 exon 10.

CONCLUSION

By using the CRISPR system we were able to cleave and introduce a therapeutic cDNA into murine TNNT2 exon 10, thus correcting a cluster of mutations present in this HCM-associated gene. This opens the doors to the development of a new genetic therapeutical approach to HCM. Hereafter, this project will aim to test the CRISPR/Cas system in an HCM animal model, to assess a future possible application in patients.

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Rui Ribeiro

Parallel Oral Session Parallel Oral Session

RESTORING THE HEARTBEAT: TRACKING CARDIOMYOCYTE PROLIFERATION

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AIM

This work aims at a detailed characterization of the morphometric dynamics and cell cycle status of cardiomyocytes (CMs), along mouse ontogeny.

INTRODUCTION

Cardiomyocytes (CMs) are the major structural constituents of the heart and are severely affected in a heart failure situation. Through embryogenesis, mononucleated diploid CMs intensively proliferate but shortly after birth undergo terminal differentiation, which comprises sarcomere assembly and morphological rearrangement, from polygonal to rod shape. This correlates with CMs' cell cycle withdrawal and with cytokinesis blockage, culminating preferentially in binucleation or polyploidy (according to the species) and ceasing of proliferation. Subsequently, cardiac growth results from cell hypertrophy. While the mammalian heart has been considered a post-mitotic organ, devoid of intrinsic proliferative capacity, recent reports on CMs renewal in the adult murine and human hearts have challenged the longstanding dogma. However, the cellular renewal's magnitude remains elusive due to tissue complexity, hurdles in isolating adult CMs and unavailability of specific CMs surface markers.

METHODS

In the present work, a novel protocol for CMs isolation was implemented and combined with immunocytochemistry and ImageStreamX to characterize and quantify CMs morphometrics and nuclei dynamics. Immunohistochemistry was performed to assess CMs' cell cycle status and proliferation within the heart.

RESULTS

The optimized isolation protocol renders high yield of well-preserved CMs. Our quantitative analysis indicate a shift in CMs' morphometry: along ontogeny CMs undergo elongation with minimal change in their width, acquiring rod-shape morphology. We also detected the presence of a small fraction of round-shaped CMs at later ontogenic stages. Regarding CMs' nuclei dynamics, our data shows multinucleation as starting around E17.5 that ultimately leads to cells with up to four nuclei at P10. Concerning CMs' cell cycle status evaluation, we observed a gradual decrease of the proliferating CMs during ontogeny, within the different heart regions analyzed.

CONCLUSION

In this study, by combining a novel CMs isolation protocol with imaging flow cytometry we were able to successfully obtain CMs' suspensions and collect quantitative data and imagery, enabling a wide range of novel experiments. This approach constitutes a robust tool to study small cell subsets because it permits the analysis of high number of cells per sample. During ontogeny, CMs gradually cease to proliferate as they phenotypically mature. The identification of a stable and small fraction of immature and putatively mitotic competent CMs within heart is thus of potential therapeutic interest.

PS 132

Sílvia Rodrigues

Parallel Oral Session Parallel Oral Session

DISSECTING CARDIOGENIC MECHANISMS ACTIVATED UPON NEONATAL CARDIAC INJURY

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AIM

This work aims to characterize the neonatal regenerative response in the mammalian heart by the evaluation of cellular/extracellular matrix dynamics including morphometric analysis of the cardiomyocytes' population.

INTRODUCTION

Cardiovascular diseases are the leading cause of death in the modern World [1] and until the past century the mammalian heart was considered a non-regenerative organ. Porrello et al. recently reported that 1-day-old neonatal mice successfully regenerate the heart after apex surgical resection, forming new muscle without generating a scar which contrasts with the extensive fibrosis observed after post-natal day (P)7 [2] and throughout adulthood [3]. However, the signals that determine this shift of regenerative-to-repair capacity after birth and the mechanisms activated following neonatal response to injury are largely unknown.

METHODS

The neonatal cardiac injury inflicted was the apex surgical resection of (P)1.5 mice. Hearts were harvested at different time-points post-resection and processed for histology (Masson's Trichrome stain) and immunofluorescence. Cardiomyocyte (CM) morphology and cardiac fibroblast populations were evaluated by imaging flow-cytometry (ImagestreamX Mark II, Amnis) and flow cytometry, respectively.

RESULTS

At 7 days post injury, the proliferation of CMs is augmented (cTroponin T+ pH3+ cells) in the injury group when compared to sham-operated controls. In line with this, we observed that fibronectin and tenascin-C have distinct deposition patterns and that mitotic CMs are predominantly found in the vicinity of these two extracellular matrix components. In addition, imaging flow-cytometry revealed that immature CMs (coccus-like shape) are more abundant in the apex region of resected animals. Interestingly, the area of mature CMs (rod-shaped) is higher in apex-resected hearts at 7 days post-injury, which is indicative of cellular hypertrophy, whereas at 60 days after resection, no signs of hypertrophy were detected. Alongside CM proliferation, an overall increase in fibroblast numbers following injury orchestrates the formation of a fibrotic scar in the center of the lesion.

CONCLUSION

The resolution of cardiac injury encompasses microenvironmental alterations that trigger CM and fibroblast proliferation thereby culminate, respectively, in the formation of functional myocardium as well as scar-tissue locating to the lesion core.

[1] Celermajer, D.S. et al., J Am Coll Cardiol. 2012; 60(14): 1207-1216. [2] Porrello, E.R et al., Science. 2011 Feb 25 ;331(6020):1078-80. [3] Martin-Puig , S. et al., Ann N Y Acad Sci. 2012 Apr;1254:71-81.

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Renáta Gáspár

Parallel Oral Session Parallel Oral Session

DOXORUBICIN-INDUCED CYTOTOXICITY IN PRIMARY CARDIOMYOCYTE CULTURES

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AIM

Our aims were to establish a model of DOXO-induced cardiocytotoxicity in primary cardiomyocyte cultures and use this model to test potential protective drugs.

INTRODUCTION

Doxorubicin (DOXO) is a widely used antitumor drug. However, the clinical use of DOXO is limited by its severe cardiotoxic effect that is associated with production of reactive oxygen species.

METHODS

Primary cardiomyocyte cultures were prepared from the hearts of 1-3 days old Wistar rats. To assess DOXO-induced cytotoxicity, two days old cardiomyocyte cultures were treated with various concentrations of DOXO (75-1200 ng/mL) for 24 hours. At the end of the treatment period cell viability was measured by calcein assay. In separate experiments one day old cultures were pretreated with the NO-donor SNAP, the superoxide scavenger TEMPOL, or the peroxynitrite decomposition catalyst FeTPPS for 20 hours followed by 300 ng/mL DOXO-treatment for 24 hours. The pretreatments were maintained during the DOXO treatment. At the end of the protocol calcein assay was performed to determine cell viability

RESULTS

DOXO induced dose-dependent cell death in primary neonatal rat cardiomyocytes. The 300 ng/mL concentration of DOXO resulted in an approximately 50% cell death and therefore was chosen to be used for further studies. Neither SNAP nor TEMPOL influenced cell death significantly when compared to the DOXO treated control group (100±5%). However, FeTPPS (79±2%) significantly decreased cell death induced by DOXO.

CONCLUSION

Primary neonatal rat cardiomyocyte culture is a suitable model for testing potential protective agents against DOXO-induced cardiocytotoxicity. Peroxynitrite plays a key role in DOXO-induced cellular injury.

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PS 161

André Leite Moreira

Parallel Oral Session Parallel Oral Session

ACUTE MYOCARDIAL STRETCH REDUCES CARDIAC STIFFNESS THROUGH TITIN PHOSPHORYLATION BY PKG

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AIM

To measure titin phosphorylation levels after acute stretch and testing the influence of PKG-related pathways on the adaptive diastolic response.

INTRODUCTION

Acute myocardial stretch leads to an increase in contractility and a progressive decrease in myocardial stiffness. Titin is the main determinant of passive tension at physiological sarcomere lengths and its distensibility is increased via phosphorylation of its spring elements by cGMP-dependent protein kinase (PKG). PKG can be activated by nitric oxide (NO) and natriuretic peptides (NPs), which are mediators released upon acute stretch.

METHODS

Myocardial strips dissected from left ventricle (n=10) and right atrium (n=14) of patients subjected to cardiac surgery and isolated papillary muscles from rabbit right ventricle (0,2 Hz; 30 °C) were subjected to acute myocardial stretch from 92 to 100% of Lmax (the length associated with maximal active tension). Different groups of rabbit muscles were incubated with Rp-8-Br-PET-cGMPs (PKG inhibitor, 10-6 M, n=7), LNA (NO synthase inhibitor, 10-5 M, n=8), hydroxocobalamin (NO scavenger, 10-3 M, n=8), A-71915 (natriuretic peptide receptor A (NPR-A) antagonist, 10-6 M, n=9), and the three latter drugs simultaneously (n=10). All-total titin phosphorylation was stained with Pro-Q Diamond (phospho-protein) and indexed to total-protein signals using Sypro Ruby;. Values are given as mean ± SEM and statistical significance was set to $p<0.05$.

RESULTS

After acute overload, there was a progressive decrease in passive tension over 15 minutes of stretch: 27±8% and 27±6% in human atrium and ventricular muscles respectively, and 43±2% in rabbit papillary muscles. This decrease in myocardial stiffness was attenuated by 40% after PKG inhibition. Isolated NO synthase inhibition, NO scavenging and NPR-A antagonism did not modify the adaptive diastolic response to stretch. However, a significant (29%) blunting of the effect was observed when the three interventions were exerted simultaneously. Titin phosphorylation increased significantly over the 15 minutes following myocardial stretch in both human (11±1% vs 41±8% in atrium and 27±8% vs 71±21% in ventricle muscles) and rabbit (13±2% vs 23±3%) muscles.

CONCLUSION

The progressive decrease in myocardial stiffness after acute haemodynamic overload seems to depend on PKG activity, which represents potential therapeutic target in patients with pathologically rigid myocardium. Moreover, blocking the NO and NPs systems, which converge on PKG activation, seems to attenuate this adaptive diastolic response. Titin phosphorylation, which is known to increase myocardial distensibility, is probably involved in this new myocardial response to stretch in both animals and humans.

PS 11

Kriti Tripathi

RELEVANCE OF POSTPRANDIAL GLUCOSE TO BODY MASS INDEX AND WAIST CIRCUMFERENCE IN YOUNG NORTH AND SOUTH INDIANS

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AIM

Evaluating Post prandial glucose and its relation to Waist Circumference and Body Mass Index in young apparently healthy individuals of representative populations of North and South India to determine health risks due to lifestyle diseases.

INTRODUCTION

Indians, particularly the youth are at a genetic and environmental risk of developing obesity, Type2 diabetes mellitus and other lifestyle diseases. Ethnic and geographical variations have been observed in North and South Indians.Waist Circumference(WC) mainly defines the abdominal adiposity while Body Mass Index(BMI) the total body fat. Postprandial glucose(PPG) is a good indicator of lifestyle diseases.Hence,this study aimed at evaluating PPG and its relation to WC and BMI in young apparently healthy individuals.

METHODS

The study was a community based observational study conducted in healthy young adults aged 20-30 years consisting of 50 North Indians and 50 South Indians,chosen after ethics approval and informed consent.WC, and BMI were measured following the standard method...PPG was measured using Accuchek glucometer under aseptic conditions.Asian specific BMI and WC cut offs were used for categorizing and analyzing data using Statistical Package for Social Science Version 17.0,Karl Pearsons correlation coefficient and students unpaired “t” test ($p<0.05$ considered significant).

RESULTS

The cohort of North Indians (n= 50, 22.8+2.0 yrs) and South Indians (n=50, 22.1+1.9 yrs) matched for age and sex. There was no significant difference in the BMI (p=0.31),WC (p=0.42) and PPG (p=0.42) levels in both populations. BMI correlated well with WC in both (North: r=0.410,p=0.002;South:r=0.377,p=0.003)and with PPG in the North Indians only (r=0.484,p=0.000). PPG was significantly higher in those with BMI>23 as compared to those with BMI <23 in both North and South Indians whereas WC was significantly higher in BMI >23 group in the South Indians only.

CONCLUSION

North Indian and South Indian apparently healthy youth have similar BMI, WC and PPG values. The BMI has significant influence on PPG in North Indians only. There was no correlation between WC and PPG. Abdominal adiposity (WC) increases dramatically in South Indians as compared to matched North Indians for same BMI. These findings suggest that BMI and WC are entities having different relations to PPG and cannot be used interchangeably but hold separate significance in determining health risks.

PS 37

Manuel Gonçalves Pinho

Parallel Oral Session Parallel Oral Session

BURDEN OF OPHTHALMIC PROCEDURES IN PUBLIC HOSPITALS OF PORTUGAL

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AIM

This study was performed in order to understand the burden of OP in hospitalizations registered in public hospitals in Portugal from 2000 to 2010 and to analyze the evolution on epidemiology associated to these events.

INTRODUCTION

The eye is one of the most exposed structures of the human body, however, to our best knowledge, no study was performed in order to understand trends on Ophthalmic Procedures (OP) and to access their evolution throughout time in an in-patient based population. The study of trends on OP is a valuable asset to understand their role in hospitalizations registered in the different databases and to direct medical policies and education towards the most performed procedures.

METHODS

We performed a retrospective observational study using administrative and clinical data, provided by the Central Administration of Health System of the Portuguese Ministry of Health (ACSS), from a national database. we used the Agency for Healthcare Research and Quality's Clinical Classification Software (CCS) to summarize ICD-9-CM codes into a small number of clinically meaningful groups. For each hospitalization episode identified, the following variables were analysed: age, sex, length of stay(LOS), procedures performed (coded according to ICD-9-CM and grouped in CCS categories), discharge date and status, mean costs and type of admission.

RESULTS

Hospitalizations associated to OP rose 377%, from 28.554 episodes in 2000 to 107.756 in 2010. Lens and cataract procedures were the group of OP with the highest frequency throughout the entire period, reaching 59% of all the hospitalizations analysed, of those episodes, 38% were performed in male patients. The overall median LOS associated to each group of OP decreased in all groups of OP, except for "other intraocular therapeutic procedures" where it remained equal from 2000 to 2010 (1 day). The group of OP with the highest mean charges associated were the "diagnostic procedures on eye" (6.207€).That same group of OP also presented the highest in-hospital mortality (2.69%) and median LOS (7 days). The outpatient surgeries increased in all groups of OP, e.g. lens and cataract procedures increased from 8.1% in 2000 to 92.4% in 2010.

CONCLUSION

From 2000 to 2010 hospitalizations associated to OP increased substantially. In the majority hospitalisations associated to OP the median LOS has decreased and the frequency of outpatient surgery has increased.

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Aleksandra Zdravković

Parallel Oral Session Parallel Oral Session

MEDICAL STUDENTS ATTITUDE TOWARDS HISTOLOGY KNOWLEDGE AND ITS APPLICATION IN PRE-CLINICAL AND CLINICAL MEDICINE

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AIM

The aim of this study is to examine the attitude of senior medical students towards the use of histology in their pre-clinical and future clinical medical practice.

INTRODUCTION

Histology as a basic pre-clinical subject is a necessary prerequisite for understanding the complexity of cell and tissue organization and function. Certain subjects relay heavily on medical students histology knowledge, since it represents the basis for understanding normal physiological and pathological processes within different diseases. Also, the application of histology in clinical practice is of special importance, as it is a part of needed education with numerous areas in clinical medicine.

METHODS

The study was done at the School of Medicine, University in Belgrade. A total of 370 senior medical students at their final sixth year, participated in the research. Data were collected using a short anonymous questionnaire which explored students attitude towards histology as a medical subject. Each answer was rated on a five-point Likert scale, where rank 1 referred to "I strongly disagree" and rank 5 referred to "I strongly agree". The statistical analysis was done using descriptive statistical methods.

RESULTS

: Students average grade during their studies (scale 6-10) was 8.4 ± 0.7 while the average grade in histology (scale 6-10) was 7.95 ± 1.23. Given results show that 29.7% (110) of the students agree or strongly agree that histology is an important subject for the proper understanding and mastering of physiology, pathology (90.8%(336)) and dermatology (37% (137)). Largest proportion of students (69.5% (257)) agreed or strongly agreed that histology was of great importance for their medical education, while 47.3% (175) of students agrees or strongly agrees that histology is important for future clinical medical practice. An interesting result is that 42.2% (156) of students think that they alone haven't spent enough time studying histology during their medical studies.

CONCLUSION

Taking into account the obtained results we can conclude that medical students have a positive attitude towards histology. The data show that after completing their exam in histology, students were able to use the acquired histology knowledge for successful mastering and understanding of other pre-clinical and clinical medical subjects, such as physiology, pathology, dermatology, neurology, pediatrics and surgery. This positive attitude should be used as an additional motive for further development of histology courses, with special emphasis on the practical application of acquired histology knowledge in clinical medical practice.

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Kughan

Parallel Oral Session Parallel Oral Session

HIV SURVEILLANCE IN RUSSIA-POSITIVES AND NEGATIVES AND THEIR IMPACTS ON EPIDEMIOLOGY OF HIV INFECTION

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AIM

(a)To distinguish possible advantages and disadvantages of HIV surveillance system in the Russian Federation. (b) To determine the effects of insufficient surveillance system on changes in incidence rates, prevalence rate, modes of transmission and distribution by sex of HIV infection in Nizhny Novgorod Region, Russia from 2006-2010/12. (c) To compare changes in incidence and prevalence rates of HIV infection between Nizhny Novgorod Region (city), Volga Federal Region (district) and Russian Federation (country.

INTRODUCTION

According to UNAIDS 2011, there are 2.7 million new HIV infections worldwide in 2010, which means that annual new HIV infections fell by 21% between 1997 and 2010 and improving globally. However, since 2001, HIV prevalence in Russia, Eastern Europe and Central Asia has increased by 250 percent, making the region home to the world's most rapidly expanding epidemic. It has been widely discussed in National Infectious Disease meetings in the Russian Federation that the surveillance system of HIV in this country sometimes is not sufficient and has some disadvantages.

METHODS

a)Peer-reviewed articles, conference proceedings and technical reports published from 2006-2012 were reviewed for information regarding inadequate surveillance system in Russian Federation. (b) Information regarding incidence and prevalence rate (per 100000 population), modes of transmission (percentage,%) and distribution by sex (percentage,%) of HIV infection from 2006-2010/12 for Nizhny Novgorod Region (NNR), Volga Federal Region (VFR) and Russian Federation (RF) is obtained from Centers for Disease Control and Prevention of Nizhny Novgorod Region. (c) A statistical analysis is made using software EpiInfo 7 and Microsoft Excel 2007.

RESULTS

(a) HIV surveillance system in the Russian Federation has some advantages and disadvantages. (b) From 2006-2012, incidence rates of HIV infection in NNR increased from 12.4 to 53.2 per 100000 population($p<0.001$) and in RF from 31.1 to 46.8($p<0.001$). In VFR, incidence rate during 2006-2009 increased from 31.5-41.5($p<0.001$) but decreased to 34.1 in 2010. (c) Prevalence rates of HIV infection in NNR increased from 125.6 to 225.8 per 100000 population ($p<0.001$), more drastically in RF from 244.4 to 453.4 ($p<0.001$) and in Volga FR from 283.6 to 412.1 ($p<0.001$).(d) Analysis showed that there are increases in placental (*2.3%-3.8%) and sexual pathway (*35.4% to 42.3%) of transmission whereas a decrease in parenteral pathway (*62.3% to 53.9%). *$p<0.001$ (e) Number of infected males increased from 56.3% to 60.1%($p<0.001$) and infected females decreased from 43.7% to 39.9%($p<0.001$).

CONCLUSION

Insufficient surveillance system can result in high number of incidence rates in NNR, VFR and RF and pronounced increasing trend of prevalence rate in VFR and RF from 2006-2010/12. The disadvantages in surveillance system can also be related to increases in placental and sexual modes of transmission and infected male sex distribution. As such, an improved surveillance system is needed for proper treatment, control and prevention of HIV infection.

PS 128

Joanna Radzimowska

ETIOLOGICAL AND EPIDEMIOLOGICAL ANALYSIS OF SUPERFICIAL FUNGAL INFECTIONS IN SOUTHERN POLAND, INCLUDING THEIR AGE AND GENDER-RELATED PREVALENCE- A 5-YEAR STUDY.

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Students' Scientific Group, Department of Dermatology

AIM
The aim of the study was to determine the prevalence and the clinical patterns of superficial fungal infections and dependence of their prevalence upon age and gender.

INTRODUCTION
Fungal infections of skin and nails are important social and therapeutic problem. 20 - 25% of the world's population has skin mycoses, making them one of the most frequent forms of infection. Their incidence continues to increase.

METHODS
Mycological research results of 4608 materials collected from patients from 2010 to 2014 were analyzed in Mycology Research Laboratory in Krakow. 60.7% of the materials was collected from women and 39.3% from men aged 39.8 +/- 19.6 (range 0.5 - 95 years old). The performed analysis was retrospective. The research material was collected from places pathologically changed: nails, feet, smooth skin, scalp and mucous membranes. The collected material was used to perform direct preparation and start a culture. The results were analysed using Microsoft Office Exel 2007. Continuous variables were compared with U Mann-Whitney test and categorial variables were compared with Chi-square test (p-value < 0,005).

RESULTS
Among 1765 positive cultures, dermatophytes were the most common isolates (49%), followed by yeasts (40%) and non-dermatophytic molds (9%). T. rubrum (86%) and T. mentagrophytes (10%) were the most prevalent species among dermatophytes with most frequent location on toenails and interdigital spaces of feet. The most common yeasts were C. albicans (26%), Rhodotorula spp. (18%) and C. glabrata (18%) mostly isolated from toenails and fingernails. The non-dermatophytic molds observed in the study were S. brevicaulis (40%) and Aspergillus spp. (32%). Their most frequent locations were on toenails. The distribution frequency of the positive cultures was 43% among men and 57% among women. The incidence of positive results increased with age of examined patients. Patients with confirmed yeasts infection were significantly younger in comparison to patients infected with dermatophytes and molds (p <0,001). Yeasts infections were observed more frequently among older patients. Dermatophyte infection occurred significantly more often among males than females. Yeasts infections and of mold infections occurred significantly more often among women than men.

CONCLUSION
Dermatophytes were the most frequently isolated fungi. Trichophyton rubrum. was the most frequent causative agent. Toenails were the most prevalent location. Fungal infections of skin and nails were more common among females and older patients. Fungal-yeast infections were more common among women and in younger age groups. Mold infections predominated especially among elderly females. Dermatophyte infections occurred significantly more frequently among men.

PS 162

João Vasco Santos

Parallel Oral Session Parallel Oral Session

CLINICAL AND EPIDEMIOLOGICAL BURDEN OF HIDROADENITIS SUPPURATIVA IN PORTUGAL: 1,391 PATIENTS FROM 2000 TO 2010

1,2 - Santos JV, 3 - Lanna C, 2,4 - Lisboa C, 1,2 - Costa-Pereira A, 1,2 - Freitas A

1 – Department of Health Information and Decision Sciences, Faculty of Medicine, University of Porto, Portugal; 2 – Center for Health Technology and Services Research (CINTESIS), Portugal; 3 – Faculty of Medicine, University of Rome "Tor Vergata"; 4 – Department of Microbiology, Faculty of Medicine, University of Porto, Portugal

AIM

We aimed to study the clinical and epidemiological burden of HS in an European country as Portugal, between 2000 and 2010.

INTRODUCTION

Hidroadenitis suppurativa (HS), a chronic inflammatory skin disease of the hair follicle, is an increasing epidemiological problem without a simple diagnosis and an effective treatment. Despite HS has a great impact in either personal and social or economic perspectives, few epidemiological studies been performed either in Europe or across the world.

METHODS

We performed a retrospective observational study using a national administrative database. We included all hospitalization (inpatients) and outpatient surgery episodes between 2000 and 2010 with a principal or secondary diagnosis of HS. Comorbidities and procedures were studied based on ICD-9-CM codes. Incidence was extrapolated to calculate prevalence (prevalence = incidence x duration), postulating HS duration of 20 years.

RESULTS

A total of 1,391 patients with HS between 2000 and 2010 resorted to Portuguese hospitals for hospitalization or ambulatory surgery, leading to 1,037 and 670 episodes, respectively. It represents a mean incidence rate of 1.2 patients per 100,000 years and a mean hospitalization rate of 0.7 hospitalizations/100,000 in-habitants/year which lead to an estimate of 0.09% of HS prevalence. Male: female ratio was of 1:1.7 and patients between 20 and 39 years had more HS incidence. Tobacco use, hypertension, diabetes and obesity were the most associated comorbidities and surgery was performed in 88% of patients.

CONCLUSION

Our study, the first nationwide hospital-based or during a long time period to our knowledge, estimated a prevalence of 0.09% in Portugal, similarly to US.

PS 25

Blaz Kacijan

Parallel Oral Session Parallel Oral Session

KINEMATIC ANALYSIS OF THE KNEE JOINT WITH ANTERIOR CRUCIATE LIGAMENT INJURY OR KNEE OSTEOARTHROSIS USING 3-D LASER TRIANGULATION

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AIM

The aim of our study was to determine the suitability of noninvasive laser triangulation method for quantitative assessement of translation of the tibia relative to femur during the movement of healthy and pathologically changed knees. We wanted to determine the possibility of quantifying the degree of instability of the knee joint with laser triangulation method used in patients with varying degrees of instability with respect to pathology.

INTRODUCTION

To assess the instability of the knee joint a number of clinical tests are being used. Routinely used clinical tests lack objectivity as their interpretation depends on the investigator. More objective mechanical tests require an invasive procedure and only enable evaluation in a fixed knee position. Thus there is a need for an objective noninvasive method for the quantification of the degree of translation of the tibia relative to femur. Laser triangulaton method allows us to measure a surface profile with high accuracy, which could help us assess the translation of the tibia.

METHODS

The study included 17 patients with clinically proven pathology of the knee joint. Using the laser triangulation method we measured hysteresis values during the open kinetic chaim movement of the knee joint. The values were then computer-processed and statistically analyzed.

RESULTS

We performed 1382 measurements. 688 (49,8%) withouth weights and 694 (50,2%) with weights. 331 (24,0%) measurements were performed on healthy knees, 259 (18,7%) on knees with anterior cruciate ligament (ACL) injury, 706 (51,1%) on osteoarthrotic knees and 86 (6,2%) on knees with total endoprotesis (TEP) implantation. Average value of normalised hysteresis on measurements withouth weights was 16±15 for healthy knees, 16±13 for knees with ACL injury, 6±7 for osteoarthrotic knees and 3±3, for knees with TEP. Average value of normalised hysteresis on measurements with weights was 18±13, 20±12, 6±6 and 3±1 for healthy knees, knees with ACL injury, osteoarthrotic knees and knees with TEP respectively.

CONCLUSION

Our findings suggest clinically appropriate reapetability of the laser triangulation method used for measuring the translation of the tibia relative to femur. We were unable to demonstrate increased translation of the tibia relative to femur in the knees with ACL injury in comparison to healthy knees. The laser triangulation method has proven lesser translation of the tibia relative to femur in osteoarthrotic knees and knees with TEP implantation in comparison to healthy knees.

PS 64

Anna Kot

Parallel Oral Session Parallel Oral Session

ENDOVASCULAR TREATMENT FOR CEREBRAL ARTERIOVENOUS MALFORMATION: PREDICTORS OF MULTIPLE STAGE TREATMENT.

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AIM

We tried to establish if there is any association between AVM localization or feeding arteries and endovascular treatment stages.

INTRODUCTION

Arteriovenous malformations (AVMs) are generally asymptomatic, but still can manifested as seizures, focal deficits and most often pain or bleeding. There are three main types of treatment: surgery, endovascular and radiotherapy which can be use simultaneously to improve outcomes. Patients with large, complex AVMs oftenly require multi-stage embolization for accurate and total closure of the malformation. The factors associated with need of multi-stage endovascular treatment are still not well described. In every case localization, feeding arteries and drainage are important to qualify to proper treatment.

METHODS

Study group consisted of 68 patients treated with endovascular procedure for AVM in Department of Neurosurgery and Neurotraumatology Jagiellonian University Medical College. We reviewed patients medical and radiological records with special attention to malformation location, feeding artery patterns, venous drainage. Each patient was evaluated using Spetzler-Martine score. We used t-test and $\chi 2$-test as appropriate to determine factors associated with requirement of multiple-stage embolization.

RESULTS

Twenty-three patients (33.82%) required multiple stage embolization. The need of multiple-stage treatment was associated with AVM being feed by anterior cerebral artery (22.73 vs. 6.98%; p=0.04) and cerebellar arteries (22.73 vs. 6.98%; p=0.04). Patients that had AVM in parietal region were less likely to be in need of multiple-stage treatment (10.00 vs. 43.90%; p=0.008). What is interesting female patients were also less likely to need multiple-stage treatment (21.74% vs. 44.44%; p=0.04).

CONCLUSION

AVM that possesses feeding arteries originating from anterior cerebral artery and cerebellar arteries more often require multi-stage embolization. Patients with AVM located in parietal region are less likely to need multi-stage embolization.

PS 69

Alicja Lachowska

Parallel Oral Session Parallel Oral Session

TIME IS (NOT?) A GREAT HEALER - DAY OF SURGERY AFFECTS LENGTH OF STAY.

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AIM
The aim of the study was to analyze how the day of the week on which a surgery is performed affects postoperative length of stay.

INTRODUCTION
It is well established for now that factors like late night procedures or weekend admittance have great influence on mortality, morbidity and hospitalization. However, effects connected with the day of the week on which a surgery is performed have not been thoroughly investigated. Our objective is a deeper examination of such effects.

METHODS
This is a retrospective study of a database of one surgical department containing all procedures from January 2005 to September 2013. Three groups of patients who underwent procedures with 2, 3 and 4 days median length of stay (LOS were isolated). Exploratory data analysis was performed to identify patterns of LOS depending on the day of the week a surgery was done. Chi-square test, logistic regression and Kruskal-Wallis test were used to determine significance of observed effects. The regression model included patients' demographics, comorbidities, a surgery duration and postoperative complications. For a better understanding of observed patterns four homogenous cohorts were carefully selected and analysed: elective cholecystectomy and emergent appendectomy, both of them open and laparoscopic.

RESULTS
Patients who underwent a procedure with 2 days median LOS (n=8559), when operated on Friday, are less frequently discharged within the first 3 postoperative days (OR=1.99, $p<0.001$). Similar effects were observed respectively for 3 days median LOS (n=912), Thursday patients, 4 postoperative days (OR=2.51, $p=0.001$) and for 4 days median LOS (n=1224), Wednesday patients, 5 postoperative days (OR=1.86, $p=0.008$). As for more specific groups, this effect was proved in case of laparoscopic cholecystectomy and open appendectomy but was not observed in case of laparoscopic appendectomy. Our results were inconclusive for open cholecystectomy. Moreover, Friday open appendectomy patients stay in the hospital for 0.64 days longer on average ($p=0.026$) and are less frequently discharged within the first 3 postoperative days (OR=2.49, $p=0.011$).

CONCLUSION
Since in our setting Sunday is not a day of standard discharge, there should be one-day delay of administrative nature for patients supposed to be discharged on Sunday according to a standard LOS for a specific procedure. However, a significant part of patients has even longer delay than expected when their early postoperative period contains weekend. Furthermore, Friday appendectomy patients have significantly longer hospitalization, probably due to late week surgery and weekend recovery.

PS 88

Jiang Bochao

Parallel Oral Session Parallel Oral Session

PREDICTORS OF SURVIVAL IN AMPULLARY CARCINOMAS

1- Bochao Jiang., 2- Choo Su Pin

1-Yong Loo Lin School of Medicine, National University of Singapore, 2- National Cancer Centre Singapore

AIM

To identify the main prognostic factors for disease recurrence after surgical resection of ampullary carcinomas, better characterize the patient profile in South East Asia, and potentially guide clinical decision making.

INTRODUCTION

Ampullary carcinomas (AC) are rare cancers that arise from the vicinity of the ampulla of Vater. There is increasing evidence to suggest that AC are biologically more analogous to intestinal rather than pancreaticobiliary carcinomas. True ACs have a better prognosis than periampullary carcinomas of pancreaticobiliary origin. This study aims to identify the main prognostic factors for disease recurrence after surgical resection with a detailed analysis of clinical, pathological and immunohistochemical parameters, so as to better characterize the patient profile in South East Asia, potentially aid in the selection of patients who may benefit from adjuvant therapy, thus helping to guide clinical decision making.

METHODS

A total of 71 patients diagnosed and treated at National Cancer Centre Singapore from 1999 to 2014 were included in the study and analysed retrospectively. All patients had histologically proven ampullary carcinoma and underwent potentially curative pancreaticoduodenectomy (PPPD or Whipple's procedure). Overall survival (OS) and relapse-free survival were estimated using the Kaplan-Meier method (Log-rank test) and Cox-regression method. A two-sided p-value of <0.05 was taken as statistically significant. All statistical analyses were performed using IBM SPSS Statistics Version 21 (SPSS Inc. Chicago, IL).

RESULTS

The median age at diagnosis was 63 years, with 54.9% being males. The median relapse-free survival (RFS) and overall survival (OS) were 17.8 and 21.1 months respectively. Factors prognostic of RFS by univariate analysis included primary resection outcome (R0 vs R1), perineural invasion, lymphovascular invasion, post- operative CA 19-9 (</=37 vs >37 umol/L), and pre-operative Neutrophil: Lymphocyte ratio (</=5 vs >5). Only two variables were found to be significant when multivariate analysis was performed: Primary resection outcome (R0 vs R1) and pre- operative Neutrophil: Lymphocyte ratio.

CONCLUSION

R1 resection and elevated pre-operative Neutrophil: Lymphocyte ratio (>5) have been identified as independent prognostic factors of recurrence in ampullary carcinomas in our patient population. These results suggest potential prognostic factors beyond traditional pathological ones, which could be derived from currently available studies that are relatively limited in the South East Asian context. Results of immunohistochemical analysis with CDX2 are expected to produce meaningful predictions of recurrence and survival. We aim to validate our findings in a prospective cohort of patients.

1. Hamed MO et al, Elevated pre-operative neutrophil to lymphocyte ratio predicts disease free survival following pancreatic resection for periampullary carcinomas. 2. Niraj Kumari et al, Intestinal and pancreatobiliary differentiation in periampullary carcinoma: the role of immunohistochemistry , Human Pathology (2013) 44, 2213–2219 3. Jonathan T Carter et al, Tumors of the Ampulla of Vater: Histopathologic Classification and Predictors of Survival, American College of Surgeons Vol. 207, No. 2, August 2008 4. C.A.M Sommerville et al, Survival Analysis After Pancreatic Resection for Ampullary and Pancreatic Head Carcinoma: An Analysis of Clinicopathological Factors, Journal of Surgical Oncology 2009; 100:651–656

Leong Fun Loon (Research Coordinator, National Cancer Centre Singapore)

PS 93

Anna Ł askawska

Parallel Oral Session Parallel Oral Session

PATIENTS' VIEWS ON THE ENHANCED RECOVERY AFTER SURGERY PROGRAMME.

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AIM

The aim of this study was to evaluate the perception of ERAS protocol implementation from patients` side.

INTRODUCTION

The Enhanced Recovery After Surgery is a programme of comprehensive perioperative care of patients undergoing elective surgery.

METHODS

The study included 87 patients (44 men and 43 women) operated in 2nd Department of Surgery JUMC between October 2014 and February 2015. The patients completed pre- and postoperative questionnaire. In the questionnaire we used ten-point scale to evaluate importance of the individual ERAS principles. Two weeks after discharge the patients also answered yes or no for several questions during telephone conversation.

RESULTS

The most important elements of ERAS protocol according to the patients were 'ability to contact the doctor after the discharge' (mean 9.64), 'perioperative counseling by nurse, anesthetist and surgeon' (mean 9.46), 'to be completely free of pain' (mean 9.41), 'using the least invasive techniques during operation' (mean 9.39). 'To start eating and drinking as soon as possible' and 'reducing the number of drips' were evaluated as the least important (mean 6.95 and 4.25 respectively). At the discharge, what changed the most was the evaluation of avoiding bowel preparation preoperatively (mean 5.56 at admission vs 7.17 at discharge). The patients had the greatest difficulty with 'abdominal pain, including pain around the wound' (median 6), and the least with 'ability to move ' (median 3). Two weeks after discharge the patients noted that they appreciate this form of care (98.81%) and they would recommend ERAS programme to others (98.81%). 4 patients (4.76%) felt the need for longer hospital stay.

CONCLUSION

The patients have positively evaluated ERAS program. Their opinion did not change after surgery. Also, 2 weeks after discharge, the patients confirmed their favorable opinions on this protocol. In postoperative period the patients had the biggest problem with abdominal pain.

PS 118

Daromir Godula

Parallel Oral Session Parallel Oral Session

INFLUENCE OF TIME PERIOD ON THE NUMBER OF ADMISSIONS TO ER AND PATIENTS' OUTCOMES

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AIM

The aim of our study was to evaluate whether knowledge of time periods with highest number of patients presenting symptoms of acute abdomen could help to provide proportional number of staff on-call and available operating rooms (OR).

INTRODUCTION

Emergency rooms (ER) are available for the patients 24/7. A significant part of admitted patients are those with symptoms of acute abdomen. They require urgent surgical consultations, radiological examination and often an operation.

METHODS

Medical records of patients admitted to 3rd Department of General Surgery JUMC in Cracow, Poland between January 2012 and December 2012 were collected retrospectively. The Department provides surgical on-calls 24/7. A group of patients admitted in the emergency setting due to symptoms of acute abdomen was selected. The aim of our paperwork was to analyze the cause of admission, basic epidemiological data and its correlation with days of the week and time of admission (all patients were divided in 3 groups: I - 8.00-15.59, II - 16.00-23.59, III - 0.00-7.59).

RESULTS

During the study period 2294 patients were treated in our Department. 561 of them (50,5% males) were admitted in emergency setting with symptoms of acute abdomen. In this group median age was 59 years (range: 15-100). The highest number of patients (96) was admitted on Sunday and the lowest (68) on Wednesday. This value was close to reaching statistical significance (p=0,052). Day of the week does not have influence on complications, mortality or length of stay. The least number of patients was admitted between 8.00-15.59 (15,7%), followed by 0.00-7.59 (38,9%) and 16.00-23.59 (45,4%). Median time from admission to surgical ward and the beginning of the procedure was the longest in the group I (337 min. vs. 228 and 239 in groups II and III respectively, p=0,01). Time from admission to ER to transfer to surgical ward was constant regardless to day of the week or the time.

CONCLUSION

Number of patients admitted to ER with symptoms of acute abdomen depends on the time. The least number was admitted between 8.00-15.59. It could be caused by the fact that patients with acute abdomen firstly seek for medical advice in their family doctor's office which office hours cover forementioned time period. Moreover, patients admitted in that time period had to longer wait for the operation. It may be due to elective procedures performed in OR at the same time. Although, it was not associated with higher mortality or morbidity. Day of the week may have influence on expected number of admitted patients however it does not affect the outcomes.

POSTER PRESENTATION

PS 97

Konstantin Dotsenko

Poster Presentation Poster Presentation

HYPOCHOLESTEROLEMIA IN NAÏVE HIV-INFECTED PATIENTS AND ITS ROLE IN THE DEVELOPMENT OF BACTERIAL COMPLICATIONS

Konstantin Dotsenko, Ekaterina Kukhta, Dmitry Aliaxeychik
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AIM

To evaluate the role of total cholesterol (TC) level as a prognostic factor at immunodeficiency conditions.

INTRODUCTION

HIV-infected patients with different comorbid pathologies (community-acquired pneumonia), who are not placed on ART, are characterized by dyslipidemia. This phenomenon might appear to have prognostic value.

METHODS

The levels of total cholesterol in patients with HIV-infection and HIV-infection in combination with community-acquired pneumonia (2009-2013) were analyzed. The cohort included 179 patients with HIV-infection who were not receiving ART (group 1), 192 patients with bacterial pneumonia and HIV-infection (group 2). The levels of TC in different clinical stages of HIV-infection and different clinical variants of community-acquired pneumonia were compared. The data were processed by statistical methods using the program "SPSS 20.0"; Chi-squared tests and multivariable linear/logistic regression.

RESULTS

Among HIV-infected patients, 67% were men, mean age 32 (18-46). Among HIV-infected patients with pneumonia – 58% men, mean age 34.5 (22-47). The duration of establishing the diagnosis on HIV-infection in the groups was 6 (3-9) and 5 (2-8) years respectively. Patients with severe pneumonia had significantly lower TC level than the patients with mild community-acquired pneumonia (3.11±0.78 mmol/L vs. 4.74±0.84 mmol/L, $p<0.01$ up to 45 years and 4.33±0.89 mmol/l vs. 5.09±0.98 mmol/l, $p<0.01$ after 45 years). Risk of severe pneumonia with concomitant hypocholesterolemia increased by 1.9-fold in patients up to 45 years old, and 1.2-fold in patients after 45 years. (OR 1.9, $p=0.001$; OR 1.2, $p=0.01$). Patients at 1-2 stages of HIV infection had higher TC level (4.62±0.18 mmol/L), CD4+-lymphocytes (269±22.53 cells/mL) and lower viral load (68431.53±22173.98 copies/mcL) than patients at 3-4 stages of HIV-infection (4.04±0.24 mmol/L, 199.56±26.51 cells/mL, 148639.62±64462.02 copies/mcL respectively). The risk of severe bacterial complications at 3-4 stages of HIV-infection increased by 2-fold in the presence of hypocholesterolemia (OR 2.0; $p=0.001$).

CONCLUSION

The results of the study indicate that total cholesterol level can be used as a criterion for the assessment of bacterial complications severity.

PS 107

Szymon Ciuk

Poster Presentation Poster Presentation

PRIMARY SCLEROSING CHOLANGITIS AND AUTOIMMUNE HEPATITIS – ARE THERE SIMILARITIES ?

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AIM

The aim of the study was to compare the clinical manifestation of these two autoimmune diseases affecting alimentary system in terms of possible similarities.

INTRODUCTION

Primary sclerosing cholangitis (PSC) is a progressive disease of the bile ducts that causes inflammation and subsequent obstruction of bile ducts both inside and outside the liver, which can ultimately lead to, liver failure, cirrhosis of the liver and liver cancer. It is characterized with elevated level of alkaline phosphatase and gamma-glutamyl transpeptidase. There is a male preponderance with mean age of 40 years. Autoimmune hepatitis (AIH) is an immune-mediated liver disorder characterized by female preponderance, elevated transaminase and immunoglobulin G levels, seropositivity for autoantibodies and interface hepatitis. There is a female preponderance with mean age of 40-50 years. Bile duct imaging (MRCP being a gold standard), liver biopsy is rarely diagnostic.

METHODS

The results of current laboratory tests and data accessed from the hospital date base were analyzed and compared between the groups. The study consisted of two groups: patients with PSC (N=18: men (M)=10, women (W)=4) and with AIH (N=18: men (M)=6, women (W)=12) hospitalized in 2013 at the Department of Gastroenterology and Hepatology, University Hospital, Krakow. All the laboratory tests were performed in hospital laboratory. The level of gammaglutamylo transpeptidase, alkaline phosphatase ,alanine and aspartate transaminases, bilirubin, albumines, autoimmune antibodies (ANA, ASMA, anti-LKM, AMA) and INR were taken into consideration. The antibodies were detected by ELISA method. Diagnostic imaging of liver (ultrasound, computed tomography) and hepatobiliary tract (magnetic resonance cholangio- pancreatography) were also obtained. Liver function was evaluated basing on biochemical parameters. Data on patients' response to treatment was collected and analysed.

RESULTS

Mean age of diagnosis in AIH group (30.3 +/- 14.6 years) was significantly higher than in PSC patients (26.5 +/- 9.1 years). AIH was more common among females (W=67%) as compared to PSC patients (W=22%), where men are predominantly affected. Cirrhosis was seen more often in the group of AIH patients (N=3 vs N=0). ALT and AST (IU/L) elevation was much better pronounced in group of AIH patients, however the difference was not statistically significant. ALP level was higher in the group of PSC patients compared to AIH and the p value reached the level of statistical significance (p = 0,001). The level of liver and biliary tract involvement was more pronounced in PSC group (N=18, 100% vs AIH: N=2, 11.1%).

CONCLUSION

Findings are consistent with the fact, that AIH affects mainly hepatocytes (ALT and AST are markers of hapatocellular injury) whereas PSC leads to cholestasis, which is manifested by ALP and GGTP elevation. PSC and AIH usually cause serious modifications within the liver and biliary system, Since the patomechanism of each unit is different, their contribution to liver damage also varies. That is why long-time prognosis, as well as complications, differ among the patients. There are several similarities, but these two units are mainly different from each other.

PS 123

Lynn Debels

Poster Presentation Poster Presentation

NONINVASIVE ASSESSMENT OF NONALCOHOLIC FATTY LIVER DISEASE IN TYPE 2 DIABETES PATIENTS

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AIM
Getting a better perspective of the distribution of NAFLD in patients with T2DM by using existing scores to screen for liver disease.

INTRODUCTION
Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in industrialized countries. Patients with type 2 diabetes mellitus (T2DM) are at a higher risk of developing NAFLD and they are more likely to evolve to more severe disease. Until today noninvasive tools to screen and differentiate for NAFLD severity are unavailable.

METHODS
Patients with T2DM presenting at the diabetes clinic of the UZ Brussel were evaluated by noninvasive routine screening. Different scores were calculated with the obtained parameters.

RESULTS
44 patients (25 male, 19 female) between 37 and 65 years of age were included. All met the criteria of metabolic syndrome. 82% of them had NAFLD diagnosed by ultrasound. Patients were more likely to have more severe disease when having eleveted liver enzymes. There was no correlation with age. The correlation with insulin therapy was negative. The different tested scores were positively correlated with each other.

CONCLUSION
Increased awareness of the magnitude of significant liver disease in T2DM patients is nessecary. The next step in our research will be performing liver biopsies and validate a score or develop a new specific score for T2DM patients.

PS 124

Tetyana Yaroshyk

Poster Presentation Poster Presentation

ENDOTHELIAL DYSFUNCTION AS A PREDICTOR OF GASTRIC-ESOPHAGEAL REFLUX DEVELOPMENT IN PATIENTS WITH COPD

1-Dudka I.V., 2-Yaroshyk T.V.
Dudka I.V.

AIM

detect the degree of development and role of endothelial dysfunction in occurrence and progress of GERD in patients with COPD.

INTRODUCTION

A number of studies have found are liable risk of chronic obstructive pulmonary disease (COPD) development in patients with gastroesophageal reflux disease (GERD) (67%) and statistically reliable association between GERD exacerbation and COPD exacerbation. Nitrogenmonoxide (NO) is a leading neuromediator of the autonomous system of the esophagus. Itensures regulation of the inferior esophageal sphincter (IES) tonus, and its relaxation by guanylatcyclase mechanism in particular. Recently, the role of nitrositive stress in pathogenesis of COPD exacerbations has been emphasized, and its evidence is increase of expired NO metabolites concentration. Intensified formation of peroxynitrite due to NO generation by activated inducible NO-synthase (iNOS) of the alveolar macrophages is an important aspect of harmful action and intensification of inflammatory process in case of COPD.

METHODS

40 patients with COPD II stage (GOLD 2 B)have been examined, including 10 patients without comorbid pathology (the 1st group), 10 individuals with endoscopically negative GERD (the 2nd group), 10 – with endoscopically positive non-erosive GERD (the 3rd group), 10 – with endoscopically positive erosive GERD (the 4th group) . the control group included 10 practically healthy individuals (PHI). The presence of ED was evaluated by the content of NO metabolites in the blood, iNOS activity by means of immune fluorescence analysis (IFA) method. Antiaggregate, anticoagulate and fibrinolytic activity of the endothelium was studied as well.

RESULTS

97,8% examined patients with COPD presented a considerable increase of NO in the blood as compared to the index of PHI ($p<0,05$). Patients with comorbid course of COPD and GERD showed more considerable increase of NO content in the blood (within 1,6-2,0 times, $p<0,05$), than in patients with isolated course of COPD (on 42%) ($p<0,05$). Patients with COPD and comorbid endoscopically negative GERD presented NO content in the blood higher than that in patients with isolated course of COPD on 17,4% ($p<0,05$), and patients with COPD and comorbid endoscopically positive GERD – on 82,4% than in patients of the 3rd group and 95,4% in patients of the 4th group respectively ($p<0,05$). iNOS activity in patients with COPD was considerably higher and kept to the above regularity: it was 2,6 times higher in the 1st group of patients than those PHI ($p<0,05$), the 2nd group – 2,8 times higher ($p<0,05$), 3rd group– 3,0 times higher ($p<0,05$), in the 4th group – 3,5 times higher with the reliable difference between the groups ($p<0,05$). The indices of spontaneous ($p<0,05$) and induced adenosine diphosphate aggregation of platelets ($p<0,05$) increased in all the patients with COPD. AT III content in the blood decreased: in patients of the 1st group on 20,3% as compared to PHI ($p<0,05$), the 2nd group – on 23,6% ($p<0,05$), the 3rd group – on 27% ($p<0,05$), the 4th group – 32,6% ($p<0,05$); fibrinolytic activity of the endothelium decreased (by decrease of potential plasminogen activity within the range 12-25%, total fibrinolytic activity – 18-20% and enzymatic fibrinolytic activity – 32-35%, all $p<0,05$).

CONCLUSION

1. COPD exacerbation is accompanied by iNOS activation and considerable NO hyperproduction. 2. Intensification of NO-dependent relaxation ofIESin patients with COPD, together with found imbalance of aggregation blood properties, the system of coagulation hemostasis and fibrinolysis factors, are important links of potential development of endoscopically positive GERD.

PS 127

Karolina Jaworska

Poster Presentation Poster Presentation

A RETROSPECTIVE ANALYSIS OF THE PATIENTS WITH COELIAC DISEASE AND PATIENTS WITH DUHRING'S DISEASE.

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AIM

The goal of study was to find difference between Duhring's disease and coeliac disease.

INTRODUCTION

Duhring's disease (DD) and coeliac disease (CD) belong to malabsorprion syndrome. In both diseases there is an abnormal immune system reaction to gluten and in both of them IgAEmA and IgTGA antibodies are found.

METHODS

We retrospectively analyzed randomly found medical histories of patients with DD (13 cases) and CD (40 cases), being treated in Outpatient Department of the Gastroenterology and Hepatology Clinic of the University Hospital in Cracow. Databases concerned the gender, age, age of the disease onset, skin manifestation, histopathological tests – concerning Marsh clasification, biochemical tests, coexisting diseases, occurrence of antibodies IgAEmA and IgTGA and adherence to a gluten-free diet. Presence of diarrhea, abdominal pain and other symptoms was also checked. Familial occurrence of DD and CD was analyzed wherever possible. Statistical analysis was performed using STATISTICA software. Data were considered significant for results with probability less than 0,05.

RESULTS

The vast majority of patients with DD and CD suffered also from other diseases. In one third of patients DD and CD coexisted autoimmune diseases. 23,5% patients with DD and 15% of patients with CD suffered from osteoporosis. There was no difference in mean age between patients with DD and CD. Mean age of the disease diagnosis was 35 +/- 23 years in patients with DD and 29 +/- 23 years in patients with CD. Both of diseases occur more frequently in women. We have not found any correlations between biochemical and morphology parameters and clinical manifestation.

CONCLUSION

CD and DD occur more frequently in women. In adulthood CD is diagnosed significantly earlier than DD. In one third of both diseases coexist autoimmune disorders.

PS 129

Justyna Maciejiczek

Poster Presentation Poster Presentation

THE EFFECT OF RS1421085 POLYMORPHISM OF THE FTO GENE ON THE PROGRESS OF HYPERTENSION IN THE FRAMEWORK OF OVERWEIGHT AND OBESITY.

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AIM
Demonstration of a potential association between rs1421085 polymorphism of the FTO gene and incidence of hypertension and overweight and obesity in consecutive patients presenting to a primary care physician in Public Health Centre.

INTRODUCTION
Genetic factors play an important role in the pathogenesis of obesity and hypertension. Many researches point towards the participation of FTO gene in both pathologies. The role of rs1421085 polymorphism still remains controversial.

METHODS
The study included a total of 391 patients from the area ofthe southern Poland, who in turn reported to the Public Health Centre. The patients were divided into 3 groups based on their waist circumference score (control group, group of overweight patients and obese patients). Genotyping was carried out using fluorescently labeled probe and readily prepared single nucleotide polymorphism kits - TaqMan Pre-designed SNP Genotyping Assay (Applied Biosystems). For statistical analysis we used Statistica 9.0 program.

RESULTS
Prevalence of overweight and obesity judged by the circumference of waist score was 71,35%, for 24,04% of which constituted overweight people and 47,31% obese. In the serum of overweight and obese people were found out statistically significant higher levels of glucose concentration, insulin, mean arterial pressure and lipid profile components in comparison to people from control group ($p<0,001$). In the control group no genotype showed correlation with higher average systolic pressure – statistically expressed as $p\geq0,05$. In groups of overweight and obese homozygotes for the T allele exhibit higher average systolic pressure than the other genotypes ($p <0.05$).

CONCLUSION
1. Obesity and hypertension are closely associated. 2. Polymorphism rs1421085 of the FTO gene doesn't show a statistically significant correlation with the prevalence of overweight and obesity in the population of the southern Poland. 3. The probability of exhibiting hypertension in a person who is a TT homozygote within the 1421085 polymorphism is statistically significantly higher than in other genotypes, in particular when the person is overweight or obese.

PS 135

Michał Moździerz

Poster Presentation Poster Presentation

IS CREATINE KINASE – MB ISOFORM A PROGNOSTIC FACTOR IN ACS BORDERLINE PATIENTS?

TOMASZ WITASZEK, Katarzyna Borowiecka, Katarzyna Jarek, Michal Moździerz

Supervisor: Bartłomiej Guzik MD, PhD Dep. of Interventional Cardiology; Faculty of Medicine; UJ; John Paul II Hospital; Chair: Prof. Krzysztof Żmudka MD, PhD, FESC;

AIM

The aim of the study was to investigate influence of the result of Creatine Kinase – MB isoform (CKMB) on prognosis of patients presenting themselves in Emergency Room (ER) with chest pain.

INTRODUCTION

According to the most recent ESC guidelines, Acute Coronary Syndrome (ACS) diagnosis should be made based on Troponin T (TNT) only and with its rise or fall pattern. This shortened ACS diagnostic algorithm - however, as a marker with high sensitivity and lower specificity, it led to overdiagnosing of ACS in emergency room. Therefore, a search for more specific biomarker in patients without ACS is a necessity.

METHODS

Out of all patients, who showed up to ER with chest pain between mid 2012 – mid 2013, we excluded those diagnosed and treated with ACS (STEMI, intermediate and high risk NSTEMI). We took interest solely in borderline patients, without clinical diagnosis of ACS made on ER. Out of 448 patients who met inclusion criteria, we've managed to perform follow-up in 362 of them (80%) and mailed out a questionnaire to the rest. Mean follow-up time was 1 year and 8 months. In statistical analysis we divided patients depending on TNT (cutoff 0,014ng/ml) and CKMB (cutoff 24U/l) results.

RESULTS

51,5% patients were male, 65% had arterial hypertension, 17% had diabetes, 40% had hypercholesterolemia, 3% had prior stroke, 18,5% had prior ACS, 18,3% prior PCI, 7,4% prior CABG, 11% had multilevel atherosclerosis. 27,6% of patients had positive TNT level and 41,3% had positive CKMB level. Subgroups: TNT+CKMB+ 12,3%, TNT-CKMB- 42,3%, TNT+CKMB- 15,4%, TNT-CKMB+ 29%. There was a correlation with mortality in a group with positive TNT (8,3% vs 3,0%; p=0,03) as well as in a group with positive CKMB (7,3% vs 2,3%; p=0,02). There was no such correlation in regard of MACCE occurence. What was the most interesting, comparing both groups with negative TnT, CKMB correlated very strongly with mortality (6.2% vs 0.6%; p =0.007). Both negative values TNT-CKMB- have very high negative predictive value of 99.37%.

CONCLUSION

CKMB is a strong prognostic marker for patients presenting with chest pain but without ACS diagnosis. Both negative values (TNT and CKMB) have high NPV.

PS 5

Pavle Banović

Poster Presentation Poster Presentation

EXPERIMENTAL FIBROSARCOMA IN THE SYRIAN GOLDEN HAMSTER INDUCED BY INOCULATION OF CELL CULTURE BHK-21/C13 AS A MODEL FOR TESTING THE POTENTIAL ANTI-TUMOR EFFECT OF MEBENDAZOLE

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AIM

The aim of this experiment is studying fibrosarcoma inducted by inoculation of cell culture BHK-21/C13 as a model of local tumor for testing antitumor drugs, and investigating potential antitumor effects of mebendazole on inducted fibrosarcoma.

INTRODUCTION

Cell culture BHK-21/C13 today is used for production vaccines against rabies, because of characteristics like spontaneous tumorogenesis it may present reliable model for research in experimental oncology. Mebendazole has been recently proved to be effective against some types of tumors in vivo as in vitro.

METHODS

Adult Syrian golden hamsters were inoculated with a suspension of tumorogenic baby hamster kidney (BHK) cells by subcutaneous injection. On day 5 after inoculation, 30% of LD50 doses for small rodents of mebendazole were given orally for 6 days, and the same dose of mebendazole suspended in 10% dimethyl sulfoxide were injected intraperitoneally into the one group of hamsters for 3 days. 19 days after inoculation of BHK cells animals were sacrificed and samples of tumor were excised, processed, described and analyzed.

RESULTS

Induced fibrosarcoma has shown 100% expression and virulence. Experimental groups with oral application of mebendazole were shown regressive changes in tumor volume, tumor cell structure and organization, while named characteristic of experimental group with intraperitoneal application of mebendazole showed smallest differences from tumors in control group.

CONCLUSION

Model of inducted fibrosarcoma has been shown as highly reproducible, with local infiltration and high level of expression and virulence. Mebendazole has showed clear antitumor effect via oral application.

PS 13

Pedro Medeiros

Poster Presentation Poster Presentation

ALTERNATIVE SPLICING MODULATION IN CELLULAR MODELS OF CANCER: ASSESSMENT OF POTENTIAL THERAPEUTIC EFFECT

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AIM

In this project, we aim to assess the effect of specific CLK inhibition in cancer cells splicing patterns, quantitatively evaluating alternative splicing events of multiple genes already described in the literature as directly associated to tumorigenesis.

INTRODUCTION

Alternative Splicing (AS) is a cellular process that enables the synthesis of different products from a single gene, whose regulation is highly complex. Cancer cells take advantage of this flexibility to produce proteins that promote growth and survival. Many of the isoforms produced this way are developmentally regulated and are preferentially re-expressed in tumors. CDC2-like kinases (CLKs) are a specific group of kinases that phosphorylate splicing regulating proteins, whose expression is frequently changed in cancer. To achieve our goal, we will use an innovative technique: the NanoString nCounter System. This is a new method that provides a direct digital readout of each mRNA and its relative abundance, without requiring cDNA synthesis or enzymatic reactions. Among its advantages, it allows a multiplexed multiple pathways analysis in a single tube, without amplification, which becomes less expensive and more reliable in quantification, than the usual method (real time RT-qPCR), when needed to analyze an elevated number of samples and of genes/alternative events.

METHODS

Cancer RCC cell lines (human clear cell renal carcinoma) with increased expression of CLK1 are being compared with similar cancer cell lines without elevated CLK1. CLK1 is selectively inhibited either chemically or by RNAi, in vitro. Purified RNA from treated cells and controls is submitted to NanoString nCounter analysis for cancer associated splicing events specific probes. The design of the probes for each gene was directed to allow distinguishing the competitor isoforms that have antagonist functions in cancer. Afterwards, these results are evaluated and correlated with cancer progression mechanisms with a bioinformatics approach.

RESULTS

The preliminary results are very encouraging in the evaluation that the nCounter System fits the analysis of cancer-associated AS events with advantage over other alternative available techniques.

CONCLUSION

In recent decades, cancer research has mainly focused on the study of genetic alterations, however, it has become clear that epigenetic changes, including changes in transcription and processing of transcripts, also play an important role in cancer development and thus should be the direction of future studies. We hope to demonstrate that the nCounter System can be used to assess splicing patterns of a wide range of different genes and AS events, filling the existing gap between genomic sequencing and specific qPCR measurement of mRNA expression, and to evaluate if CLKs inhibition in cancers that have increased expression of these enzymes may change the isoforms expression pattern resulting from AS, and thus help to stop tumor progression, potentially by identifying new therapeutic strategies for cancer.

Gabinete de Apoio à Investigação Científica, Tecnológica e Inovação (Faculdade de Medicina da Universidade de Lisboa), Bolsa Gulbenkian/FMUL de Investigação Científica, Fundação para a Ciência e a Tecnologia

PS 22

Wout Devlies

Poster Presentation Poster Presentation

‘CANCER IN PREGNANCY’: EFFECTS OF PRENATAL EXPOSURE TO CANCER TREATMENT ON INTRA-UTERINE GROWTH RESTRICTION (IUGR). DEVELOPMENT OF A XENOGRAFT MODEL TO ESTABLISH INSIGHTS IN THE UNDERLYING PATHOPHYSIOLOGIC MECHANISMS OF IUGR.

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AIM

In this study we want to create a suitable murine xenograft model to examine the possible effect of chemotherapy on the placental tissue related to IUGR.

INTRODUCTION

Cancer in pregnancy has a prevalence of 1 in 1000 to 2000 pregnancies. Previous studies of Amant et al. (2010, 2012) found the developmental outcome of such children to be overall reassuring. However, intra-uterine growth restriction (IUGR), which places infants at significant risks concerning perinatal morbidity/mortality, was exceptionally frequent. The mechanism underlying IUGR following ‘in utero’ exposure to cancer treatment is up till now unexplored.

METHODS

The test population consisted of groups of six ovariectomised, immunodefficient, and hormonally stimulated mice. A biomarker panel was established to evaluate factors concerning angiogenesis, proliferation, inflammation, DNA damage and apoptosis. Factors were analyzed by flow cytometry, immunohistochemistry and -fluorescence, enzyme-linked immunosorbent assay (Elisa) and polymerase chain reaction (PCR) analysis on urine, blood and the engrafted placental tissue.

RESULTS

Four test phases with engraftment of first-and third trimester human placenta were conducted. Comparing the placental tissue before and after engraftment, we found preserved structure and histological features. Also, the PCR analysis showed preserved genetic characteristics of the primary engrafted placental tissue. Specifically Flk1, eNOS, IGF-1, and PGF showed a stable expression, Flt1 and IGF-2 were slightly upregulated after engraftment. HCG secretion, evaluated by ELISA in the urine and blood, was present for 3 weeks.

CONCLUSION

We created a murine xenograft model to evaluate the effect of chemotherapy on human placental tissue. Our preliminary data showed preservation of the placental tissue before and after engraftment, and mimicking of the histological features. The engrafted placental tissue retained its active function for 3 weeks. This established murine xenograft model will be useful to examine the effect of cancer treatment, moreover it could serve as a model for other fetal toxicity studies.

Bernstein I. et al. Morbidity and mortality among very-low-birth-weight neonates with intrauterine growth restriction, Am J Obstet Gynecol, Vol 182, 1, 1, 198 – 206

PS 23

Henrique Duarte

Poster Presentation Poster Presentation

PHOSPHORYLATION OF THE ESTROGEN RECEPTOR ALPHA IN BREAST CANCER: THE HUNT FOR THE ELUSIVE KINASE

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AIM

To identify the kinase responsible for ERα T594 C-terminal phosphorylation.

INTRODUCTION

The emergence and progress of breast cancer is strongly associated with elevated levels of steroid hormones that act upon tumour cells through their respective receptors. The overexpression of the nuclear Estrogen Receptor α (ERα) plays a key role in breast malignancy. Recently, phosphorylation of the Threonine-594 (T594) residue of ERα C-terminal region has been reported as crucial for receptor's activity and protein-protein interaction. However, the identity of the responsible kinase remains unveiled.

METHODS

Two parallel sets of experiments were conducted. 1st Set: Hydrophobic Interaction-Fast Protein Liquid Chromatography (HI-FPLC) was performed on fresh cell extracts of HEK293 cell line. Eluted fractions were incubated, at 37◦C, with magnesium, natural ERα ligand 17β-Estradiol (E2), ATP and ERα Ligand-Binding Domain (LBD), purified from previously transformed TOP10 E. coli cells. Incubation products were immunoblotted with antibodies against both total and T594 phosphorylated ERα. 2nd Set: PJ69 yeast strain expressing either wild-type (wt) ERα LBD or its T594A mutated version, which cannot be phosphorylated at the 594 position, were grown in the presence or absence of E2 and ERα competitive antagonist and 4-Hydroxytamoxifen (4-HOTAM). Cell extracts from treated yeast cultures were immunoblotted as described above.

RESULTS

1st Set: ERα T594 phosphorylation was detected in FPLC lately eluted fractions. 2nd Set: Differential wt ERα T594 phosphorylation in yeast cells showed to be ligand-dependent. Phosphorylation on ERα T594A was not detected. While both E2 acted as a powerful phosphorylation trigger, 4-HOTAM prevented ERα phosphorylation.

CONCLUSION

1st Set: Detection of kinase activity in FPLC lately eluted fractions provides valuable insights into the enzyme's isoelectric point and its ability to establish strong ionic interactions. Moreover, mass spectrometry can now be performed on these fractions to identify the kinase. 2nd Set: ERα phosphorylation in yeast further narrows down the number of kinase candidates, since the yeast and human kinome greatly, but not totally, overlap. Additionally, differential phosphorylation of ERα T594 appears to be ligand-dependent. Upon E2 binding, conformational changes in ERα expose T594 to phosphorylation, similarly to what happens, at great extent, in breast cancer cells due to ERα overexpression. On the other hand, ERα structure upon 4-HOTAM binding makes the T594 residue sterically inaccessible, preventing receptor phosphorylation and subsequent activation. In conclusion, Here, a novel molecular mechanism linking ERα T594 phosphorylation and receptor differential conformation upon ligand binding is proposed. Furthermore, new insights into the identity of the enzyme responsible for the ERα T594 phosphorylation indicate this kinase as a promising molecular target in breast cancer therapy.

PS 58

Maria Salazar

Poster Presentation Poster Presentation

BROWNING OF WHITE ADIPOCYTES: MELANOCORTINS AS ESSENTIAL PLAYERS

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AIM

The aim of this study was to investigate the ability of melanocortin neuropeptides to transdifferentiate murine 3T3-L1 white adipocytes in beige/brown adipocytes.

INTRODUCTION

Nowadays there is an increased prevalence of obesity which constitutes a major risk factor for several co-morbidities including diabetes, atherosclerosis, cardiovascular diseases and cancer (Cummins et al., 2014). Obesity results from an accumulation of white adipose tissue (WAT), specialized in energy storage. White adipocytes are characterized by a single large lipid droplet, nucleus at the periphery, minimal cytoplasm, few mitochondria and expression of the hallmark gene resistin (Giordano et al., 2014). In contrast, brown adipose tissue (BAT) perform thermogenesis, burning fat to produce heat via an uncoupled metabolism (Chechi et al., 2013). Brown adipocytes have multiple lipid droplets, many mitochondria and express characteristic genes such as uncoupling protein 1 (UCP-1). UCP-1 is located at the inner mitochondrial membrane and dissipates the proton gradient generated in oxidative phosphorylation (Keipert et al., 2014). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) is a master regulator of mitochondrial biogenesis and respiration thus being also highly expressed in BAT (Ventura-Clapier et al., 2008). In humans, this tissue was initially identified in newborns, but recent evidence suggests that adults also have active brown adipose tissue. (Virtanen et al., 2009) In addition, beige or brown-like adipocytes were discovered within WAT (Young et al., 1984). Beige adipocytes are very similar to brown adipocytes and may arise by transdifferentiation of white adipocytes when subjected to certain conditions, such as chronic β-adrenergic stimulation and exposure to cold (Wu et al., 2012). Melanocortin neuropeptides have an important role in food intake and energy balance. They derive from the polypeptide pro-opiomelanocortin (POMC) that is expressed in hypothalamus, pituitary and several peripheral tissues. POMC proteolytic cleavage originates the adrenocorticotropic hormone (ACTH), α, β and γ-melanocyte stimulating hormone (MSH) that are recognized by five membrane melanocortin receptors (MCRs) designated MC1R to MC5R (Cooray et al., 2011). In white adipocytes, it is known that the melanocortin neuropeptides have a lipolytic effect mediated by PKA - dependent activation of HSL and PLIN1, and also inhibit fatty acid re-esterification by an ERK-dependent pathway (Rodrigues et al., 2013). Melanocortins also promote thermogenesis in brown adipocytes (Iwen et al., 2008), but their role in the transdifferentiation of white to beige/brown adipocytes is unknown.

METHODS

Cell Culture: 3T3-L1 pre-adipocytes were maintained in culture with DMEM supplemented with 10% fetal calf serum and subsequently differentiated by incubation with DMEM/F12 containing 10% fetal bovine serum (FBS), 10µg/ml insulin (INS), 250nM dexamethasone and 0,5mM 3-isobutyl-1-methylxanthine during four days. Cells were then allowed to differentiate for more four days in medium with DMEM/F12, FBS and INS. Real - time PCR: Differentiated adipocytes were incubated with 1µM α-MSH for 4h (controls were treated with vehicle). For ERK1/2 inhibition, cells were treated with 10µM UO126 for 30 minutes, before the α-MSH stimuli. Extraction of total RNA was performed using PureLink RNA Mini Kit (Ambion) and cDNA was synthesized by GRS RT-PCR Kit (Grisp) using 1µg of total RNA. Real-time PCR was carried out with SYBR® Select Master Mix (Life Technologies) using the StepOne™ Real-Time PCR System (Applied Biosystems) and specific primers for the mouse cDNA sequence of UCP-1, PGC-1α and resistin. Mitochondria Biogenesis: Binding of the fluorochrome 10-N-nonyl acridine orange (NAO) to mitochondria membrane was measured. For this, after 24h of α-MSH treatment, cells were fixed in 4%PFA and incubated with 1µM NAO for 30min. Cells were then lysed and fluorescence was measured with a 495 excitation and 520 emission filter set.

RESULTS

Real-time PCR results revealed that fully differentiated 3T3-L1 adipocytes stimulated for 4-hours with α-MSH have a significant increased expression of UCP-1 gene in about 20-fold over control and PGC-1α in almost 2-fold. Additionally, α-MSH treated adipocytes present a decrease of 50% on the expression of resistin gene. This downregulation is attenuated by ERK1/2 inhibition. However, cell treatment with the ERK1/2 inhibitor prior to α-MSH stimuli had no effect on UCP-1 mRNA expression. NAO fluorescence measurements

showed that 3T3-L1 adipocytes, stimulated with α-MSH for 24h, present a significantly higher (*P<0,05) content of mitochondria. In accordance, melanocortin treatment also increases the total oxygen consumption rates (OCR) and improves uncoupled OCR in 3T3-L1 cells.

CONCLUSION

Melanocortin treatment with α-MSH prompts brown/beige features in 3T3-L1 adipocytes, decreasing expression of resistin, a white adipocyte hallmark gene and increasing expression of UCP-1 and PGC-1α, characteristic of beige/brown adipocytes. In fact, BAT expresses substantial amounts of UCP-1 and has a mitochondria rich content, and consequently a higher level of PGC-1α expression. In agreement, we found that α-MSH promotes mitochondrial biogenesis and it is known that BAT has a higher mitochondrial density than WAT. These data correlates with the increased OCR and uncoupled OCR promoted by alpha-MSH. It is known that resistin levels are elevated when adipocytes have higher lipid content. Indeed, ERK1/2 downregulation of resistin mRNA correlates with previous data showing that ERK1/2 mediate the inhibition of fatty acid re-esterification promoted by α-MSH treatment (Rodrigues et al., 2013). Nevertheless, the role of α-MSH on the browning effect seems to be regulated by other pathway. Further studies will be needed to clarify this mechanism. In conclusion, the melanocortin α-MSH induces beige/brown adipocyte characteristics in 3T3-L1 adipocytes.

PS 67

Katarzyna Tyrak

Poster Presentation Poster Presentation

DENSITY OF DENDRITIC CELLS EXPRESSING CD1A AND DC-LAMP IN CUTANEOUS MELANOCYTIC LESIONS.

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AIM
The study was designed to evaluate the density of DCs in tissues taken from patients with one of the following diagnosis- dysplastic nevus, melanoma in situ, invasive melanoma (stage III or IV according to Clark scale) or metastatic melanoma.

INTRODUCTION
Cutaneous melanoma is considered to be one of the most immunogenic tumors. Dendritic cells (DCs) play a key role in immunological reactions, as they are responsible for presenting antigens and evoking immune response. DC cells display considerable heterogeneity in phenotype, location and function.

METHODS
Archival tissue samples from 78 patients with one of the above-mentioned diagnosis were subjected to immunohistochemistry with two different antibodies against dendritic cells - CD1a and DC-LAMP. DCs were counted under a light microscope per 1mm2 both in epidermis and dermis within the fields with the highest density of DCs. Statistics were calculated in Statistica Programme 10.0.

RESULTS
The significant differences in density of CD1a-positive as well as DC-LAMP-positive dendritic cells ($p<0,05$) were observed between the four groups. Median value for DCs in metastatic melanoma was lower than median values for DCs in each of the other stages of melanocytic lesions ($p<0,05$). There were no statistically important differences in CD1a and DC-LAMP expression with reference to the gender of a patient.

CONCLUSION
In the present study we evaluated the density of the DCs expressing CD1a and the maturation marker DC-LAMP. Our results suggest that density of DCs corresponds with the stage of melanocytic lesions and could probably correlate with their progression. Next step of research should concentrate on finding factors, which stimulate or inhibit the migration and maturation of dendritic cells in these four conditions. Differences in density of CD1a-positive and DC-LAMP-positive dendritic cells in the evolution of melanocytic lesions might be regarded as a proof of distinct functional immune response and could be of prognostic importance. Therefore the phenotype, location and function of dendritic cells in cutaneous melanoma warrant further studies.

Neagu M, Constantin C and Zurac S. Immune Parameters in The Prognosis and Therapy Monitoring of Cutaneous Melanoma Patients: Experience, Role and Limitations. Hindawi Publishing Corporation BioMed Research International 2013: Article ID 107940 Ladanyi A,

PS 74

Maria Leticia Carraro

Poster Presentation Poster Presentation

PROMISING NEW ANTITUMOR CHIRAL DERIVATIVES OF XANTHONES: SYNTHESIS AND EVALUATION OF ENANTIOMERIC PURITY BY HPLC

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AIM

Synthesize new promising antitumor chiral derivatives of xanthones (CDX) as single enantiomers with good yields. Evaluate the enantiomeric purity of the synthesized CDX by HPLC using a chiral stationary phase (CSP).

INTRODUCTION

Xanthones are, under medicinal chemistry point of view, very motivating bioactive compounds associated with a large spectrum of biological and pharmacological activities.1,2 Recently, our group has described the ability of CDX to inhibit the growth of three human tumor cell lines, namely A375-C5 (melanoma), MCF-7 (breast adenocarcinoma), and NCI-H460 (non-small cell lung cancer).4 Some of them exhibited enantioselectivity results and interesting dose-dependent growth inhibitory effects on the evaluated cell lines. An interesting example of high enantioselectivity for MCF-7 and NCI-H460 cell lines was observed with the enantiomers of a CDX synthesized by coupling the 2-carboxy-6-methoxyxanthone (XCar2) with both enantiomers of the chiral amine α-dimethylbenzylamine.

METHODS

Synthesis: Coupling reactions between the chemical building block XCar2 and both enantiomers of four chiral amines, using the reagent O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethylluronium tetrafluoroborate (TBTU). Enantiomeric purity: Determination of the enantiomeric excess (e.e.) by HPLC on (S,S)-Whelk-O1® CSP using ACN:MeOH (50:50 v/v) as mobile phase with flow rate of 1 mL/min. The analyses were performed at room temperature in an isocratic mode and UV detection (254 nm).

RESULTS

Eight CDXs were synthesized at room temperature after 20-30 min with yields of 90-94%. All the enantiomeric mixtures of CDX were enantioseparated by chiral HPLC with very high enantioselectivity and resolution. The e.e. for all the CDX was measured, achieving values higher than 99%.

CONCLUSION

New amide CDX were obtained with good yields, short reaction times and no racemization. The HPLC method was successfully employed to measuring the e.e. for all the CDX. The evaluation of growth inhibitory activity on human tumor cell lines of the synthesized CDX is in progress.

1Pinto, M., Sousa, M.; Nascimento, M. Curr. Med. Chem., 2005, 12, 2517. 2Masters, K-S.; Bräse, S. Chem. Rev., 2012, 112, 3717. 3Fernandes, C.; Oliveira, L.; Tiritan, M. E.; Leitao, L.; Pozzi, A.; Noronha-Matos, J. B.; Correia-de-Sá, P.; Pinto, M. M., Eur.

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PS 85

Mário Fontes

Poster Presentation Poster Presentation

BEER POLYPHENOLS CAN MODULATE ANGIOGENIC AND METABOLIC PATHWAYS IN T2 DIABETES

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AIM

Identify the discrepant molecular mechanisms of metabolic and angiogenic complications within the organs of T2DM mice and evaluate the effect of beer polyphenols consumption in these pathways.

INTRODUCTION

Type 2 Diabetes is a chronic metabolic disease with an increasing incidence and it is a leading cause of morbidity and mortality. Besides the changes in metabolic patterns, diabetes presents disturbances in vascular homeostasis in several organs, represented by the angiogenic paradox. Beer polyphenols as xanthohumol (XN) and 8-prenylnaringenin (8-PN) modulate angiogenesis, having the potential to affect these pathologic processes.

METHODS

C57Bl/6 mice were divided in 5 groups: standard diet, high fat diet (HFD), HFD and ethanol, HFD and XN, and HFD and 8-PN. The HFD groups developed T2DM after 20 weeks with this regimen. Body weight and plasma glucose were evaluated during this period and glucose and insulin tolerance tests were perfomed. Microvessel density (MVD) was assessed by immunochemistry for CD31. Angiogenic and metabolic pathways were quantified by Western blotting (Wb). Statistically significance was assessed by ANOVA test followed by Bonferroni correction using triple assays.

RESULTS

Body weight and plasma glucose levels were higher in HFD groups compared to control and the supplementation with XN and 8-PN decreased these levels. The same rationale was followed in glucose and insulin tolerance tests. An increase was observed in MVD in the kidney of HFD animals, as well as a reduction of the neovascularization in the left ventricle (LV). Phenolic treatment led to a significant reduction in neovessel formation in kidney and a slight increase of endothelial staining in LV. These results were confirmed by Wb. We found lower levels of pACC/ACC in liver in HFD group in comparison with control group. In HFD and ethanol or with XN groups these levels were even lower. In HFD and 8-PN group, we found higher levels than in HFD group but lower than control group. PFKFB3 levels in liver were higher in HFD group when compared to control group. 8-PN attenuated these augment and XN was capable of completely prevent it.

CONCLUSION

XN and 8-PN are known by their roles in modulating angiogenesis. These beer polyphenols can also have interesting effects in metabolic diseases like T2DM. ACC is a key enzyme in diabetes, expressing the imbalance in lipid and carbohydrates pathways. Decreased phosphorylation of ACC in liver as we found in our model represents enhanced fatty acids synthesis. 8-PN seems to have the potential to prevent at least partially this disproportion. PFKFB3 plays a leading role in angiogenesis, since it regulates glycolysis which is essential in proliferating cells like tip cells of forming vessels. Since we have disturbances in vascular homeostasis in T2DM, we found higher levels of PFKFB3 in liver in HFD group. As in the last example, 8-PN partially revoke these changes. XN was completely capable of preventing this dysregulation. XN and 8-PN were also capable of attenuating the angiogenic paradox in LV and kidney. Beer polyphenols can modulate angiogenic and metabolic pathways in T2DM, having a potential role in preventing diabetes complications.

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Gertjan A.C. Rasschaert

Poster Presentation Poster Presentation

SCREENING FOR MODULATION OF EXOSOME RELEASE BY CANCER CELLS

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AIM

Establish a cheap and readily screening method for potential inhibitors of exosome release by cancer cells. Reveal essential accessories of the exosome secretion pathway. Discover a unique compound to prevent further metastasis.

INTRODUCTION

Molecular mechanisms behind exosome biogenesis and secretion are poorly understood. An essential role is dedicated to the small GTPase Rab27B, a regulator of vesicle exocytosis that delivers pro-invasive signals for increased tumor size, invasiveness and metastasis in estrogen receptor positive breast cancer cell lines, both in vitro and in vivo. In vitro MCF-7 GFP-Rab27B cell lines are used to hunt down the influence of a series of compounds on exosome secretion. V-ATPase inhibitors, bafilomycin A1 and concanamycin A, because of demonstrated presence of V-ATPase subunits on GFP-Rab27B vesicles. Cytoskeleton inhibitors, cytochalasin D and nocodazole to undermine potential intracellular vesicle transport routes.

METHODS

MTT assays were performed to study the influence of our compounds on cell proliferation. To analyze the effect of our compounds on exosome secretion, the design consist of two legs. Quantitative leg: nanoparticle tracking analysis is performed on isolated particles from conditioned medium of treated cells to study the difference in total particle secretion. Morphological leg: fluorescence microscopy is performed on treated cells to observe changes in intracellular localization of GFP-Rab27B vesicles containing the exosomes.

RESULTS

All used compounds display reversible lowering in total measured particle concentration (40-70%). Individual reversible inhibition of V-ATPase activity and cytoskeleton morphology demonstrates that the substrates control peripheral localization of Rab27B vesicles. This suggests the particle lowering observed in the quantitative leg is due to reduction in exosome secretion.

CONCLUSION

Inhibiting V-ATPase activity and interfering cytoskeleton architecture by administration of drugs is potentially linked to a lowering of exosome secretion in estrogen receptor positive breast cancer. The positive screening of these inhibitors reveals essential accessories in the exosome releasing pathway and proposes new perspectives for potential prevention of further metastasis in the future. We established a readily and cheap screening method for inhibitors of exosome release in cancer cells.

1) Hendrix, A., et al., Effect of the secretory small GTPase Rab27B on breast cancer growth, invasion, and metastasis. J Natl Cancer Inst, 2010. 102(12): p. 866-80
2.) Hendrix, A., et al., The secretory small GTPase Rab27B as a marker for breast cancer p

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Joana Moreira

Poster Presentation Poster Presentation

BAICALEIN DERIVATIVES WITH CASPASE MODULATORY ACTIVITY

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AIM

synthesis Baicalein derivatives with caspase modulatory activity

INTRODUCTION

Flavonoids represent an outstanding class of naturally occurring compounds with interesting biological activities, being the antitumor effect one of the most reported in the literature [1].The antitumor activity of flavonoids is associated with, at least in part, to their ability to induce apoptosis by affecting the expression or activity of a wide variety of molecules involved in apoptosis pathways, namely the caspase family proteins [2]. Recently, as result of the search for new caspase modulators by our group a baicalein prenylated derivative has been identified as caspase-7 activator [3].

METHODS

microwave synthesis and yeast cell based assays

RESULTS

These studies support the hypothesis that the alkylation of the flavone scaffold could represent a promising strategy to obtain anticancer compounds that act by activating caspases. In the present work, the synthesis of two alkylated derivatives has been achieved by the reaction of baicalein with alkyl halides in alkaline medium under microwave irradiation. The synthesized compounds were characterized by NMR techniques (1H NMR, 13C NMR, HSQC and HMBC). Their ability to modulate caspases-3, and 7 activity was evaluated using yeast cell based assays [3].

CONCLUSION

were obtained two derivatives of baicalein as activators of procaspase-7

[1]. Batra, P.; Sharma, A. K., Anti-cancer potential of flavonoids: recent trends and future perspectives. 3 Biotech, 2013, 3 (6), 439-459. [2]. Ravishankar, D.; Rajora, A. K.; Greco, F.; Osborn, H. M., Flavonoids as prospective compounds for anti-cancer

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Ana Rita Moreira

Poster Presentation Poster Presentation

USE OF DRYING METHODS TO PRODUCE PROLIPOSOMES FOR DELIVERY OF A XANTHONE WITH GLIOMA CELL LINES GROWTH INHIBITORY ACTIVITY

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AIM

To develop a proliposomal system to encapsulate and deliver a xanthonic compound (LEM2) with glioma cell lines growth inhibitory activity.

INTRODUCTION

Proliposomes are dry, free-flowing granular products composed of drug and phospholipids which, on hydration, convert into vesicular structures [1]. They emerge from the necessity to overcome the stability problems related to liposomes, vesicular systems that mimic biological membranes. Although liposomes have been widely explored for the delivery of drugs [2], its storage for a long period is limited due to poor chemical and physical stability [3]. Preparation of proliposomes is a promising strategy to formulate liposome dispersions. Several studies have shown the potential of proliposomes to be delivered in different administration routes and for a wide range of biological activities. However, the applicability of proliposomes has not yet been explored for molecules with potential antitumor activity.

METHODS

LEM2 was synthesised from the building blocks 3,4,5-trimethoxytoluene and 2-methoxybenzoyl in four reactional steps. Proliposomes were prepared with egg phosphatidylcholine (EPC) and cholesterol (CHOL) as the lipid part and mannitol as the carrier material, employing two different drying methods: freeze drying and spray drying. Proliposomal formulations were studied in varying molar ratios of EPC and CHOL, varying weight ratios of lipid part and mannitol and different drug percentages. The proliposomal powders were hydrated in order to generate liposomes.

RESULTS

LEM2 was successfully synthesized with a yield of 64%. The optimized proliposomal formulation had a molar ratio of EPC to CHOL of 3 to 1, a weight ratio of mannitol to lipid of 10 to 1 and 2% of LEM2. It was also observed that proliposomes under hydration did indeed generate liposomes.

CONCLUSION

This study has shown the suitability of both freeze and spray drying to produce proliposomes which on hydration produce liposomes encapsulating LEM2. Studies with proliposomes should continue to emerge since it is an area of interest and more investigation is required so they could have a clinical application.

1. Payne, N. I.; Timmins, P., et al. Journal of pharmaceutical sciences. 1986, 75, 4, 325-329. 2. Tripathi, G.; Chaurasiya, K., et al. International Journal of Current Pharmaceutical Research. 2013, 5, 3, 4-14. 3. Payne, N. I.; Browning, I., et al. Journa

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Vanessa Botelho

Poster Presentation Poster Presentation

ROLE OF MACROAUTOPHAGY OVEREXPRESSION IN OXIDIZED PROTEINS ACCUMULATION

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AIM

To provide evidence on the crosstalk between two major protein turnover systems, the proteasome and lysosomal autophagy. To do so, proteasome activity and carbonylated proteins levels will be assessed in condition of macroatophagy upregulation.

INTRODUCTION

Aging is a fundamental process that represents a major risk factor with respect to the development of important human pathologies, including cancer, neurodegenerative, and cardiovascular diseases. It is characterized by progressive physiological integrity loss, leading to impaired function and increased vulnerability to death (Lopez-Otin et al., 2013). Collecting evidence back up the view that many of the changes observed during aging are consequence of oxidative damage (Levine et al., 2001). Oxidative stress, resulting from an imbalance between oxidants and anti-oxidants in favor of the former, leads to a disruption of redox signaling and control and/or molecular damage (Sies, H., 2015). An important category of biomolecules susceptible to oxidation are proteins (Castro et al., 2013), whose resulting in structural changes that antedate to loss of function (Tamarit et al., 2012). A relevant oxidative protein modification is carbonylation, a non-enzymatic irreversible event and a common feature of aged cells. Carbonylated proteins have been used as a severe oxidative damage marker as they can become dysfunctional and accumulate as insoluble protein aggregates that alter cellular functionality (Castro et al., 2012). To cope with carbonylated proteins and to prevent their accumulation, cells employ the proteasome, the main non-lysosomal structure. Proteasome is a complex catalytic system that exhibits two predominant forms, the 20S catalytic core, which is ATP and ubiquitin tagging independent, and the 26S form that combines the 20S core and at least one 19S regulatory cap.(Jung et al., 2009) The 20S proteasome form has been shown to be the main site for oxidized protein degradation; however, if the degrading rate is inferior to carbonylated proteins formation rate, protein aggregates form and even inhibit the proteasome (Castro et al., 2013) (Nowotny et al., 2014). In a previous study, oxidative stress challenged cells accumulated oxidized proteins followed by insoluble protein aggregates in a time-dependent manner. Furthermore aggregates lead to proliferation impairment and proteasome activity diminishment (Castro et al., 2012). Recently, we found macroautophagy to be upregulated under these conditions, possibly as a compensatory mechanism. As a variety of data suggest the existence of a close relationship between the proteasome, autophagy and pathogenesis of many diseases (Wojcik, 2013). The two main cellular degradative systems, it is relevant to understand how they cooperate when oxidized proteins form. To maintain homeostasis, autophagy is triggered as a selective, lysosomal led intracellular components digestion system, which is highly regulated by a (limited) number of autophagy-related proteins (Wen et al., 2013). A lipidated form of LC3, LC3-II, has been shown to be an autophagosomal marker in mammals, and has been used to study autophagy (Tanida et al., 2004) The binding of LC3 to PE is essential for the hemifusion of lipid membranes and is suggested to drive the expansion of the autophagosome (Johansen et al., 2014). Taking advantage of our pre-established stress model (Castro et al., 2012), and using a plasmid for LC3 overexpression, we decided to address the hypothesis that macroautophagy overexpression compensate, proteasome inhibition and, promote oxidized proteins clearance.

METHODS

2. Material and Methods 2.1 Plasmid Purification The isolation of pure plasmid was performed using the Hi Pure Plasmid Midiprep kit (Invitrogen). The obtained plasmid DNA was used for transfection experiments. 2.2 Cell Culture HeLa cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 2mM glutamine in 5% CO2/humified atmosphere at 37 ◦C, until 70–80% confluence was attained. Cells were transiently transfected with 0.4µg pcDNA3.1 hLC3B-mCherry using Lipofectamine 2000 (Invitrogen). 2.3 Neutral Red/Cell Proliferation Hela cells were submitted to oxidation by adding 100, 150, 200, 250 and 500µM of H2O2 for 3 and 24 hours in multiwell microplate of 96 wells. After the treatments, the medium was discarded and 200 µL of neutral red (33 µg/mL) was dissolved in complete medium and added to each well. After incubation for 2 hours at 37°C, cells were washed with PBS solution 0.1M and placed with 50% ethanol containing 1% (v/v) acetic acid. The absorbance was measured at 540 nm using a multiwell microplate reader (Infinite® 200-TECAN). The same protocol was followed for transiently transfected HeLa cells expressing hLC3B-mCherry under oxidation (100µM H2O2). Subsequently, cells without treatment were run under identical conditions

and served as controls. 2.4 Cell Treatment and Cellular Extracts preparation HeLa cells transiently transfected with hLC3B-mCherry for 18 hours, were incubated with 100μM H2O2 for 3 and 24 hours. One hour prior to H2O2 submission, cells were treated with 1μM 3-MA (macroautophagy inhibitor) and 1μM Lactacystin (Proteasome inhibitor) (Invitrogen) (ongoing experiments). The concentrations selection was based on product datasheet and literature (Castro et al., 2012). Cells were washed with ice-cold PBS and solubilized in lysis buffer (glycerol l12.5%, 6.25mMTris, pH6.8, SDS 5%,bromophenol blue 0.08%). Lysates were sonicated and protein concentrations were determined using the Bradford protein assay (BioRad) (Bradford, MM., 1976) 2.5 Immunoblotting Extracts were boiled at 65 ºC for 20min and 95ºC for 5min. The same volume (15 μl) from each sample was loaded and resolved in a 12% SDS-PAGE gel. Proteins were blotted onto Hybond membranes (Amersham Biosciences Europe) (equal concentration of protein in each lane was confirmed by staining the membrane with Ponceau solution). When the experiment was performed to detect protein carbonylation, the membranes were directly derivatized with 2,4-dinitrophenyl hydrazine (DNPH, Sigma Aldrich). Briefly, after the transfer, the membranes were equilibrated in TBS (Trisbuffersaline)/ 20%methanol, washed for 5 min in 10% trifluoroacetic acid (TFA,VWR), incubated for 10min with 5 mM DNPH/TFA (10%) in the dark, washed with TFA (10%) to remove the excess of DNPH, and finally washed again five times (5 min each) with 50% methanol. Following this, membranes were blocked with 5% BSA/TBST (Tris buffer saline with Tween 20, 0.1%) for 1 h. After blocking, the membranes were probed with rabbit IgG anti-DNP (Sigma) at a 1:10,000 dilution as the primary antibody, anti-p62 (Sigma) and anti-atg5 (Abcam) at a 1:1000, anti-LC3 (Novus Biol) at 1:2500. Chemiluminescent detection was performed using the Clarity Western ECL SUBS (BioRad) and visualized in Chemidoc TM XRS (BioRad Laboratories), after incubation with a secondary HRP-conjugated antibody (1:5000, Jackson ImmunoResearch). 2.5 Fluorescence microscopy HeLa cells transiently transfected with hLC3B-mCherry, were cultured on glass coverslips and after the treatments (2.2) cells were fixed with 4% paraformaldehyde, permeabilized for 5 min with 1% Triton X-100 and blocked with 5% bovine serum albumin. Immunodetection of p62 was carried out by incubating cells with the anti-p62 antibody (1:1000, Sigma) overnight at 4 ºC and a secondary antibody Alexa 568 (Molecular Probes). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and the images were captured with an ApoTome fluorescence microscope (Zeiss).

RESULTS

3. Results To evaluate the proliferation rate, HeLa cells were submitted to oxidative conditions using hydrogen peroxide at various concentrations and analyzed in a time frame from 3 to 24 hours (see Materials and methods for details). Viabilities were high for every group at 3h (above 65%), however at 24h, notable differences between control and treated cells were shown, except for 100 μM treatment that remained above 60%, suggesting a non-cytotoxic concentration. The same protocol was followed but with HeLa cells overexpressing hLC3B-mCherry under 100μM H2O2 challenge. We verified that transfected cells without H2O2 preserved viability above 90% between 3h and 24h. Different results were obtained for H2O2 challenged non-transfected and transfected cells, where a decrease in viablity to 70% and 55% at 24 hours respectively was observed. Preliminary results showed successfully transfected cells with LC3B plasmid. Confirmation was achieved by Western Blot and immunocytochemistry by verifying other autophagy markers such as p62 and atg5 (Itakura et al., 2011; Mehrpour et al., 2010). Unexpectantly, we noticed that after 3h of oxidative stress autophagy overexpressing cells exhibited twice as much carbonylated protein, compared to non-transfected stress submitted cells.

CONCLUSION

4. Discussion/Conclusion All cells have surveillance systems that regulate the quality of proteins and organelles, to eliminate misfolded, malfunctioning or damaged components (Choi et al., 2014). On this setting, as proteasomal degradation and autophagy perform a remarkable array of physiologically important cellular tasks, their loss may lead to functional impairment pathological situations(Tanaka et al., 2014). Sounding evidence supports a critical role for the two main cellular proteolytic systems in quality control and in maintenance of cellular homeostasis (Wong et al., 2010). Accumulation of oxidized (carbonylated) proteins is a widely used biomarker of oxidative stress, a hallmark of cellular and organismal aging and also in age-related diseases (Baraibar et al., 2013). Reported an eddition, it has been commonly used to analyze the degree of damage to proteins under oxidative stress conditions (Tamarit et al., 2012). Growing evidence supports the idea that at different levels, signaling pathways act upstream of the autophagy machinery and regulate, the formation and the maturation of autophagosomes, and their fusion with lysosomes (Mehrpour et al., 2010). The existence of these different levels of regulation also suggest different ways of autophagy modulate, such as overexpressing LC3B(Shvets et al., 2008), inhibiting signaling pathways (3-MA)(Huang et al., 2013), monitoring involved proteins (Atg5, p62)(Lu et al., 2014), inhibiting proteasome (Lactacystin) (Castro et al., 2012),and protein carbonylated detection (Tamarit et al., 2012) as we are using in our research. In this study, resuls reflect the proteasome autophagy axis hypothesis. However, other, ongoing, studies should follow to better support the crosstalk between the two major systems, and enlighten the regulation of how carbonylated proteins formation and degradation.

References Bradford, MM. (1976). A rapid and sensitive method forn the quantitation of microgramа quantities of protein utilizing the principle of protein_dye binding. Analytical biochemistry,1976 May 7;72:248-54. Baraibar, M. A., Ladouce, R., & Friguet,

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Gabriela Moreira

Poster Presentation Poster Presentation

PROLIPOSOMES AS PACLITAXEL DELIVERY SYSTEMS: A NEW APPROACH FOR CANCER THERAPY

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AIM

Development and characterization of a proliposomal formulation containing paclitaxel.

INTRODUCTION

Cancer is a disease with a high mortality rate. Chemotherapy is challenging because the cytotoxic agents are not specific to cancer cells and cause toxicity in healthy tissues. To overcome such drawbacks strategies involving nanodelivery systems have been developed, being liposomes one of the most used. Liposomes are biocompatible and can enhance the efficacy of chemotherapeutics, reducing side effects, which make them suitable systems for cancer treatment [1]. Paclitaxel appears to be a promising antineoplastic agent. Its inclusion in liposomes has proved to be a good approach to improving their antitumor efficacy [2]. Although the potentials of liposomes their application as therapeutic agent is still challenged by its physical and chemical instabilities in aqueous dispersions for long-term storage. Proliposomes constitute a potential alternative. They are dry free-flowing particles that can form a liposomal suspension when are hydrated [3]. Compared with conventional liposomes, proliposomes exhibit higher physical stability and have potential to be exploited in several routes of administration [4].

METHODS

Proliposomes were produced by lyophilization using a formulation with a molar ratio of phosphatidylcholine and cholesterol 3:1 and mannitol as carrier material. Formulations with different percentages of paclitaxel were produced. The obtained proliposomes were analysed by differential scanning calorimetry. After hydration, zeta potential, particle size, polydispersity and drug encapsulation efficiency of the resulting liposomes were evaluated.

RESULTS

The liposomes reconstituted by hydration from proliposome powders had a mean diameter in the range of 500 nm, zeta potential < -30 mV and revealed be capable of encapsulating the drug.

CONCLUSION

Lyophilization showed to be an effective method to produce proliposomes, which presented high stability. The composition of proliposomes influences the properties of powder and the caracteristics of liposomes formed after hydration. More studies are necessary to optimize the formulation and make it more suitable as potential drug delivery system.

[1] Gentile, E., Cilurzo, F., Di Marzio, L., Carafa, M., Ventura, C. A., Wolfram, J., Paolino, D. and Celia, C. (2013), Liposomal chemotherapeutics. Future Oncology, 9 (12),1849-1859. [2] Crosasso, P., Ceruti, M., Brusa, P., Arpicco, S., Dosio, F. and Cat

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Agostinho Lemos

Poster Presentation Poster Presentation

DEVELOPMENT OF NEW PROMISING DRUG CANDIDATES AS P53 ACTIVATORS BASED ON A XANTHONE SCAFFOLD

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AIM

Based on xanthone scaffold of distinct building blocks and on known p53:MDM2 inhibitors, a molecular hybridization strategy was followed to obtain new promising aminoxanthone derivatives as potential disruptors of p53:MDM2 interaction with drug-like prope

INTRODUCTION

The growth-suppressive activity of p53 is regulated by interaction with two negative modulator proteins, MDM2 and MDMX, which are overexpressed in about half of all human tumors. Inhibiting p53:MDM2/X interaction may constitute an appealing strategy for cancer therapy. Based on crystallographic structures of p53 in complex with MDM2 and MDMX, several classes of small-molecule inhibitors have been identified [1]. Xanthone derivatives have been described as promising antitumor agents with the ability to target p53:MDM2 interaction. In fact, the natural xanthones, α-mangostin and gambogic acid, and the synthetic derivative pyranoxanthone 1 (LEM1) exhibited potent cytotoxic activity against several human tumor cell lines accompanied by an inhibitory effect on p53:MDM2 interaction [2, 3]. Together, xanthone has revealed to be promising scaffold for the identification of p53 activators.

METHODS

In this work, a library of aminated xanthones was synthesized by reductive amination of three xanthonic building blocks. Docking simulation studies were performed in order to predict and rank these derivatives according to their binding affinity towards MDM2. Their ability to disrupt p53:MDM2 interaction was assessed by yeast-screening assays

RESULTS

The synthesis and structural elucidation of the aminated xanthones will be presented. Docking studies have shown these derivatives presented a higher binding affinity to MDM2 than the respective building blocks. Their inhibitory activity to disrupt p53:MDM2 interaction using yeast-screening assays is still ongoing.

CONCLUSION

These results may contribute to the study of the most favorable structural requirements in order to obtain novel and potent inhibitors of p53:MDM2 interaction with an anti-tumor effectiveness.

[1] Khoury, K., et al. (2011), The p53-MDM2/MDMX axis - A chemotype perspective. Med Chem Comm, 2(4), 246-260. [2] Leão, M., et al. (2013), α-Mangostin and Gambogic Acid as Potential Inhibitors of the p53–MDM2 Interaction Revealed by a Yeast Approach. Jou

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Rita Rocha

Poster Presentation Poster Presentation

THE ROLE OF BMP7 IN WOUND HEALING IN DIABETES TYPE I

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AIM

This study evaluated the role of BMP7 in wound healing in the skin of diabetic mice type 1 and non-diabetics.

INTRODUCTION

Diabetic foot ulcer remains one of the most serious secondary complications in diabetic patients with an impact not only for them, but also in health care costs. Neuropathy, ischemia, and vascular diseases affect the development of ulcer. Deregulation levels of inflammatory factors, growth factors and proteins of the family of transforming growth factor β (TGF-β) as the bone morphogenic protein 7 (BMP7) has been associated with the difficulty in wound healing in diabetes. The balance between these two molecular signaling pathway TGF-β / BMP7 may be a new therapeutic target for wound healing in diabetic patients.

METHODS

The type I diabetes was induced by streptozotocin in mice controls and transgenic mice that partially express the BMP7 gene (BMP7 +/-). The transcription of BMP7 mRNA and TGF-B1 mRNA by qRT-PCR was analyzed at day 0 and day 10, post-wounding, using skin biopsies. Through histological analysis (H & E and Masson's Trichrome) the organization of the extracellular matrix, inflammation and collagen deposition was also evaluated. The presence of blood vessels in tissue, was evaluated with CD31+ staining by immunohistochemistry.

RESULTS

Between the non-diabetic heterozygous BMP7 mice (BMP7+/-) and non-diabetic wild type mice (WT),there was no statistically significant difference in wound healing at day 10 . In the presence of diabetes, the BMP7+/- mice (DM BMP7) had the worst healing than the diabetic WT mice, at day 10. At day 0, non-diabetic BMP7 mice had a decrease in the transcription of the BMP7 mRNA compared to non-diabetic WT mice. In the presence of diabetes, at day 10 post-wounding, the transcription of BMP7 mRNA was higher in BMP7 mice (DM BMP7) compared to diabetic WT mice (DM WT). At day 0 the transcription of the TGF-β mRNA was higher in the diabetic BMP7 compared to non-diabetic BMP7 and diabetic WT. At day 10, there are no statistically significant differences between groups. At day 0 and day 10, the extracellular matrix of the skin was better organized in the WT mice (diabetic and non-diabetic) compared to BMP7 mice (diabetic and non-diabetic). The same was observed in the inflammatory process. At day 0, the collagen deposition was higher in non-diabetic mice (both in WT and BMP7) compared to diabetic mice (both in WT and BMP7). At day 10, diabetic BMP7 mice had more collagen deposition compared to diabetic WT mice. The number of the skin blood vessels was higher in non-diabetic BMP7 mice compared to non-diabetic WT mice, at day 0.

CONCLUSION

This study showed that the decreased levels of BMP7 (heterozygous) can influence skin wound healing since it increases collagen deposition and angiogenesis.

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Fatime Hawchar

Poster Presentation Poster Presentation

ROLE OF PEROXYNITRITE IN RAPID VENTRICULAR PACING-INDUCED POSTCONDITIONING

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AIM

The current study was taken to see if elevation of peroxynitrite level, already proven in the mechanism of IPost, plays a role in the mechanism of RVP. In addition, possible RVP-activated downstream cardioprotective signaling pathways were investigated.

INTRODUCTION

Our research group has found previously that rapid ventricular pacing (RVP) performed at the beginning of reperfusion reduces ischemic injury, similarly to ischemic postconditioning (IPost). The molecular mechanism of RVP is not elucidated.

METHODS

Hearts isolated from male Wistar rats were perfused according to Langendorff. In the ischemia-reperfusion (I/R) control group 15 minutes of equilibration was followed by 30 minutes of regional index ischemia and 7 minutes of reperfusion. Postconditioning was induced by applying 6x10 s /10 s global ischemia/reperfusion (IPost) or 6x10 s 600/min rapid ventricular pacing/spontaneous rhythm (RVP) at the onset of reperfusion. At the end of reperfusion hearts were frozen and powdered in liquid nitrogen. Peroxynitrite production was quantified by measuring cardiac 3-nitrotyrosine with ELISA. Activation of RISK and SAFE pathways was examined by Western blot.

RESULTS

Both IPost and RVP significantly increased cardiac 3-nitrotyrosine (2.45±0.43 and 2.73±0.49 ng/mg, respectively) versus I/R control (1.25±0.24 ng/mg, $p<0.05$). Neither RISK (Erk1, Erk2, Akt), nor SAFE (STAT3) pathways were significantly activated in IPost and RVP groups compared to I/R.

CONCLUSION

As IPost, RVP also increases cardiac 3-nitrotyrosine suggesting that peroxynitrite may play a role in RVP-induced postconditioning. Since neither RISK nor SAFE is activated during RVP-induced cardioprotection, further experiments are needed to elucidate the exact molecular mechanisms.

Rapid ventricular pacing-induced postconditioning attenuates reperfusion injury: effects on peroxynitrite, RISK and SAFE pathways, Pipicz et al., BJP (2015)

Supervisors: Márton Pipicz M.D. PhD student, Tamás Csont M.D. Ph.D. associate professor

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Pedro C. Nunes

Poster Presentation Poster Presentation

CAN HYPOXIA HAVE AN EFFECT ON THE CHEMORESISTANCE OF COLORECTAL CANCER?

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AIM

The aim of this work focuses on the study of the effect of hypoxia in the chemoresistance profile of colorectal cancer. For that, the expression and localization of resistance proteins MRP-1 (multidrug resistance protein 1), (PGP) (P-glycoprotein) and LRP

INTRODUCTION

In the world, colorectal cancer (CRC) is the third cancer with most incidence in men and the second in women. In Portugal CRC incidence is almost six thousand new cases per year. Clinical studies show that tumor hypoxia is associated to a chemoresistance of CRC to therapy. In normal conditions, resistance proteins upregulation is identified as one of the reasons for the lower rate of drug cellular response. One of the causes is the membranar expression of these resistance proteins, causing chemotherapeutic drugs extrusion. However, it remains unknown the effect of hypoxia on the expression of these proteins in human colorectal cancer cell lines.

METHODS

The three human colorectal cancer cell lines (WiDr, C2BBe1 and LS1034) were cultured in normoxia (18% O2) and hypoxia (2% O2) conditions for 2 and 48 hours. Total expression of LRP and total and membrane expression of MRP-1 and PGP were evaluated by flow cytometry.

RESULTS

In normoxia condition we observe that C2BBe1 cells have the higher expression of LRP, while LS1034 cells reveal a high presence of PGP. When C2BBe1 cell line is exposed to hypoxia conditions, a gradual decrease of total resistance proteins expression is obtained accompanied by a simultaneously increase of membranar expression of these proteins. Regarding LS1034 in hypoxia, it was observed an increase of total LRP and PGP, while an increase of membranar MRP-1 expression was observed. In WiDr cell line, it wasn't observed a significant variation of the presence of these proteins.

CONCLUSION

In normoxia conditions, C2BBe1 cell line appears to be the most resistant cell line, due to high presence of LRP, comparatively to the other cell lines. LS1034 cell line also has a higher resistant profile comparing with WiDr cell line due to the higher expression of PGP. Relatively to hypoxia effects in C2BBe1 we observed a decrease of the total presence of resistance proteins, however, we also observed a translocation of these proteins to the cellular membrane, providing a greater resistant profile. In LS1034 cell line, we realized that variations are not too proeminent, nevertheless it was observed an increase of the LRP and PGP expression. So, we can assume that hypoxia induces an increase of chemoresistance in LS1034. In summary, the results allow us to conclude that hypoxia induces chemorresistance in human colon cancer cells by the increase of resistance proteins. These results also demonstrate that CRC response to hypoxia stimuli depends on the colon cancer cell line.

PS 141

Mafalda Carrington

Poster Presentation Poster Presentation

PROGNOSIS AND PREDICTIVE FACTORS OF EARLY-RECURRENCE IN COLORECTAL CANCER

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AIM
We aimed to identify the predictive factors of early recurrences of colorectal cancer, define prognostic factors and evaluate potential differences amongst patients with colon and rectum primary tumors that could support a separate analysis.

INTRODUCTION
We aimed to identify the predictive factors of early recurrences of colorectal cancer, define prognostic factors and evaluate potential differences amongst patients with colon and rectum primary tumors that could support a separate analysis.

METHODS
We selected 102 consecutive patients with colorectal carcinoma recurrence after a curative resection, from March 2011 to December 2013. Clinicopathological characteristics and survival rates were compared between rectum and colon primary tumors, as well as between early (<12 months) and late recurrence patients.

RESULTS
Thirty-two percent and 68% of the patients had early and late recurrences, respectively. Preoperative albumin <38g/L (OR: 8.196; 95%CI 2.123 - 32.258; p=0.002) and pT4 (OR: 2.950; 95%CI 1.003 - 8.696; p=0.049) were proven to be independent predictive factors for early recurrence. Overall and progression-free survival curves were better in the late-recurrence group (p<0.05) and in patients who had undergone resection of their recurrent lesion (p<0.01). Multivariate analysis by tumor location revealed that emergency presentation (OR: 14.706; 95%CI 1.333 - 166.667; p=0.028), preoperative albumin <38g/L (OR: 4.444; 95%CI 1.299 - 15.385; p=0.017) and Jass IV (OR: 2.639; 95%CI 1.167 - 5.988; p=0.020) were independently associated with colon primary cancer. These tumors had more abdominal lymph node recurrences, while lung recurrences predominated in the rectum (p=0.006 and p=0.013, respectively).

CONCLUSION
We conclude that a recurrence before 12 months is significantly associated with a worse prognosis and that preoperative albumin <38g/L and pT4 staging is a predictor of an early recurrence after total resection. Furthermore, a colon primary location was independently associated with three recognized worse prognostic factors in colorectal cancer, although location did not influence the prognosis. More intensive primary tumor treatment and follow-up for patients with these risk factors is recommended in order to prevent relapse within one year after surgery, and therefore worse progression and survival outcomes.

PS 145

Nuno Almeida

Poster Presentation Poster Presentation

COMBINATION OF PHOTODYNAMIC THERAPY WITH ACETYLSALICYLIC ACID: A POSSIBLE APPROACH TO ENHANCE TREATMENT IN HUMAN COLON AND ESOPHAGUS CANCER CELLS?

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AIM

Evaluate the possible synergic effect of acetylsalicylic acid combined with photodynamic therapy in WiDr and OE19 cell lines.

INTRODUCTION

Photodynamic therapy is a minimally invasive treatment modality and is approved for the treatment of many diseases, namely macular degeneration, skin diseases and certain types of cancer and continues to expand its list of applications over time. Its action occurs through excitation of a photosensitizer agent with an appropriate wavelength to their area of maximum light absorption, forming reactive oxygen species that could lead to death of tumor cells by direct action by apoptosis, necrosis or autophagy, by damaging the tumoral blood vessels or by promoting an innate or adaptive immune response. Non steroidal anti inflammatory drugs (NSAID's) have a great clinical utility, which results from the ability of these molecules have activity to inhibit cyclooxygenases (COX). Within the NSAID's is acetylsalicylic acid, commonly known as aspirin, a non-specific inhibitor of COX, which inhibits COX-1 and COX-2. The use of cyclooxygenase inhibitors such as acetylsalicylic acid in combination with photodynamic therapy could be a way to decrease promotion of tumor angiogenesis, cell proliferation and to promote cell death by apoptosis increasing the overall effectiveness of treatment.

METHODS

The WiDr cell line was cultured in DMEM medium supplemented with 5% fetal bovine serum (FBS), 1% antibiotics and sodium piruvate, while OE19 cell line was propagated in culture with RPMI also with 5% FBS, 1% antibiotics and sodium piruvate. For the studies of proliferation evaluation 80.000 cells/well were plated in 48 multiwell plates, with the addition of a photosensitizer , previously synthetized by us, at the concentrations of 5 nM, 50 nM, 200nM and 500 nM past 24h. Subsequently the plates were irradiated with a flow 7,5mW / cm2 to achieve 10J, followed by the addition of aspirin at the concentrations of 2,5 mM and 10 mM. After 24 hours cell proliferation was assessed by the MTT assay. For the flow cytometry studies, the cell cultures were stained with Annexin V/Propidium iodide-FITC probe for evaluating cell death type, JC-1 probe for evaluating mitochondrial membrane potential, Propidium iodide for evaluating cell cycle, DHE probe for evaluating the presence of superoxide ion, DCF probe for evaluating the presence for peroxides and finally Mercury orange probe for evaluating the glutathione activity.

RESULTS

Following the MTT reduction assay, the results suggest that the combination of photodynamic therapy with acetylsalicylic acid diminishes both cancer cells lines proliferation in a manner dependent of the concentrations of the photosensitizer. In flow cytometry, the results show that necrosis is the major cell death type, in a manner dependent of photosensitizer concentration, although cell death by apoptosis also occurs. In terms of cell cycle, there is a measurable retention in G0 in some conditions. The presence of reactive oxygen species seems to increase in a manner dependent of photosensitizer concentrations and the activity of GSH seems not to be highly affected by it. Finally the membrane mitochondrial potential seems to increase.

CONCLUSION

The experiments performed suggest that the combination of photodynamic therapy with acetylsalicylic acid decrease both cell lines (WiDr and OE19) proliferation with more efficacy than photodynamic therapy and aspirin per se. With flow cytometry there is also possible to see that the combination has also a better efficacy in the promotion of cell death compared with the solo methods. Furthermore it appears that the OE19 cell line is the most susceptible to this of approach. Therefore, these preliminary results are promising and encourage further studies.

PS 146

Sara Petronilho

Poster Presentation Poster Presentation

ANTITUMOR ACTIVITY OF SPLICING INHIBITOR PLADIENOLIDE B IN ERYTHROLEUKEMIA – A STUDY IN CELL LINES

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AIM

To study the antineoplastic effect of Pladienolide B in two erythroleukemia cell lines (K562 and HEL), particularly: To evaluate its effect in cell growth and viability; To perform a cell death and cell cycle analysis, to better elucidate its mechanism of

INTRODUCTION

The splicing of pre-mRNA into functional mRNA, carried out by the spliceosome, represents a crucial step for the cell's genetic expression. Alternative splicing is an extremely flexible point of gene control, of which cancer cells often take advantage, producing proteins that promote growth and survival. Recently, several splicing inhibitors, such as Pla-B, have been tested as antineoplastic agents in different malignancies, with promising results. Mutations in some of the spliceosome's components have been identified in several hematological malignancies, including MDS and acute myeloid leukemia (AML), constituting a potential therapeutic target to be explored.

METHODS

K562 and HEL cells were incubated in the absence or presence of increasing concentrations of Pla-B in single dose (from 0.25 to 100 nM) and in daily administration (for 0.5 nM). Cell viability and density were evaluated using the trypan blue method. Cell death was determined by optical microscopy (May-Grunwald Giemsa staining) and flow cytometry (FC). Cell cycle analysis was evaluated by FC, using a PI/RNAse solution. DNA sequencing was performed to assess the presence of SF3B1 mutations in exons 14 and 15.

RESULTS

Treatment with Pla-B significantly decreased the viability and proliferation of the K562 and HEL cells in a time, concentration and administration schedule dependent manner. HEL cells were more sensible to Pla-B than K562 cells (after 72 h of incubation the IC50 was 1.5 nM and 25 nM, respectively). SF3B1 mutations in exons 14 or 15 were not detected in any cell model used. Pla-B induced cell death preferentially by apoptosis and an accumulation of cells in the G0/G1 phase of the cell cycle.

CONCLUSION

Our results show that Pla-B induces a cytotoxic and cytostatic effect in both cell lines. Differences in drug response in our two models may be due to different cell genetic backgrounds. K562 cells present the BCR-ABL fusion gene which has been associated with increased expression of proteins involved in splicing and with alternative splicing, while HEL cells bear the JAK2 V617F mutation. SF3B1 mutations in exons 14 or 15 were not detected in any cell line, suggesting that the observed cytotoxic effect is not dependent on this spliceosome mutation. Mutations in other genes involved in the splicing process which could influence drug-response were not excluded in our models. Pla-B could represent a new therapeutic approach in the treatment of erythroleukemia. However, its efficacy may be dependent on cell genetic profile.

PS 149

Sara Veiga

Poster Presentation Poster Presentation

RELEVANCE OF MICRORNAS EXPRESSION IN RESPONSE TO TARGETED THERAPIES IN CHROMIC MYELOID LEUKEMIA

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AIM

Our goal was to evaluate the influence of the expression levels of miR-21, miR-125b and miR-155 in CML cell lines sensible and resistant to Imatinib, as well as the therapeutic potential of new drugs such as Bortezomib, Parthenolide and Everolimus.

INTRODUCTION

Imatinib is, at present, the first-choice treatment for chronic myeloid leukemia (CML) patients. Despite the impressive success of treatment obtained with this drug, some patients show incomplete response or resistance to Imatinib. The BCR-ABL oncoprotein, resulting from the BCR-ABL fusion gene, characteristic of CML, is able to activate multiple signaling pathways, including the NF-kB, mTOR and the proteasome pathways, which may provide new therapeutic targeted alternatives in cases of resistance to Imatinib. Furthermore, the altered expression of microRNAs, small RNA molecules that regulate gene expression, may influence the sensitivity and/or acquisition of resistance to therapy.

METHODS

In this work we used two CML cell lines, one sensitive to Imatinib (K562 cells) and another resistant to Imatinib (K562 RC cells). Cell viability was assessed by rezasurine assay and cell death by flow cytometry (annexin V and propidium iodide) and through optic microscopy (May-Grünwald-Giemsa staining). The expression of phosphorylated NF-kB, ubiquitin conjugates, and cell cycle analysis was performed by flow cytometry. The expression levels of AKT and AKT activation were analyzed by Western Blot. MiRNAs expression was performed by qRT-PCR using commercial kits.

RESULTS

Initially we observed that K562 RC cells showed an increased expression of miR-21 and miR-125b and a decreased expression of miR-155 in relation to K562 cells. Bortezomib, Parthenolide and Everolimus were able to induce a decrease in cell viability in a time-, dose- and sensitivity to Imatinib -dependent manner. Parthenolide and Bortezomib induced a stronger cytotoxic effect on K562 RC cells, while Everolimus shows a higher cytotoxic effect on K562 cells. These differences may be related, among others, with miRNAs expression levels. Moreover, these compounds induce cell death predominantly by apoptosis and also induce cell cycle arrest. Additionally, it was also observed that treatment with these compounds was also able to modulate the expression of these miRNAs .

CONCLUSION

In summary, miR-21, miR-125b and miR-155 expression levels could provide new biomarkers predictive of response to TKI and/or new targeted drugs such as Bortezomib, Parthenolide and Everolimus in CML. Moreover, these drugs may be new therapeutic approaches in CML .

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PS 150

Mariana Ribeiro

Poster Presentation Poster Presentation

METHIONINE SYNTHASE POLYMORPHISM INFLUENCE P15 AND P16 METHYLATION STATUS

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AIM

In this context, we investigated the relevance of one polymorphism in MTR gene (rs1805087) and its possible correlation with the development of MDS, as well as its influence in p15 and p16 methylation status.

INTRODUCTION

Genetic background, namely single nucleotide polymorphisms, and epigenetic abnormalities, may affect the individual risk for haematological malignancies development, including Myelodysplastic Syndromes (MDS). The epigenetic pathway driven by DNA methylation can be influenced by inadequate intake of folate, methionine or vitamin B12, which are responsible for the supply of methyl groups for DNA methylation. As so, single nucleotide polymorphisms in genes that encode these dietary nutrients' metabolism, such as methionine synthase (MTR), can affect DNA methylation status and contributing to the individual susceptibility to develop MDS. Besides that, tumor suppressor genes such as P15 and P16 are frequently hypermethylated in MDS, playing a role in the development of this haematological neoplasia.

METHODS

This study included 63 patients diagnosed with MDS and 98 healthy controls (CTL). The genetic profile of MTR (rs1805087) was assessed by Tetra-primer-ARMS-PCR. The strength of association between the polymorphism and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%). The localized methylation status was analysed through P15 and P16 methylation profile by MS-PCR, which was performed using two sets of primers, one for methylated DNA and other for unmethylated DNA. The statistical analysis was carried out by, $\chi 2$ test and Fisher exact test ($p < 0.05$).

RESULTS

For MTR, our results show a predominance of the frequency of allele A in controls and in MDS patients (CTL=83,7%; MDS=83,3%). As to genotype, AA is the most prevalent in both controls and patients (CTL=68,4%; MDS=69,9%) and GG genotype has the lowest prevalence (CTL=1%; MDS=2,9%). Our results also suggest that, even though there was no significance between the genotypes and disease risk, we observed a prevalence of the AA genotype for patients who had lower survival rates. Concerning the methylation profiles for MDS patients, our results show a correlation between the MTR GG genotype and the susceptibility to present the P16 gene methylated (p=0,003) or both, P15 and P16 (p=0,0108).

CONCLUSION

Our results suggest that MTR's genetic variant rs1805087 influence the methylation status of P15 and P16 tumor suppressor genes. However, these results need to be confirmed in larger systematic and prospective studies.

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Sara Isabel Fernandes

Poster Presentation Poster Presentation

POLYMORPHISMS IN TRANSPORTER AND METABOLIZING PROTEINS - ROLE IN MONOCLONAL GAMMOPATHIES' DEVELOPMENT

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AIM

In this work, we investigated the influence of MDR1 (rs1045642), MRP1 (rs4148330), OCTN2 (rs274561) and UCK2 (rs2185268) polymorphisms as risk factors for myeloid neoplasias development, namely MM and MGUS, as well as their possibility of prognostic risk

INTRODUCTION

Polymorphisms in transporter and metabolizing proteins may play a role in the predisposition for the development of hematological neoplasias, namely Multiple Myeloma (MM) and Monoclonal Gammopathy of Undetermined Significance (MGUS), and can influence drugs' efficacy. ABC (ATP-binding proteins), SLC (solute carrier) transporters and metabolizing proteins recognize endogenous and exogenous substrates, and are involved in their transport across intra- and extracellular membranes as well as in their metabolization/activation. Therefore, genetic variability in proteins MDR1, MRP1 (efflux transporters), OCTN2 (influx transporter) and UCK2 (metabolizing protein) can influence the development, progression and therapy response in neoplastic malignancies.

METHODS

This study enrolled 145 samples from patients diagnosed with monoclonal gammopathies (MG) (including 77 MM, 68 MGUS) and 164 healthy controls (CTL). The genetic profiles of MDR1, MRP1, OCTN2 and UCK2 were accessed by RFLP-PCR and ARMS-PCR. The strength of association between polymorphisms and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%) and the influence of these polymorphisms in patients' overall survival (OS) was assessed by Kaplan Meier analysis.

RESULTS

Our results display no deviations in allelic and genotypic frequencies from the expected values. On one hand, the OR analysis of polymorphic variants from MDR1 revealed that CT genotype is a risk factor for the development of MG, with an OR of 1.84-fold (CI95% 1.02–3.32, p=0.043). Besides that, CT genotype from MDR1 results in an increased risk for MM development of 2.27-fold (CI95% 1.09–4.70, p=0.028). On the other hand, allele T from MDR1 is a protector factor for the development of MM with an OR of 0.41-fold (CI95% 0.19-0.88, p=0.023). Despite MRP1 genetic variants show increased tendencies for the development of MG, in particular MM, the CI95% has no reliability for sustained conclusions. The Kaplan Meier analysis shows that MM patients with GG genotype from UCK2, relatively to GT or TT genotypes, have a decreased OS in about 11 months (GG – 31.1±3.8 months; TT or GT – 42.8±0.8 months; p=0.017).

CONCLUSION

Our results suggest a contribution of these genetic polymorphisms, mainly the genetic profiles, in monoclonal gammopathies' development and survival. However, their role in therapy response remains unknown. To clarify these issues a larger systematic and prospective studies will be needed.

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PS 173

Jelena Spasic

Poster Presentation Poster Presentation

IMMOBILIZATION OF ESCHERICHIA COLI WHOLE-CELL 4-OXALOCROTONATE (4-OT) BIOCATALYST FOR IMPROVED BIOTRANSFORMATION PROCESS

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AIM

The aim of this study was to improve biocatalytic production of 4-nitro-3-phenyl-butanal with two strategies: biocatalyst immobilization with alginate and carrageenan and product recovery using Amberlite XAD-2 polymeric adsorbent.

INTRODUCTION

β-nitrostyrene and its derivatives, products of Michael-type addition reaction, have antimicrobial activity and are often used in pharmaceutical industry as precursors for amino alcohols. Whole-cell system based on recombinantly expressed 4-oxalcrotonate tautomerase (4-OT) has been developed and shown to be effective biocatalyst of asymmetric Michael addition of acetaldehyde to β-nitrostyrene (Narancic T, 2013). Although biocatalyst was very efficient, improvement in terms of recycling of biocatalyst was necessary.

METHODS

Whole cells were immobilized in 4% alginate gel and beads of 1 and 2 mm were obtained. Liquefaction of the beads core was performed by addition of sodium citrate making them capsules. Whole cells were also immobilized in 1.5% carrageenan and cubes were made. Reaction was followed by depletion of beta-nitrostyrene by reduction of absorbance at 320 nm. Reaction product was recovered from aqueous reaction buffer by addition of XAD-2 resin.

RESULTS

With whole-cell immobilization, production of 4-nitro-3-phenyl-butanal was improved. Alginate beads and whole-cells showed activity at pH 7.4 and pH 9, while at pH 4 only beads were active while cells lysed, and all further experiments were carried out at pH 7. Carrageenan cubes and alginate capsules, had lower relative activity than free whole-cells and weren't stable after storage. Alginate beads had greatest relative activity in comparison to all other biocatalyst including free whole-cells, and retained 85% of activity after two months of storage. Bioprocess was further improved by using XAD-2 resin for more effective product recovery.

CONCLUSION

Alginate beads can be reused in multiple reactions making them cost-effective. By using XAD-2 resin for product recovery amount of organic solvent was reduced by 20-fold in comparison to previously reported method, making this process more environmentally friendly.

[1] Narancic T RJ, Jovanovic P, Francuski D, Bigovic M, Maslak V, Savic V, Vasiljevic B, O'Connor KE, Nikodinovic-Runic J. (2013) Highly efficient Michael-type addition of acetaldehyde to β-nitrostyrenes by whole resting cells of Escherichia coli expressi

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PS 28

Sava Kovacevic

Poster Presentation Poster Presentation

INFLUENCE OF PRENATAL DEPRIVATION OF VITAMIN C ON TEETH DEVELOPMENT IN GUINEA PIG FETUSES

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AIM

The aim of the research is to investigate the effect of prenatal vitamin C defficiency on teeth development of guinea pigs.

INTRODUCTION

Beside the perooksidative role of vitamin C it is also an essential factor in the process of collagen synthesis. Guinea pigs are precocial rodents with full prenatal dental development. Besides humans guinea pigs belong in a unique species that are not capable of synthesising vitamin C. The importance of vitamin C on tooth development has not been tested yet.

METHODS

The study encompassed 14 fertilized female albino guinea pigs. Their diet was comprised of vitamin C-free food and ad libitum water enriched with vitamin C. The 10th day of fertilization, experimental group was depleted of vitamin C. Deprivation lasted until the 50th day, after which the females were sacrified and their fetuses taken out. Viscerocranium of fetus were fixed and dehydratated, after wich they were embeded in parafin and longitudinal sections were made. Masson trichrome stain was used for histology

RESULTS

The lack of vitamin C during intrauterine development in experimental animals lead to: decresse of density and diameter of tooth pulp capilars; decresse of thickness of predentin and dentin layers; lack of dental enamel and lag of direct ossification process of the alveolar bone.

CONCLUSION

Intrauterine deprivation of vitamin C in guinea pigs led to relevant histological changes on all tooth structures and surrounding tissues.

1. Anđelković Z, Somer Lj, Danilović V. Zubi. U: Anđelković Z i sradnici. Histologija. Niš: Impressum; 2009. p. 188-190. 2. Roncević I. Digestivni sistem. U: Nikolić I, Radenković G, Todorović V. Embriologija data status; 2007. p.192-206. 3. Depew M, Tuck

PS 35

Ricardo Cardoso

Poster Presentation Poster Presentation

ACUPUNCTURE EFFECTS ON DELAYED ONSET MUSCLE SORENESS

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Fernando Pessoa University

AIM

The purpose of this study was to evaluate the effects of acupuncture on delayed onset muscle soreness.

INTRODUCTION

Delayed onset muscle soreness (DOMS) is a common form of muscle soreness, experienced by individuals who perform unaccustomed exercise as a result of exercise induced muscle damage (EIMD) (Aminian-Far et al., 2011). It typically involves an eccentric component, it peaks between 24 and 48 hours post-exercise and spontaneously disappears within 5 to 7 days (Aminian-Far et al., 2011; Torres et al., 2012). Muscle soreness can negatively interfere with the activities of daily living as well as sports performance (Barbe, Barr, 2006; Barr, Barbe, Clark, 2004). While western medicine attributes DOMS to local inflammation, due to either mechanical damage or swelling, leading to ischemia and muscle spasms, Traditional Chinese Medicine (TCM) views DOMS as localized "Qi" and "Xue" stasis that manifests as pain and soreness of the joints, muscles and/or tendons. Collectively, this is known as muscle Bi-syndrome (Xinnong, 1987). Therefore, from a theoretical standpoint, acupuncture might be an attractive, beneficial, low-cost, quick and low-risk treatment strategy for DOMS treatment, improving performance on athletes and productivity in workers.

METHODS

All research was undertaken at Hospital-School of Fernando Pessoa University and was previously approved by the Ethical Committee of Fernando Pessoa University. All participants signed an informed consent form in accordance with the Helsinki Statement being informed that they could quit at any moment without consequences and guaranteeing them anonymity. The sample of this study was comprised of a total of forty five volunteers (mean ± s age 25,38 ± 4,77 years; weight 65,76 ± 9,88 kg; height 169 ± 0,09 cm and body mass index 22,94 ± 2,51 Kg/m2) of both genders (19 males, 26 females). The subjects were randomly divided in three groups with 15 elements each using a software available at www.graphpad.com/quickcals: in a verum acupuncture group (VA), a sham acupuncture group (SA) and a control group (CG) with no loss in the follow up period. The study was divided in 3 phases: Pre-intervention phase, Exercise induced muscle damage (EIMD) protocol phase and an Intervention phase In the Pre-intervention phase the subjects, after being informed about the study design and procedures sign a consent form. After completing a screening questionnaire for biometric and personal data, as well as for detection of possible exclusion criteria, which included a history of hamstring or quadriceps muscle injury, previously medical diagnosed musculoskeletal pathologies in the lower limb or renal, cardiac, metabolic, and endocrine disorders which might inhibit the performance of physical exercise (Abad et al., 2010; Hilbert et al., 2003; Milias et al., 2005),use of anti-inflammatory AID"s, pain and muscle relaxants and individuals who had performed vigorous physical exercise within seven days prior to the protocol (Abad et al., 2010; Hilbert et al., 2003; Milias et al., 2005). Were also excluded woman's with menstruation, pregnant, participants with intense fear of needles and any type of drugs consumption (Itoh et al., 2008) and participants who drank beverages containing caffeine or alcohol in a period of less than 12 hours prior to the measurements (Hübscher et al., 2008).After being randomly assigned to one of three groups (VA vs. SA vs. CG), leg dominance was determined and baseline values established for the 6 separate main outcome measures on the non-dominant leg (Newton et al., 2012) in the following order: muscle soreness (MS), range of motion (ROM), pressure pain threshold (PPT), vertical jump (VJ) and isokinetic (average quadriceps peak torque; quadriceps peak torque/body weight) [Time 0 (T0)]. In the EIMD protocol phase the participants performed the same EIMD protocol of the Miyama and Nosaka (2004a), that has been previously shown to cause significant elevation in muscle damage indices and produce DOMS (Miyama and Nosaka, 2004a; Goodall and Howatson, 2008; Howatson et al. 2012). In this protocol subjects dropped from a height of 0.6m step and jumped upward maximally immediately after landing from the box and landed on the surface again after the vertical jump. To perform the next drop jump, subjects had to climb two steps of stair into the platform. Five sets of 20 drop jumps were performed with a 10 s interval between jumps, and a 2 min rest period was given between sets. Subjects performed drop jumps with barefoot, because it has been reported that biomechanical factors are influenced dramatically by shoes (Wit et al., 2000; Ogon et al., 2001), and comparisons between bouts were thought to be easier by eliminating the effect of shoes. In the intervention phase, a second (T1, prior to the intervention), third (T2, 20 min after the intervention), a fourth (T3, 24h after, 10 min before the second intervention) and a fifth (T4, 24h after, 20 min after the second intervention) assessments were performed. In the T1, T2 and T3 assessments, only MS, ROM, PPT were performed, as measures as VJ and isokinetic might exacerbate the muscle injury. In the T4

assessment, all T0 baseline measures were repeated. Acupuncture was performed or not, for 2 minutes immediately after T1 and T3 assessment moments, according the group they belong. In the CG they didn´t underwent acupuncture but a 2 minutes period of rest between assessments were assured as it was the necessary time to apply the acupuncture technique in the VA and SA groups. Participants in the VA were submitted to an acupuncture treatment using the "Leopard spot" technique (Greten, 2010) in the meridian conduit points of S34, S36, and H3, on the non-dominant limb with an insulin needle, in the SA they received the same technique but in three other points also on the non-dominant lower limb but that were not associated with any conduit (with no therapeutic evidence). The CG didn´t received any type of treatment. For the execution of acupuncture protocols, all participants were positioned in the supine position and received treatment at total rest. All participants were blinded regarding the treatment and didn`t know to which group they were allocated (Witt, et al., 2005). Once properly desinfect the skin, the participants were intervened in points according to the group. The points were selected by experts practitioners. The selection of points in VA was based on the Heidelberg model of Traditional Chinese Medicine (Greten, 2010). The "Leopard spot" technique consisted in 5 quick penetrations on skin (Hauer, et al., 2011; Nabeta, and Kawakita, 2002) in the selected points. The penetration depth was controlled by the size of the blade of the insulin needles (Hauer, et al., 2011). The data collected were processed using the statistical analysis software IBM SPSS Statistics 22. Descriptive measures were calculated and a Shapiro-Wilks test was used for assessment of normality and as there wasn´t a normal distribution along the groups, non-parametric tests were used. A Kruskal-Wallis test with for analysis of differences between groups in the different moments was performed along with a Friedman test with a Bonferroni post-hoc test, to assess differences within the group along the different assessment moments.

RESULTS

The protocol of EIMD showed success in inducing DOMS. When VA, SA and CG groups were compared, it was found that VA had better results in all outcome measures, especially in MS, PPT and VJ. However, SA group showed better results than CG, when MS and PPT were compared, but they were not statistically significant.

CONCLUSION

Acupuncture has been studied as a treatment for many causes of pain, being a promising treatment for DOMS because it showed that can reduce MS and improve PPT and VJ, yet with limited results in muscular power and ROM.

PS 41

Borodai Inna

Poster Presentation Poster Presentation

CORRECTION OF IMMUNOLOGICAL DISORDERS IN PATIENTS WITH HABITUAL MISCARRIAGE

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AIM

The aim of this study was to reduce reproductive losses by identifying features of immune status in HM and eleborate new methods of complex treatment of this disease.

INTRODUCTION

Protection of women reproductive function and the birth of a healthy child are important problems of modern obstetrics. Among this problems antenatal care in habitual miscarriage (HM) has great importance. The incidence of this abnormality varies from 10 to 25% of all pregnancies and is the cause of 75% of newborn morbidity. Significant success in the prevention and treatment of HM was achieved recently, but incidence of this disease tends to increase.

METHODS

Materials and methods. Immune status of 85 pregnant women with HM was studied. They were divided into 2 clinical groups depending on the treatment. I clinical group consists of 43 pregnant women with threatened miscarriage who underwent conventional therapy. II clinical group include of 42 pregnant women with threatened miscarriage who underwent combined therapy with extracted cells of placental tissue (ECPT). The levels IgG, IgM, B- and T- lymphocytes, circulating immune complexes, neutrophils phagocytic activity in serum blood were examined in the study. Women with antiphospholipid syndrome, genetic abnormalities, hyperhomocysteinemia, sexual apparatus abnormalities, infectious diseases were not included into the studied groups

RESULTS

Levels of IgG in women of gr. I increased to 9,44 g/l, while in the gr. II they grew up to 9,56 g/l ($p<0,05$) after 2 weeks of treatment. Level of IgM in the gr. I grew up to 0,79 g/l, in gr. II to 0,83 g/l. Level of B-lymphocytes (CD22+) in the gr. I elevated from 11,2% to 16,4%, while in the gr. II – from 14,1% to 48,2% ($p<0,05$). The level of T-suppressors(CD8+) grew more sharply in the gr. II (21,7±1,3% - 31,3±1,2%), compared to the gr. I – (22,1±0,6% - 26,4±0,9%). Number of T-helper cells(CD4+) decreased in gr. II – (36,0±1,5% - 24,5±0,7%), while in the gr. I – (34,5±1,4% - 27,4±0.7%) ($p<0,05$). The best results among women with the threatened of miscarriage were in the group of patients who were administered ECPT before the treatment. Term delivery were observed in 38 women of gr. II and in 29 women of gr. I. Preterm birth was noted in 4 women of gr. I, and in 2 women of gr. II ($p <0.05$). Pregnancy ended with miscarriage in 10 women in the gr. I, and in the gr. II – only in 2 cases.

CONCLUSION

The evidence of active immune correcting effect of the ECPT is more conspicuous stabilization of most indicators of neutrophils phagocytic activity in women complex treatment compared to patients, that received conventional therapy of threatened abortion. Using of ECPT in the complex treatment of HM has a strong immune correcting action that promotes prolongation of pregnancy and can significantly reduce the number of complications during pregnancy and improve birth outcomes, indicating it's high efficiency (90.5%) compared with conventional therapy (67.4%) .

PS 42

Skorbach Olena

Poster Presentation Poster Presentation

IMMUNOLOGICAL IMBALANCE IN WOMEN AFTER HYSTERECTOMY

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AIM

The aim of this study was to investigate immunological processes in women with uterine myoma after hysterectomy.

INTRODUCTION

Hysterectomy is the most common surgery in gynecological practice. Postoperative changes in the ovaries leading to their reduced production of sex hormones, which play an important role in the regulation of immune responses. However, the lack of a common pathogenic concepts of the various manifestations of abuse adaptation deficit estrogen effects on a woman's body leads to the necessity to search new etiopathogenic approaches.

METHODS

The study included 60 women aged 40-51 years. The basic (I) group consisted of 30 women who underwent abdominal supracervical hysterectomy without removal of both ovaries. The control group (II) consisted of 30 apparently healthy women. Hormonal concentration in serum blood was determined by enzyme immunoassay using test kits manufacturing company "Alkor Bio" (St. Petersburg, Russia). Cytokine concentrations in serum blood were determined by ELISA using commercial test kits produced by "Protein contour" (St. Petersburg, Russia). Fas-L was determined by using a reagent kit company «Medsystems» (Austria).

RESULTS

It was found a significant increase of gonadotropic hormones (follicle-stimulating and luteinizing) against of decreasing of sex hormones (estradiol, progesterone) [$p<0.05$] in women after hysterectomy compared to the women of group I. More significant changes in the concentrations of cytokines in the dynamics were observed at definition of TNF-α, γ-IFN and Fas-L. The concentration of TNF-α in women of gr. I was significantly increased in almost 4 times compared to gr. II [from 0.81±0.09 pg/ml to 3.01±0.13 pg/ml, $p<0.05$]. The concentration of γ-IFN in the serum of patient's gr. II increased almost in 3.5 times in comparison with indicators of gr. I [from 8.8±0.07 pg/ml to 2.8±0.09 pg/ml, $p<0.05$]. It was observed increasing of Fas-L in patients after hysterectomy in 4 times compared with the control group [from 0.12±0.03 to 0.49±0.03 pg/ml., $p<0.05$].

CONCLUSION

Thus, estrogen deficiency that occurs in women after hysterectomy can influence immune system and lead to increasing of concentrations of γ-IFN, that entails an elevate of other cytokines – TNF-α, as well as a marker of apoptosis Fas-L.

PS 43

Diana Tarawneh

Poster Presentation Poster Presentation

PECULIARITIES OF FAMILY HISTORY IN INFERTILE WOMEN WITH INEFFECTIVE ASSISTED REPRODUCTIVE TREATMENT

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AIM

Optimization of patients' preparation to ART in order to increase the efficiency and reduce complications in stimulation of superovulation and in the further course of the pregnancy.

INTRODUCTION

Stimulation of superovulation in assisted reproductive treatment (ART) is a factor associated with activation of microthrombogenesis, yet there usually should be aggravating factors to implement them. These factors include inherited or acquired predisposition to thrombosis. Furthermore, ART ineffectiveness can be related to a disorder of microcirculation and development of microthrombi in endometrium and chorion at the early stages of pregnancy. One of the clinical guidelines aimed at identifying hereditary defects of hemostasis, when preparing the patients to in vitro fertilization, is the assessment of family history of thrombotic conditions: venous thrombosis in family members younger than 50 years, early heart attacks and strokes, signs of venous thromboembolism, varicose veins.

METHODS

The study involved 60 women with unsuccessful attempts of in vitro fertilization in history – I group and II group (control group) 30 healthy women. Clinical examination included a detailed study of family history of thrombotic conditions. All patients were examined for antiphospholipid syndrome (acquired thrombocytopathy) and hereditary forms of thrombocytopathy. Laboratory diagnosis of antiphospholipid syndrome (APS) includes determination of lupus anticoagulant and antiphospholipid antibodies by ELISA - method. Functional coagulologic methods were employed to exclude possible deficiency of natural anticoagulants, namely AT III and protein C. Assessment of thrombophilia severity was carried out by assessing the level of direct markers-complexes, such as thrombin-antithrombin complex (TAT) and D-dimer.

RESULTS

Patients of I gr. were found to have a higher percentage of compromised thrombotic history - 33%, as compared to 10% in the control gr. The structure of family history of thrombotic conditions was as follows: myocardial infarction in 30%, hemorrhagic and/or ischemic stroke - 28%, pulmonary embolism - 10%, varicose veins and venous thrombosis - 32%. Examination of hemostatic system revealed hypercoagulable syndrome due to increased activity of internal coagulation mechanism factors and enhanced functional activity of thrombocytes (FAT) in the I gr. in 45% women, while 20% of women were found to have significant impairments in protein C system.The number of patients with increased FAT in the II gr. comprised only 10% and none of them had disorders in protein C system. Hyperhomocysteinemia was observed in 31.6% patients of the I gr. and in 20% patients of the II gr. Circulating lupus anticoagulant (CLA) was found in 41.7% women of the I gr. CLA was observed in 16.7% patients of the II gr.

CONCLUSION

Reduction in hypercoagulation factors in the 2nd group might have contributed to successful fertilization after IVF. In order to increase ART effectiveness and reduce the risk of thrombotic complications, it is recommended to provide a thorough study of family history of thrombotic conditions followed by diagnosis of hereditary and acquired thrombophilia.

PS 70

Mário Sousa Pimenta

Poster Presentation Poster Presentation

ANTI-ANGIOGENIC PROPERTIES OF RED RASPBERRY EXTRACT ARE MEDIATED BY REDUCED CELLULAR PROLIFERATION AND MIGRATION ABILITY OF HUMAN ENDOTHELIAL CELLS

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AIM

This investigation addressed the hypothesis that red raspberry extract exerts a potent anti-angiogenic effect through their phenolic compounds.

INTRODUCTION

Angiogenesis plays a critical role in many diseases. As a hallmark of slow damage accumulation diseases such as cancer or diabetes, it still urges to address potential inhibitors of the phenomena. Polyphenols are a class of natural compounds whose potential as antioxidant molecules and inhibitors of chronic, neurodegenerative diseases and cancer has been reported over time. Recent in vitro and in vivo studies have reported reduced cellular proliferation upon cancer cell lines, albeit being elusive due to lack of a deep analysis of the signalling pathways involved in the phenomena and a broad analysis of endothelium mediated effects.

METHODS

Human microvascular endothelial cells (HMVECs) were used to determine the influence of red raspberry extract on cellular viability (MTS assay), proliferation (BrdU incorporation), migration (injury assay) and TLS formation (Matrigel assay). HMVECs cultures were established and treatments were performed during 24h, at the concentrations of 10, 25, 50 and 100 µg gallic acid equivalents (GAE)/mL and compared with a control group (culture medium with extract excipient). Western blot analysis of protein expression in cell lysates was performed in order to determine the subcellular underpinnings related to the previous results observed.

RESULTS

Red raspberry phenolic compounds reduced cellular viability and proliferation in a dose-dependent manner. The concentrations of 50 and 100 µg EAG/mL exhibit higher levels of cytotoxicity when compared with controls, thus limiting the broad spectrum of different extract concentrations analysis. A significant reduction in the percentage of proliferating cells was found at 50 and 100 µg EAG/mL, corroborating the MTS results. Phenolic compounds induced a significant abrogation of human endothelial cells ability to migrate to injured area, even at low concentrations. The total TLS number decreased in a dose dependent-manner towards higher extract concentrations. Likewise, the number of branching points per unit of area was also significantly diminished at all concentrations and varies in a dose dependent-manner, albeit paradoxically, towards higher test concentrations the ratio free TLS / total TLS suffered an increase.

CONCLUSION

Our results strongly suggest that phenolic compounds present in red raspberry extract inhibit angiogenesis through suppression of proliferation and migration of endothelial cells. At higher test concentrations, the reduction of branching points per unit of area and the increase of free TLS/total TLS ratio prompt us to the inability of connection of filopodia extensions between adjacent cells. Further studies are required to support the influence of phenolic compounds in cytoskeleton organization and its pathophysiologic consequences. Elucidating these mechanisms is crucial for the development of preventive/therapeutic strategies targeting pathological conditions where angiogenesis is exacerbated.

PS 77

Ernesto Ruivo

Poster Presentation Poster Presentation

HOW ARE EMBRYONIC AND INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC MYOCYTES PROTECTED AGAINST SIMULATED ISCHEMIA/REPERFUSION IN THE PRESENCE OF A NO DONOR

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AIM

In this project we had 2 main goals: identification of stem cell derived cardiomyocytes via detection of specific cardiac cell markers and secondly, test the response of these cells to simulated ischemia/reperfusion (SI/R)-induced cell death.

INTRODUCTION

Embryonic and induced pluripotent stem cell (ESC and iPSC, respectively)-derived cardiac myocytes are potential cell sources for the study of cardioprotective mechanisms and therapy.

METHODS

Mouse embryoid bodies (EBs) were seeded onto gelatin coated surface and were kept in growth medium under normoxic conditions (at 37ºC, 5% CO2) for 8-16 days. EBs were dissociated by the application of 4 different digestion procedures. Cells were then labeled with cTnI and VCAM-1 antibodies, being the immunopositive cells detected by flow cytometry for the precise identification of cardiac committed cells. In a separate experiment, mouse EBs (HM1, IPS 3.4, IPS 4.1) were subjected to 150 min SI in the presence of the NO-donor SNAP (10-6 M) or vehicle, followed by 120 min R. After SI/R, viability of cells was assessed by propidium iodine staining. Then, one set of full EBs were measured with plate reader, while another set of EBs were digested and cells were immunolabeled (cTnI and VCAM) and analyzed by flow cytometry.

RESULTS

The intracellular antigen cTnI was remarkably expressed in both day-8 and -16 with all types of digestions. The highest cTnI expression was registered on day-8 (75.31% of total cells) with application of trypsin. The cell surface antigen VCAM-1 was not detectable after trypsin digestion. However, the application of collagenase type IV on day-16 resulted in the highest ratio of VCAM-1 positive cells (41.76%). IPS (line 3.4) EBs and VCAM positive cells were more sensitive for SI/R injury than IPS 4.1 cell line. Interestingly, SNAP protected full EBs from SI/R, but not immunopositive cardiac myocytes isolated from the EBs.

CONCLUSION

We successfuly identified and validated the expression of intracellular and cell surface antigens present in stem cell derived cardiomyocytes Moreover, we concluded that the cardiocytoprotective NO donor protects full EBs against SI/R injury, but not the cardiac myocytes in the EBs, suggesting that iPS-derived cardiac myocytes at the current development stage are not suitable for testing cardiocytoprotective mechanisms.

PS 100

Emilija Djuric

Poster Presentation Poster Presentation

THE EFFECT OF ALLOPREGNANOLONE ON LOCOMOTOR ACTIVITY OF RATS

Emilija Đurić
Assist. Prof. Aleksandra Rašić Marković

AIM

The aim of this stydy was to investigate locomotor response to allopregnanolone in adult female rats.

INTRODUCTION

Allopregnanolone is a neuroactive steroid that has stimulative, anxiolytic, and antidepressant effects. Fluctuations in the levels of allopregnanolone seem to play an important role in various neuropsychiatric disorders.

METHODS

Adult female Wistar albino rats were divided into following groups: 1. Saline-treated (C); 2. Allopregnanolone (20 mg/kg i.p.: A group). Rats were tested in automated locomotor activity monitors (48 cm long × 48 cm wide × 40 cm high; Experimetria Ltd, Budapest, Hungary) housed in light-proof, sound-attenuating room. All experiments were performed between 8:00 A.M. and 4:30 P.M. using animals maintained on a 12 hr light/dark cycle from 6:00 A.M. to 6:00 P.M. The room was lit by a 12-W infrared bulb. Activity monitors were used to quantify horizontal and vertical activity (rearing). As rats moved within the activity monitors, infrared beam interruptions were automatically recorded as activity counts, and these data was translated by the Experimetria software to horizontal distance traveled (in mm), which was used as the measure of locomotor activity. Independent measures included total ambulation distance traveled, ambulation time (total time spent in motion), number of rearing events and thigmotaxis (i.e. walking close the walls). Thigmotaxis was assessed by the ratio of the distance covered less than 2.5 cm away from the walls to the total distance covered, expressed as a percentage.

RESULTS

Allopregnanolone (20 mg/kg) 20 min after administration, significantly decreased ambulation distance ($p < 0.01$), ambulation time ($p < 0.01$) and number of rearings ($p < 0.01$) in A compared to the C group. Thigmotaxis was not affected by i.p. injection of allopregnanolone ($p > 0.05$).

CONCLUSION

Allopregnanolone in dose 20 mg/kg, 20 min after administration significantly decreased vertical and horizontal activity in rats, but did not affect thigmotaxis in open field test.

PS 148

Carina Brito

Poster Presentation Poster Presentation

ARGINASE 1 AND INOS EXPRESSION AT SYSTEMIC LEVEL AND FETOMATERNAL INTERFACE DURING TOXOPLASMA GONDII INFECTION

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AIM

Our study focused on the effects of T. gondii infection on Arg-1 and iNOS expression at the maternal-fetal interface and systemic level (Spleen and Peritoneal exudate cells) using the mice model.

INTRODUCTION

Toxoplasma gondii (T. gondii), is considered to be the world's most successful zoonotic parasite, causing congenital toxoplasmosis, the second most common fetal intrauterine infection, leading to toxoplasmosis, often causing neonatal mortality, spontaneous abortion and blindness [1]. In the last years, scientific breakthroughs have been made in understanding the immune response to toxoplasmosis, but there is still lack knowledge about which immune players are responsible for the pathology associated with this infection during pregnancy, specifically at the maternal-fetal interface. Macrophages have a variety of activation states, being able to adapt their functions to environmental changes of cytokines, described as innate (TLR ligation), classical/M1 (TLR ligation with IFN-gamma) or alternative/M2 (IL-4Ralpha ligation) [2]. Thus, while classical macrophage activation is able to control replication through induction of inducible nitric oxide synthase (iNOS), alternative activation can control parasite replication through induction of arginase 1 (Arg-1) and depletion of arginine [3].

METHODS

Infection of BALB/c and C57Bl/6 mice, resistant and susceptible strains respectively to T. gondii infection and the use of a type II strain of T. gondii allowed the follow-up of pregnancy. In vivo infection was performed using viable tachyzoites obtained from in vitro infected-confluent human foreskin fibroblasts. Pregnancy outcome was evaluated both in infected and control animals by the quantification of normal and resorbed implantation sites. The evaluation of parasite loads in the organs was done by quantitative Real Time-PCR using Taqman probes (qRT-PCR). Arg-1 and NOS expression was evaluated by immunohistochemistry.

RESULTS

A decreased parasite load is observed in spleen cells from pregnant BALB\c or C57BL/6 mice compared with non-pregnant animals. The parasite load obtained in PEC presented a different pattern probably due to different cell population's composition when compared to spleen cells. Immunohistochemical analysis indicated an increased expression of Arg-1 in the decidua from infected animals (BALB\c and C57Bl/6) compared to controls, while iNOS expression is increased in controls compared with infected animals.

CONCLUSION

Our results suggest that pregnancy is somehow able to protect the animals against infection with T.gondii. However, these results are not statistically significant and increased number of samples must be analyzed. Immunohistochemical analyses tells us that in mice infected with T. gondii, the activation of macrophages changes from M1 profile (classic) to an M2 profile (Alternative) . To confirm these results more precise techniques will be used, such as RT-PCR and Western Blotting.

[1] Li XL, Wei HX, Zhang H, et al. A meta-analysis on risks of adverse pregnancy outcomes in Toxoplasma gondii infection. PLoS One 2014; 9: e97775. [2] Brown MB, von Chamier M, Allam AB, et al. M1/M2 macrophage polarity in normal and complicated pregnancy

PS 160

Salah Izat Shokry

Poster Presentation Poster Presentation

THE LEVEL OF THE MAIN PRO-INFLAMMATORY INTERLEUKINS IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS AT DIFFERENT STAGES OF THE DISEASE DEVELOPMENT

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AIM

The goal of our research was to study the level of the main pro-inflammatory interleukins in children with JIA at different stages of the disease

INTRODUCTION

Pro-inflammatory interleukins are major pathogenic factor in the development of juvenile idiopathic arthritis (JIA). Its increased level reflects disease activity and justifies the purpose of immunobiological therapy. One of the pressing issues remains valid, the optimal choice starting drug therapy to prevent the base of its ineffectiveness.

METHODS

The study involved 32 children (23 girls, 9 boys) within 2-18 years with JIA, who were hospitalized in the clinic of SE "Institute for children and adolescents health care of NMSA Ukraine" and 34 healthy children for control group. The duration of the disease up to 6 months was in 23,4%; up to 12 months - in 12,9%; up to 24 months – in 21,3%; more than 24 months - in 42,5% of patients. Interleukins content (IL1β, IL6, TNF-α) was studied by ELISA. For the statistic processing of the material Stagraphics 3.0, parametric and non-parametric criteria were used.

RESULTS

There is in children with JIA revealed significantly elevated mean levels of cytokines. Increased mean level of IL-1β was more frequency in patients with polyarticular JIA variant than with oligoarthritis ($p<0,05$); level of IL-1β was increasing during increasing duration of the disease ($p<0,05$) and did not depend on the sex and age of patients. The mean level of IL-6 was higher at patients with polyarticular JIA female ($p<0,05$) and did not depend on the duration of the disease. TNF-α content was highest in boys ($p<0,05$), in patients with disease duration less than 24 months and was comparable in patients with different types of disease. The high correlation between IL-1β and IL-6 ($r=0,68$, $p<001$), IL-1β and TNF-α ($r=0,71$, $p<001$) in patients with disease duration maximum 12 months were found. Independence value have revealed in patients with JIA. Least TNF-α content was noted in female patients with the highest levels of IL-6. It is in this patient's group obviously not effective initial therapy by TNF blockers.

CONCLUSION

A cytokine level in patients with JIA has a very elevated, but varies depending on the sex, variants and duration disease. Changing the content of cytokines in the blood is an important prerequisite for the selection of biological therapy.

PS 8

João Ramos

Poster Presentation Poster Presentation

MORPHOMETRIC ANALYSES OF BRIAN ATROPHY IN TYPE 2 DIABETES: EVIDENCE FROM BOTH T1 AND T2 MRI

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AIM
We aimed to study the regional distribution of brain atrophy in DM2, with an emphasis on vascular anomalies, analyzing both T1 and T2-weighted MRI scans.

INTRODUCTION
Type 2 diabetes (DM2) is associated with brain atrophy and microvascular pathology. However, the available literature is poor concerning the distribution of brain atrophy in DM2. We aimed to study the regional distribution of brain atrophy in DM2, with an emphasis on vascular anomalies, analyzing both T1 and T2-weighted MRI scans.

METHODS
This study used magnetic resonance imaging (MRI) scans in 34 participants with DM2 and 42 participants without DM2. VBM was used to study the distribution of atrophy in DM2. T1-weighted scans were analyzed with the usual intent of identifying anatomical abnormalities in brain tissue, whereas T2-weighted scans were used to assess both anatomical and vascular disturbances.

RESULTS
T1-VBM showed a bilateral distribution of atrophy, affecting mainly the sub-lobar and insular areas, but also the occipital and temporal lobes. T2-VBM showed similar patterns, namely atrophy in the limbic lobe, as well as in the sub-lobar, insular and temporal areas, all bilaterally. An ANOVA test allowed to visualize the overlapping areas of both analyses.

CONCLUSION
In conclusion, we found that gray-matter atrophy in DM2 patients is bilaterally distributed in the sub-lobar and insular areas, as well as in the temporal and occipital lobes and limbic system.

PS 12

Divya Goil

Poster Presentation Poster Presentation

EFFECTS OF MATERNAL HYPOTHYROIDISM ON PREGNANCY OUTCOME: A RECORD BASED COMPARATIVE STUDY.

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2-Professor and HOD of Preventive and Social Medicine, 3,4,5,6,7-MBBS Students

AIM

• The study aims to determine the proportion of maternal hypothyroidism among women visiting the tertiary care hospital under study.
• To assess the effects of maternal hypothyroidism on maternal and neonatal health and compare the occurrence of different

INTRODUCTION

More than 136 million women give birth every year. About 20 million of them experience pregnancy related illness. There is evidence that pregnancy exerts a stressful impact on thyroid gland, causing alteration of thyroid function. Consequently, thyroid disorders are amongst the most common endocrine problems in pregnant women. Improving the well-being of mothers and infants is an important public health goal for every country as it determines the health of the next generation. According to some studies, associated feto-maternal outcomes were increased risk of abortion, low birth weight, premature delivery, anaemia, preeclampsia, placental abruption, postpartum haemorrhage (PPH) and gestational diabetes. In contrast, other studies have suggested that maternal hypothyroidism is not associated with a consistent pattern of adverse outcome. Therefore, further scientific study is required to correlate the two.

METHODS

This is a Retrospective Comparative study carried out in February 2014 at Kasturba Medical College Hospital, Attavar. A pretested proforma was used to collect data on 80 hypothyroid (H) and 160 euthyroid (E) pregnant women. It consisted of 4 sections containing antenatal details, maternal and neonatal outcomes and obstetric details, respectively. The records of pregnant women attending the antenatal care facility in 2013 were studied. The collected data was analysed using SPSS v.11.5. For qualitative data, Chi-square test was used and $p<0.05$ was taken as statistically significant.

RESULTS

A total of 445 obstetric cases had been investigated for thyroid function. Of these, 80 (18%) were found to be hypothyroid (avg TSH: 6.972) whereas 330 (74.2%) were euthyroid (avg TSH: 2.395). The predominant maternal complications observed were anaemia (H-36.3%, E-26.3%, p=0.11), preeclampsia (H-18.8%,E-11.9%,p=0.15), abruptio placenta(H-5%, E-3.8%,p-0.648), GDM (H-11.4%, E-7.4%, p=0.317), PPH (H-8.8%, E-13.1%, p=0.648) and cardiac dysfunction (H-3.8%, E-2.5%, p=0.570). The predominant fetal complications observed were abortion (H-22.5%, E-13.8%, p=0.086), breech presentation (H-23.8%, E-15%, p=0.096), low birth weight (H-10%, E-5.6%, p=0.213), premature delivery (H-8.8%, 6.3%, p=0.477) and respiratory fetal distress (H-12.5%, E-8.8%, p=0.361).

CONCLUSION

Amongst the fetal complications, abortion (22.5%) and breech presentation (23.8%) showed a favourable trend and amongst the maternal complications, anaemia (36.3%) and preeclampsia (18.8%) were found to be most predominant in hypothyroid women. Although similar complications were also found in women of euthyroid status, percentages of complications were found to be considerably higher in hypothyroid pregnant women suggesting that inclusion of routine thyroid screening during the antenatal period as well as timely management of the same in order to possibly reduce risk of various pregnancy related complications, should be carried out.

1. 10 facts on maternal health – WHO [Updated on August 2013] Available from: http://www.who.int/features/factfiles/maternal_health/en/index.html 2. Glinoer D, 2004 The regulation of thyroid function during normal pregnancy: importance of the iodine nutri

PS 36

Magdalena Czerżyńska

Poster Presentation Poster Presentation

CONVENTIONAL RADIOGRAPHY. DENTAL FILLING PIXEL BRIGHTNESS.

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AIM

The assessment of level of brightness of each image pixel during a X-Ray imaging different permanent dental restorations.

INTRODUCTION

Dental X-Ray is the precisest and the fastest method to diagnose secondary caries. Conventional radiography, because of availability and small dose of radiation which is given to patient, is still popular in dental offices

METHODS

Twelve probes of different dental materials were made and radiated. Forty eight measurements ware made using variable voltage 70-75kV.Constant conditions: scratter grid, FFD (Focus Film Distance=100cm). Results were worked out with computer program – Stata.

RESULTS

It was proved that the type of dental filling material has an influence on pixel brightness ($p<0.001$). The value of voltage alternating current has an effect on pixel brightness ($p<0.001$) every twelve probes of different materials

CONCLUSION

The level of RTG radiation absorption determinates the brightness of pixel and Grayscale. The higher level of radiation absorption is, the higher brightness of pixel is. Creating dental materials, make use of permanent restorations, which have reasonable level of radiation absorption could improve diagnostic quality of radiographs.

PS 39

Dynnik Oleksandra

Poster Presentation Poster Presentation

CHARACTER OF ANTHROPOMETRIC PARAMETERS AND SEXUAL DEVELOPMENT OF GIRLS WITH ABNORMAL UTERINE BLEEDING

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AIM

The aim of our work was to determine the features of physical and sexual development of girls with AUB

INTRODUCTION

All biological functions of the child's organism are somehow associated with parameters of its body, as it is the most important integral criterion for growth and development. Metamorphoses, occurring with a teenager in a relatively short period of time, require considerable strain of all the systems in his organism. Over the last years these phenomena are regarded in clinical medicine as functional disorders, including functional pubertal disorders (FPD), to which belongs abnormal uterine bleeding of puberty (AUB P). Even in the normal course of puberty the teenager's body undergoes a considerable tension, caused by physiological changes. Naturally, in case of delay, precocity or disorders in synchronicity of the body adaptive tension of the organism systems in adolescents is enhanced significantly. In general, these variants of the course of physiological development at puberty in the clinical practice are defined as dysontogenesis and can serve as a cause of AUB.

METHODS

331 adolescent girls with AUB P, aged 11-18, were under observation (ranging in age from 11 to14 years, n=173 and from 15 to 18 years, n= 158). Study of the anthropometric data has revealed that in 57.1% (n=189)body weight corresponded to the physiological norm, overweight was observed in 26.3% (n=87), including 9.1% of patients with obesity, and body weight deficiency was recorded in 16.6% (n=55)of our patients.

RESULTS

It should be noted that overweight and obesity were typical for the younger girls, and body weight deficiency was characteristic of the senior patients (p1,2 <0,01). Deviations in the growth parameters have been revealed in more than a third of patients (37.5%). In 30.2% they are registered within SDS ± 1, and in 17,3% -SDS> ± 2. Tall stature has been found significantly more frequent in patients of a younger group (p <0,01). As regards sexual development, its precocity has been established in 44.4% of a younger age group, with respect to a calendar age. Retardation of sexual development was observed in 5,7% (p <0,01) of patients from the group of senior adolescents, which was significantly more frequent than in the population. Early menarche was recorded in 12.7% of patients. This is significantly more often than healthy peers. At physiological body weight bleeding most often occurs with menarche, and in patients with impaired physical development in the period of menstrual function.

CONCLUSION

We can assume that the occurrence of uterine bleeding at physiological anthropometric parameters associated with the breakdown of adaptive capabilities, and when violations - most likely due to discoordination regulatory mechanisms. Adolescents with deviations in anthropometric parameters and sexual development disturbances are at risk for the formation of menstrual function disorders, the most serious of which is AUB at puberty. They should be under long-term medical supervision for carrying out sanative, therapeutic and preventive measures.

PS 54

Leila Azimi

Poster Presentation Poster Presentation

ANTIBIOTIC PRESCRIBED AND THE RESULTS OF ANTIBIOTIC SUSCEPTIBILITY TESTING IN BURN PATIENTS

Leila Azimi 1*, Somayeh Soleymanzadeh-Moghadam 2 , Laleh Amani 1, Faranak Alinejad1, Aida Rastegar Lari 3 , Abdolaziz Rastegar Lari 1

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AIM

The aim of this study was to determine relationship between antibiotics prescribed and the results of antibiotic susceptibility testing in microbial laboratory in teaching burn hospital in Tehran, Iran.

INTRODUCTION

Burns and subsequent consequences are a global problem. Burn wound infection is one of the most common causes of death in burn injuries. Further, burn patients are at high risk for nosocomial infection. Thus, infection control in burn patients especially in the first 5 days after hospitalization is important. Infection control during this time period can prevent morbidity and mortality in these patients. Appropriate and accurate antibiotic prescription can be considered an important factor in increasing the awareness of patients about proper antibiotic use.

METHODS

In this cross sectional study, we consider Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus aureus as most important cause of nosocomial infection especially in burn patients. 525 strains of Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus aureus were isolated from 335 hospitalized burn patients. Identification of the strains was performed by biochemistry and microbiological tests. Antibiotic susceptibility tests were performed according to CLSI guide line for each bacteria genus. The records were audited to find the antibiotic used.

RESULTS

The results indicated that P. aeruginosa is the most prevalent Gram negative bacteria. The results of this study showed some antibiotics prescribed like, imipenem, amikacin, ciprofoloxacin and cefepime regardless of the antibiotics susceptibility responds in the lab.

CONCLUSION

Infection control is very important in burn care units, because burn wound infection is one of the main causes of morbidity and mortality among burn patients. Thus, the appropriate prescription of antibiotics can be helpful, but unreasonable prescription can have detrimental consequences, including greater expenses to patients and community alike.

PS 96

marjan rashidan

Poster Presentation Poster Presentation

EVALUATION OF PREVALENCE AGENTS OF COMMUNITY-ACQUIRED URINARY TRACT INFECTION AND ANTIBIOTIC RESISTANCE IN PATIENTS ADMITTED TO LABAFINEJD HOSPITAL IN TEHRAN, IRAN

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AIM

This study was undertaken to compare the prevalence of uropathogens and their antimicrobial resistance in Community-Acquired Urinary Tract Infection (CA-UTI) admitted to Labafinejd hospital in Tehran, Iran.

INTRODUCTION

Urinary tract infection is one of the most common bacterial infections in outpatient settings. Increased antimicrobial resistance of uropathogens is a matter of global public health concern. Treatment of CA-UTI depends on both prevalence and antimicrobial resistance of causative bacteria at specific geographical areas.

METHODS

This survey was carried during a six month period on patients with urinary tract infection at Labafinejad hospital and Pediatric Infectious Research Center of Shahid Beheshti University in 2014. A total number of 275urine samples were sent to laboratory of microbiology and identified by bacterial standard methods. Antimicrobial resistance tests were done by disk diffusion agar according to criteria recommended by the National Committee for Clinical Laboratory Standards (CLSI 2014).

RESULTS

Of the 275 bacterial isolates the most frequent isolates were Escherichia coli (76%), enterococci (15.6%), Klebsiella pneumonia (5%), Citrobacter spp. (1.4%), proteus mirabilis (1.09%), Enterobacter spp. (0.3%), and Edwardsiella spp (0.3%). The results indicated that among Enterobacteriaceae, E.coli isolates resistant to most antibiotics, Nalidixic acid (76%) and the least resistance to Meropenem (4.7%). Of Gram-positive cocci, 86% of enterococci were Tetracycline resistance and 100% sensitive to Ampicilin and Penicilin.

CONCLUSION

The findings of this study reveal that E. coli is the predominant pathogen of this infection. According to the continuous changing in bacterial isolated from patients with UTI and antibiotic resistance patterns, it is recommended that bacterial resistance patterns in populations are evaluated in any region annually.

MALE'S KNOWLEDGE AND ATTITUDE ABOUT DOMESTIC VIOLENCE

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AIM

The aim of the study is to understand male's attitude behaviour and knowledge about domestic violence.

INTRODUCTION

Domestic violence is a global phenomenon without national, economic, religious, geographic or cultural borders. Domestic violence can be defined as a pattern of behavior in any relationship that is used to gain or maintain power and control over a family member. This abuse can happen in multiple forms including physical, emotional, and mental. Previous researches show that domestic violence is mostly performed by males. WHO estimates that 30% of women who have been in a relationship have experienced some form of physical or sexual violence by their partner an 38% of murders of women are committed by an intimate partner.

METHODS

This is a descriptive study. Data was collected from male officers and nurses who work in two hospitals located in Diyarbakır and Istanbul and male students from a college in Istanbul, using a standard questionnaire(Q54).Data was analyzed by SPSS 11.0 with Spearman-Correlation , Chi-Square tests and significance level was accepted as 0.05.

RESULTS

Volunteer 414 males were interviewed [32.3±9,4year(median=30.0,min=17,max=63)]. Data was collected two different cities in terms of socioeconomy and culture. Forty two percent of participants have never been married.Fifty seven percent of participants had a university degree or higher, 8% of participants had a middle school education or less.Forty percent of the married males stated that psychological violence must not be performed ever and 60% of them stated that it might be performed under some certain circumstances. Majority of participant (80%) stated that they did not approve the physical violence while 20% of them stated that it can be performed under some certain circumstances. Half of them who thought physical violence can never be performed, stated that psychological violence can be performed.Beside this, 83% of participants informed that they had experienced physical abuse by their parents at least once during their childhood, 11% of these, performed verbal abuse on their wives. Minority of participants (1.5%) who had never experienced physical violence during their childhood, performed psychological violence on their wives(p X2=0.0005). Also, 90 % of the ones who have been subjected to violence at least once during their childhood have informed that they applied physical violence to someone else in the future.Nearly 40% of participants have stated that they do not concern insulting as violence, 46.4 % do not concern scolding as violence , 48.1 % do not concern restriction of freedom as violence.In addition of these, 29 % of participants have stated that they agree with the idea of " women should not be working since it impacts the unity of the family"

CONCLUSION

Most of the participants don't state some kind of behaviour as a violence which are normally violence such as insult, scolding,restriction of freedoms. In addition to that, majority of participants especially who had experienced physical abuse by their parents at least once during their childhood that are tend to be performing psychological violence and they even do not think psychological violence as violence.Also some of the participant think about working or studying of womens affect unity of family in a bad way, that's why women's shouldn't work or study. In order to fix these problems, awareness of violence and education during childhood would be beneficial.

1-)Council of Europe, 2002; BMA 1998; British Home Office Research Study, 1999 2-)Global and regional estimates of violence against women: prevalence and health effects of intimate partner violence and non-partner sexual violence, WHO, 2013

Prof. Dr. Suleyman Goren, Assist. Prof. Dr. Fuat Nihat Ozaydin

PS 140

Daniel S. Joffe

Poster Presentation Poster Presentation

AUTOMATIC DETECTION OF ARTERIOSCLEROSIS OF CAROTID ARTERIES ON DENTAL PANORAMIC X-RAYS.

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1 - Master Student at the University of Twente 2 - Supervisor at the University of Twente 3 - Dentist at Tandzorg Steenwijk (Steenwijk Dental Clinic)

AIM

To create a computerised automatic detection of arteriosclerosis of carotid arteries on digital dental panoramic X-rays

INTRODUCTION

Arteriosclerosis of carotid arteries is a major cause of strokes. Several studies in the past have already shown that arteriosclerosis of carotid arteries can be seen on dental Panoramic X-rays. However, as the origin, diagnosis, impact and treatment of arteriosclerosis in the neck area is not in any way a dental problem or does it have any dental implications, the finding of such an anomaly is mostly ignored in the full dental field, ranging from education on the subject to clinical identification by dental clinicians.

METHODS

The purpose of this study was to see if arteriosclerosis of the carotid arteries could be detected automatically by computer analysis of digital panoramic X-rays. Based on thirty digital panoramic x-rays provided and diagnosed initially by the VU Medical Centre of Amsterdam, department of Maxillofacial Surgery, Mathlab software was used to automatically obtain the regions of interest. Mainly on pixel values, regional maxima and minima and the interconnectivity of structures.

RESULTS

It turned out that the Mathlab software could be programmed to have a high sensitivity for detecting structures in the region where arteriosclerosis of carotid arteries anomalies appear on digital panoramic x-rays.

CONCLUSION

Further fine tuning of the software is needed for better specificity; primarily because the sample was limited to thirty images only, but secondarily as the golden standard for confirming arteriosclerosis of the carotid artery diagnosis is the ultrasound examination of the neck, data from such examination should be fed back so the software could self learn to optimise itself for a more specific recognition.

Assistance provided by Dr. H.S. Brand and the VU medical centre of Amsterdam, department of Maxillofacial surgery was of the utmost importance to even begin this study.

PS 165

Iana Fedorenko

Poster Presentation Poster Presentation

THE INFLUENCE OF RISK FACTORS OF NON-COMMUNICABLE DISEASES ON MEDICAL STUDENTS IN UKRAINE

Iana Fedorenko
PhD, professor Haliyenko Ludmila

AIM

Is to determine the health status of medical students and to define the lifestyle and risk of chronic non-communicable diseases of medical students.

INTRODUCTION

From the health of medical students is largely dependent future performance of the functions of preserving and promoting health of population. So, it is necessary to correct the lifestyle of students which on 50% causes the formation of public health.

METHODS

Conducted literature review and questionnaires of 128 respondents, 80 of them women and 48 are men; average age 20.5 years.

RESULTS

The research work showed us that frequency of NCD among medical students (100 respondents) 21.8 for diseases of the digestive system, 18.0 - respiratory diseases, 14.1 - cardiovascular diseases, 11.7 - diseases of the genitourinary system, 10.9 - allergic disease. It was found that only 53.9% of respondents feel themselves good. Although, during the year only 7.8% were healthy 25.8% did not ask for a medical help at all. Regularly visit doctors 1-2 times per year - 65.6% of respondents; 4 or more times per year seek treatment – 8.6%. After analysis of the incidence was found that 75% of respondents have illness 1-2 times during the year. The risk of non-communicable diseases in the last year was 17.2%. The main reasons in which students not appeals for a treatment (100 respondents) are the difficulties arising from absence on lesson (70.1±1.2), distance of profile health institution (61.7±1.2), own medical knowledge (34.9±1.3), the idea that the disease "will go" itself (14.0±0.9). The groups of the risk of diabetes and latent forms of hypertension are 2.4% and 3.9%, respectively 36.7% of respondents observed had pain in the heart. 51.5% noted a sharp sudden dizziness. 60.2% felt nervous and mental overload. Stressful situations during the study noted 72.8%. Character of a feed of medical students, showed that 46.0% have insufficient and dangerous multiplicity meals (1-2 times), 73.2% do not have breakfast, 30.9% do not have lunch and 94.0% have a late dinner in the range of 7-12 p.m. It was found that 11% of respondents smoke very often. 6,3% students very often drink alcohol. 28.9% of respondents who regularly doing exercises and sport. 14.1% of respondents classified to special medical groups. 12.5% are on the dispensary accounting. 16.4% of respondents' close relatives suffering from chronic non-communicable diseases. Based on respondents' reasons for dissatisfaction about their health at the first place they noted "Stress situations" (65%), on the second place – "Mental overload during the studying" (61%), the third – "Non-rational schedule" (55%).

CONCLUSION

There are common risk factors NCD among the students Ukraine. Unhealthy lifestyles of students make a significant prevalence of diseases, including NCD. There is a tendency to increase the frequency of incidence during the years of study in University. With significant levels of morbidity of students revealed low levels of appeals for medical help (low medical activity). Promising ways to safeguard and promote the health of students, is active promotion of healthy lifestyles and reduce the prevalence of risk factors. The priority form of prevention work should be a course on new European policy - Health 2020 and the correction of risk factors of NCD and disease prevention can be achieved through an integrated approach involving motivational, social, economic, informational instruments.

PS 27

Edit Vigh

Poster Presentation Poster Presentation

BEDSIDE MONITORING OF EXPIRATION FLOW HETEROGENEITY UNDER ELECTIVE CARDIAC SURGERY

1-Edit Vigh, 2-Ferenc Petak, 2-Gergely H. Fodor, 1-Barna Babik

1-University of Szeged-Faculty of Medicine-Department of Anaesthesiology and Intensive Therapy, 2-University of Szeged-Faculty of Medicine-Department of Medical Physics and Informatics

AIM

Our study was made to measure that establishing a closer maesurement point to the alveolo-capillary surface of the capnogram how reduces this measuring mistake and how it influences the ventillation heterogeneity of the lung.

INTRODUCTION

Slope of the third phase of capnogram illustrates ventillation heterogeneity and ventillation-perfusion fitting disorders. However, the different CO2 containing virtual gas compartments' heterogenous emptying dynamics can notify properly this inhomogeneity in patients under ventillation, axial heterogenity during endotracheal tube ventillation diminishes the flow-heterogenity.

METHODS

We investigated patients (n=6) under elective cardiac surgery. CO2 levels were measured simultaneously with side-stream capnogram both from the endotracheal tube's distal part (AO) and from 0,5cm before the tube's intratracheal part (IT). Then we identificated the third phase from both methods, and the fluctuations were characterized with spectral analysis. Magnitudes given at 0,4 Hz (S0,4) were compared at different parts of the operation considering both the AO and IT datas.

RESULTS

Third phase, detected with IT, is more variable than the AO's, what was registered as the S0,4 3,24±0,4 higher levels ($p<0,05$). Elevation of PEEP makes capnogram curves' smoother, and this was seen both the IT and the AO curves' decreasement at S0,4 (-60±5%; -59±2%, $p<0,05$).Opening the chest resulted in the same way as mentioned above (-60±10%; -33±21%, $p<0,02$).

CONCLUSION

Analysis of the records show that the axial gas blending at the endotracheal tube disfigures the third phase of the capnogram. Usage of the intratracheal capnogram reduces this drawback, and this way it proves us an accurate way for the bedside registration of exspirational heterogenity.

PS 33

Fereshteh Yazdanpanah

Poster Presentation Poster Presentation

IS THERE ANY CORRELATION BETWEEN RADIOLOGIC FINDINGS AND ERADICATION OF SYMPTOMS AFTER PYLOROMYOTOMY IN HYPERTROPHIC PYLORIC STENOSIS?

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AIM

investigate the correlation between radiologic findings and eradication of symptoms after pyloromyotomy in hypertrophic pyloric stenosis

INTRODUCTION

Infantile hypertrophic pyloric stenosis (HPS) is a condition in which the antropyloric portion of the stomach becomes abnormally thichened, resulting in obstruction to gastric emptying. HPS occurs in patients aged 2-10 weeks and is the most common surgical condition in infants, with a frequency of approximately 1 to 2 cases per thousand births. Despite the frequency of this condition, it was not recognized until the late 19th century, when the detailed description of two fatal cases was reported by Hirschsprung in 1887 and published one year later. It has been shown that HPS occurs much more frequently in males than in females. The incidence rate in Caucasians is higher than that in Asians. The etiology of HPS is still unclear, undoubtedly includes environmental and genetic components. The typical presentation is projectile, non-bilious vomiting occuring at the age of 2-8 weeks and usually occurring 10-30 minutes after feeding. Although medical diagnosis can be done by palpable olive-shaped mass in the right upper quadrant, abdominal ultrasonography and barium studies are necessary to establish the diagnosis. In this study, we wanted to investigate the correlation between radiologic findings and symptoms eradication after pyloromyotomy in HPS patients.

METHODS

We retropectively studied 124 patients from March 21, 2004 to March 20, 2014. The infant patients had confirmed diagnosis of HPS and underwent pyloromyotomy at Children's hospital of Tabriz, Iran. We excluded premature infants as well as infants with febrile or septic conditions. Patients with bilious vomiting were also excluded. There were 101 boys and 23 girls, at median age of 35 days (range: 16 to 90 days) in the study patients. Diagnosis was initially established by either an ultrasound or barium meals together with clinical findings followed by intraoperative confirmation. Open pyloromyotomy was performed once the patient was adequately resuscitated and the associated metabolic derangements were corrected. Feeding was started classically 6 hours postoperatively and advanced gradually according to patients' tolerance. Patients were discharged once they tolerated full enteral feeding without vomiting. Demographic features, clinical findings, diagnostic work-up, type of operation and postoperative findings were recorded for the study patients. Ultrasonographic findings were recorded for the study patients. These included pyloric canal length (PCL) and pyloric muscle diameter (PMD). These values were calculated in the milimeter scale. Postoperative persistence of vomiting was calculated in hours and days of postoperative admission to discharge were recorded in days. The pearson correlation coefficient was used for comparative analysis of continuous variables.

RESULTS

During the study period, we had 124 patients (101 boys, 23 girls) with confirmed diagnosis of HPS who admitted to Children's Hospital of Tabriz, Iran. The male: female ratio was 4.43: 1, and the median and mean (SD) age at presentation were 35 (1.90) and 39.06 (1.42) days. According to ultrasound findings of patients, the range of pyloric canal length (PCL) was between 7.60 to 29.00 mm and the mean of this parameter was 19.54±0.324 mm. Pyloric muscle diameter (PMD) was between 2.70 to 9.00 mm and the mean of this parameter was 4.86±0.108 mm. Among the study patients, 90 patients had episodes of postoperative vomiting. Table 1 illustrates PCL, PMD and duration of postoperative vomiting and admission in the study patients. Plain abdominal radiography was done in 30 patients while 14 patients had barium swallow. PCL and PMD did not have any significant correlation with the time to vomiting persistence after pyloromyotomy (p-value=0.735 and p-value=0.812 respectively). The correlation with the duration of hospitalization were also insignificant (p-value=0.814 and p-value=0.930 respectively).

CONCLUSION

Difinitely, it would be useful to determine which patients can tolerate feeding and discharge early after pyloromyotomy and which patients cannot, so as to benefit from the significant cost saving and improved use of hospital beds and appropriate and effective service that result from early discharge and yet prevent emergency department visits and readmission for some patients and anxiety for their parents. Previous studies have shown little change in postoperative admission time on the basis of feeding regimen. Early feeding (less than four hour) after pyloromyotomy have not decreased the time to full feedings or the duration of postoperative hospitalization. Furthurmore, ad libitum feedings also had little effect on time to full feedings and to discharge. The most recent change in treatment of pyloromyotomy has been the adoption of laparascopic pyloromyotomy. A recent multi- institution, prospective, randomized trial by Hall et al. showed that the time to both full feedings and discharge was reduced by 10 hours with the laparascopic technique. However, in this study, the time to deliberate feedigs was still 18.5 hours and the duration of hospitalization after surgery was 33.6 hours. Furthurmore, two other prospective studies showed that the time to full feedings and the time to discharge were similar between the laparascopic and open techniques. Predicting early discharge on the basis of the pyloromyotomy technique is thus not feasible. Because previous studies found no effect from factors not associated with the patients themselves, we sought correlation between patient-specific radiological factors such as pyloric length (mm) and pyloric width (mm) and vomiting persistence after pyloromyotomy and duration of hospitalization. In our study, we found pylorus size had no effect on persistence of vomiting after surgery and postoperative length of stay. Our study had multiple limitations. First, it was retrospective study. Several different surgeons performed the pyloromyotomies, and the specific techniques used were based on each surgeon's preference. During the study period, the minimally invasive approach had just begun to be implemented, accounting for the low number of laparascopic pyloromyotomies. However, we do not believe that the use of different techniques would have a major effect on recovery, as shown by multiple previous studies. Another limitation of our study was that the postoperative feeding regimen was not standardized although previous studies have not shown any association between different regimens and postoperative conditions of the patients. Finally, only slightly more than 70% of the patients in our study were discharged within 24 hours. Because of these results, we believe that until a prospective study confirms these findings, patients should remain hospitalized until they tolerate full feedings after pyloromyotomy.

PS 65

Piotr Kwiatkowski

Poster Presentation Poster Presentation

INTRAOPERATIVE RUPTURE (IOR) DURING SURGICAL TREATMENT OF ANEURYSM– ANALYSIS OF INTRAOPERATIVE DATA.

Piotr Kwiatkowski, Aleksandra Wolny
Neurosurgical Student Group at the Neurosurgery Department of MSW Central Hospital in Warsaw.

AIM

Aim of the study was to analyze and assess frequency of known risk factors of intraoperative rupture (IOR) aneurysms in population of patients treated surgically for aneurysm in our department.

INTRODUCTION

There are three treatment options for patients with the cerebral aneurysm: non-surgical therapy, clipping and endovascular coiling. The surgical clipping remains the gold standard in the treatment of both ruptured and unruptured cerebral aneurysms. The Intraoperative aneurysm rupture (IOR) remains a serious complication of intracranial aneurysms treatment.

METHODS

The study was based on the protocols of surgical procedures conducted in Central Hospital MSW in Warsaw from 1995 to 2010. Patients who underwent brain aneurysm surgery were included to the analysis. Following factors were analyzed: patient age, location of aneurysm and presence of subarachnoid haemorrhage before the surgery.

RESULTS

In analyzed period of time 368 patients underwent brain aneurysm surgery. The mean age was 51 years. The incidence of IOR was 43%, majority was caused by aneurysmal manipulation (96%). Aneurysm locations were as following: middle cerebral artery (MCA) - 31%, internal carotid artery (ICA) – 31%, anterior communicating artery (ACA) - 29%. The highest rate of IOR was recorded at surgery for aneurysms of the middle cerebral artery (MCA) with 46% IORs of MCA surgeries what consist 14% of all IORs, next was internal carotid artery (ICA) with 43% and 13% and anterior communicating artery (ACoA) with 41 % and 12% IORs. IOR of ophthalmic artery aneurysms was 100% but there were only 4 patients. The incidence of ruptures shows increasing tendency with in larger aneurysms. The incidence of IOR is lower during the treatment of asymptomatic aneurysms (12 %) than those with SAH episode before.

CONCLUSION

It seems crucial to assess risk of IOR for selection of appropriate treatment methods. In case of signifficant risk of IOR we suggest operative clipping technique. The low rate of IOR in the treatment of asymptomatic aneurysms is argument supporting surgery for unruptured asymptomatic aneurysms.

PS 66

Aleksandra C. Wolny

Poster Presentation Poster Presentation

POSTOPERATIVE PSYCHOLOGICAL SYMPTOMS IN THE PATIENTS AFTER MENINGIOMA RESECTION.

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Neurosurgical Student Group at the Neurosurgery Department of MSW Central Hospital in Warsaw.

AIM

The aim of the study was to assess and compare patients outcomes after meningioma resection.

INTRODUCTION

Meningiomas are mostly benign tumours originating from the arachnoid cap cells, represent 13–26% of all intracranial tumours. They are more common in older age and in females. Five-year survival for typical meningiomas exceeds 80%, but is poorer (5-year survival <60%) in malignant and atypical meningiomas.

METHODS

: Cases involving 97 adult patients who underwent surgery for an intracranial primary meningioma between 2007 and 20012 at MSWiA Central Hospital in Warsaw and we eliminated patients with others diseases with signs may imitating signs from CNS to get the data of 56 patients . We prepared statistic with included localization of tumor and neurological signs causes by it after this we garner informations about with sings from these before operation are still present and if appear new.

RESULTS

From the cohort of meningioma patients women constitute 68% patients and men 32% and mean age of patients was 60 year. Additional post-operative signs didn't appear only in 26 patients (46%). From the frontal lobe tumor resection common symptom was a headache, which after surgery disappeared completely but after this location of surgery patients start complained that their behavior turned into excessive tearfulness and depressive and appeared sleep problems in 33% of patients. Epilepsy also was presented in 25% of these patients. The headache was the most frequent preoperative symptom from temporal lobe location of the tumor in 50% of patients but after surgery disappeared in 85% of them. Patients reported that their behavior after surgery became more impulsive and nervous and most of them was under the care of a psychiatrist. They also have sleep and memory problems. Motor aphasia in complication in 62% of patients with left parietal lobe tumor but after surgery didn't disappeared at all.

CONCLUSION

Additional symptoms after operation appearing often and it is important to consider psychiatrist diagnosis after resection tumor from frontal and temporal region. Epilepsy is common and serious postoperative complication and it should be pharmacological controlled.

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Monika Wenio

Poster Presentation Poster Presentation

INFLUENCE OF HIGH POSTOPERATIVE DRAINAGE ON EARLY OUTCOME AFTER ORTHOTOPIC HEART TRANSPLANTATION

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AIM
The purpose of this research was to assess the necessity of rethoracotomy and factors that impact the duration of postoperative respiratory therapy and Intensive Care Unit (ICU) stay.

INTRODUCTION
Heart transplantation (HTX) is the ultimate treatment option in patients with end-stage heart failure. Despite advancements in early postoptreative patients care, postoperative complications are still an issue. The risk of postoperative bleeding in this group is particularly high due to urgent heart transplant surgery. The purpose of this research was to assess the necessity of rethoracotomy and factors that impact the duration of postoperative respiratory therapy and Intensive Care Unit (ICU) stay.

METHODS
The study included 53 consecutive patients (4 females, 49 males, median age 53 years), who underwent orthotopic heart transplantation between the years 2007-2014 and survived the first day after surgery. Among other things, analysis assesses the value of chest tube drainage and the necessity for blood products transfusion within first 24 hours after the surgery. Then, we performed analysis of the impact of the above factors on mortality rate, duration of respiratory therapy and ICU stay, the total duration of hospitalization and the need for rethoracotomy during the first day.

RESULTS
Median postoperative drainage within the first 24 hours after surgery accounts for 685 ml. Statistically significant differences between the measured drainage in the first two and last two hours of analysis has been proven (p=0,000). High postoperative drainage correlates with longer respiratory therapy (p=0,046). In the group of patients who required a large amount of packed red blood cells (pRBC) transfusion, longer time of respiratory therapy was observed (p = 0.00) and also a longer duration of ICU stay (p = 0.00). Within the first 24 hours, 13.21 % of the patients required rethoracotomy. The mean drainage for the decision of rethoracotomy was 941.43 ml +/- 647.57 ml.

CONCLUSION
The study indicates that increased postoperative drainage and increased request for pRBC prolongs the time of respiratory therapy. Increased request for pRBC also prolongs the duration of ICU stay.

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Mariana Matias

Poster Presentation Poster Presentation

LOCAL RECURRENCE OF RETROPERITONEAL LIPOSARCOMAS

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AIM

The purpose of this meta-analysis was to compare and present the current insights and controversies regarding the studies recently published about LPS therapy.

INTRODUCTION

: Soft tissue sarcomas are mesenchymal neoplasms, biologically heterogeneous, relatively uncommon but with a high recurrence rate. The most common subtype is liposarcoma (LPS), which represents 24% of extremity and 45% of retroperitoneal soft tissue sarcoma. The natural behavior and outcome of LPS are dependent of the age of the patient, anatomical site and depth, size, respectability of the tumor, as well as of histology, grade, nodal disease, and distant metastasis. The clinical behavior of LPS ranges from indolent nonmetastasizing disease that grows expansively to aggressive subtypes that can recur and metastasize rapidly, with an incidence rate of 4%. Accurate diagnosis and staging using a multidisciplinary approach are important to establish appropriate management and prognosis. Surgical resection is the mainstay of curative treatment. However, complete surgical resection is often technically challenging and limited by invasion of adjacent nerves, blood vessels, and organs. Local recurrence following complete resection of primary retroperitoneal liposarcoma is a common clinical problem that frequently leads to morbidity and mortality.

METHODS

Meta-analysis

RESULTS

A major challenge in treating retroperitoneal LPS today remains the high rate of local recurrence. Even when a complete resection has been achieved, local recurrence is the main site of treatment failure. The average growth rate of a locally recurrent liposarcoma, defined as the maximal linear tumor size of the local recurrence divided by the time interval to local recurrence, may help select patients likely to benefit from repeat surgical resection. The use of adjuvant therapy, in patients with retroperitoneal LPS, to reduce the probability of recurrence and distant metastases has long been a controversial topic of discussion. One of the most important limiting factors is difficulty in delivering sufficient radiation dose because of toxicity to surrounding organs including kidneys, bowel, liver, and spinal cord. Some studies have suggested that adjuvant radiation therapy may decrease the probability of local recurrence rates in superficial trunk and extremity sarcomas. However, the efficacy of adjuvant therapy for retroperitoneal is less clear. Randomized trials using newer radiation techniques are on going, but the results were not publish yet. This techniques, including intensity modulated radiation therapy, respiratory guided therapy, proton or heavy ion radiation therapy, and stereotactic radiation therapy should permit a higher dose of radiation to be given with less normal tissue toxicity . The benefits of chemotherapy for retroperitoneal LPS are even less clear.

CONCLUSION

Complete surgical resection is still the treatment of choice for patients with initial and locally recurrent retroperitoneal liposarcoma. Incomplete resection should be undertaken only for symptomatic relief.


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