Book of Abstracts

Oncology & Molecular Biology

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PS 195

Dupuytren's fibroblasts extend and retract their processes with altered concentrations of TGF-?1

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AIM

We hypothesised that 30ng/ml of TGF-?1 would lead to shortened cell processes when compared with a concentration of 12.5ng/ml, while an absence of TGF-?1 would leave the process lengths unchanged. In addition, we hypothesised that the fibroblasts would extend and retract their process lengths in response to increasing and decreasing doses of TGF-?1.

INTRODUCTION

Dupuytren's contracture is a disorder of the hands where progressive fibrosis of the palmar fascia causes it to become thickened and shortened; initially, there is fibroblast proliferation and differentiation into myofibroblasts – cells that have been shown to have a key role in contraction of the collagen matrix – leading to digital flexion and functional impairment. Currently, surgical release is the main treatment for Dupuytren's contracture. However, there are risks and complications with surgical intervention and so less invasive therapies are being investigated, particularly those targeting the aberrant pathophysiological processes responsible for the development of the condition. One of the growth factors that has been found at high levels in Dupuytren's tissue is transforming growth-factor beta-1 (TGF-?1). Increasing doses of TGF-?1 up to 12.5ng/ml has been shown to increase the contraction force exerted by the Dupuytren's fibroblasts in a three-dimensional collagen gel, while higher doses have been shown to inhibit this. However, although changes in contractile force and myofibroblast up-regulation have been quantified, changes in Dupuytren's fibroblast morphology in response to altered concentrations of TGF-?1 have not been tested to date.

METHODS

Dupuytren's nodule fibroblasts (n=3), with flexor retinaculum fibroblasts serving as controls, were embedded in 3D fibroblast-populated, restrained collagen gels and treated with 0, 12.5 and 30ng/ml of TGF-?1. The effect of both constant exposure and increasing or decreasing concentrations on cell process' length was assessed by fixing the after both four and six hours, and then staining with Phalloidin-Alexa Fluor 488 and Hoechst. Cells were then visualised under ultraviolet light using an inverted microscope, cell process length was measured and the data were analysed using ImageJ software (1) and SPSS Statistics 17.0 (2).

RESULTS

Both Dupuytren's and flexor retinaculum fibroblasts demonstrated significantly shorter processes at concentrations of 0 and 30ng/ml of TGF-?1, compared with 12.5ng/ml. Furthermore, the lengths of the Dupuytren's fibroblast processes were significantly shorter with increasing and decreasing doses of TGF-?1 after exposure to 12.5ng/ml ($p<0.0001$). Dupuytren's fibroblasts had significantly shorter processes than their flexor retinaculum counterparts, and showed a natural tendency to retract them in the absence of TGF-?1.

CONCLUSION

A concentration of 30ng/ml TGF-?1 inhibits Dupuytren's fibroblast process extension, compared with 12.5ng/ml, while in the absence of TGF-?1 the tendency of the cells is to retract their processes. This is the first study to quantify the changes in Dupuytren's fibroblast process length and to show that Dupuytren's fibroblasts are sensitive to changing concentrations of TGF-?1. We propose that this process plays a significant role in the matrix contraction process of Dupuytren's disease. This opens up new avenues of non-surgical treatment options to be explored by inhibiting this process.

PS 234

The identification of genes regulated by androgen receptor splice variants in castrate-resistant prostate cancer

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AIM

To identify genes regulated by ARv in a cell-based model of CRPC

To show different regulation of genes by Androgen receptor full length and variants

INTRODUCTION

The current standard of care for clinically localized prostate cancer (PCa) is surgery or radiotherapy. However, patients with metastatic or recurrent disease are most often managed with androgen deprivation therapy, either alone or in combination with an anti-androgen. While androgen deprivation therapy frequently results in an initially favorable clinical response, after a period of quiescence, tumors invariably progress to the lethal phenotype of the disease referred to as castrate-resistant prostate cancer (CRPC). There is now incontrovertible evidence that the androgen receptor (AR) remains active in this stage of disease. Recent studies suggest that progression to CRPC may be due to generation of AR splice variants (ARv) lacking the ligand binding domain. The aim of this study was to identify genes regulated by ARv in a cell-based model of CRPC.

METHODS

Experiments were performed in two PCa cell line models: LNCaP (androgen-dependent) and 22Rv1 (CRPC model). An AR null cell line was used to study individual ARv. Protein expression was determined by western blot analysis. ARv transcriptional activity was determined by a Luciferase reporter assay.

RESULTS

22Rv1 cells were transfected with siRNA to knock down either all AR isoforms or just AR full length (AR-fl). Changes in the gene expression were analyzed to determine genes specifically modulated by AR-fl, ARv or all isoforms. We confirmed by western blot analysis that some genes are regulated by all AR isoforms, while others are specifically modulated by either ARv or AR-fl. We found AR-fl up-regulates Nurr-1 in the presence or absence of androgen, while ARv decreases its expression. All AR isoforms up-regulate the expression of FANCI independently of androgen stimulation. In contrast, ARv strongly down regulates PACP, while AR-fl decreases its levels in androgen-dependent manner. Finally, we found two genes specifically modulated by ARv independently of the presence of AR-fl. ARv induce PMEPA-1 and inhibits NPR-1 expression.

CONCLUSION

We show differential gene expression between AR isoforms in PCa. ARv target genes are implicated in signaling pathways central to proliferation, angiogenesis and metabolism. Further analysis of these signaling pathways may reveal potential therapeutic targets in patients who progress despite androgen deprivation therapy.

PS 235

P53 expression in endometrial carcinoma

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AIM

Study goal is to evaluate the level of p53 protein expression in endometrial carcinomas and significant correlation between elevation of p53 protein expression in cancerous tissue and certain clinical and patological factors cocerning endometrial carcinoma- patient's age, histological type, histological grade and depth of myometrial invasion.

INTRODUCTION

P53 is a protein that provides the DNA repair or the begining of cell death by apoptosis. P53 mutation is the most common genetic change that occurs in human cancers. High expression of p53 protein is considered to have higher expression in biologically aggressive tumors of different locales, including endometrial carcinoma.

METHODS

A total of 34 cases of endometrial carcinoma treated at the Surgical clinic for operative oncology, Institute of Oncology Vojvodina, were included in this study. The immunohistochemical staining for p53 protein were evaluated.

RESULTS

Immunoreactivity of p53 proteine has been demonstrated in 13 out of 34 cases (38.2%) of endometrial adenocarcinoma. Seven of these (27%) are endometrioid, and the remaining 6 (75%) non-endometrioid type of endometrial adenocarcinoma. The highest percentage of p53 expression was observed in women over the age of 60, but there were not statistical significance. Non-endometrioid type of adenocarcinoma showed positive immunoreactivity of p53 in 75% cases, in contrast to the endometrioid type in which the percentage was 27%. Increased expression of p53 protein was detected in 6 (85.7%) out of 7 grade III tumors, in 3 (20%) out of 15 tumors grade II and 4 (36.3%) out of 11 grade I tumors.

CONCLUSION

Statisticaly significant correlation has been determined between positive p53 protein expression and non-endometrioid type of carcinoma, and low degree of histological differentiation. There was no statistically significant correlation between p53 protein expression and an older age (women older than 60 years of age) and the degree of myometrial invasion.

PS 260

Investigating the genetic relationship between SMC/Condensin and Topoisomerase-II in the yeast Saccharomyces cerevisiae Genome

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AIM

This investigation assesses the potential of a synergistic effect when inhibiting both Topoisomerase-2 and Condensin, which may prove beneficial clinically in tackling cancer.

INTRODUCTION

DNA Topoisomerase II is a target for numerous anti-cancer drugs used to treat breast, lung and prostate cancers. Although highly effective, these agents produce unwanted side effects in quiescent cells, for example cardiomyopathy. Therefore an important goal of clinical research into Topoisomerase II is to maximise therapeutic efficacy whilst minimising the risk of adverse effects. One approach to this is to simultaneously target pathways that promote Topoisomerase II action specifically in cycling cells, potentially reducing the dosage of agent required for cytotoxicity substantially.

The SMC Condensin complex, the type two Topoisomerase complex and the mitotic spindle are required for chromosome segregation. The Condensin complex is thought to drive the decatenation activity of Topoisomerase-2 to ensure chromosome resolution is successfully completed. Rapidly proliferating cells are especially sensitive to loss of Condensin function. Indeed, Condensin is over-expressed in some cancer types, including breast and lung cancer. If Topoisomerase II function is compromised, requirement for Condensin function is likely to become acute.

METHODS

This project examined the genetic synthetic interactions between Topoisomerase-2 and three Condensin genes in yeast. The Condensin and Topoisomerase-2 had previously been engineered to express a modified protein containing an N-terminal 'degron' motif so that gene expression could be controlled by temperature and by galactose-driven expression of the ubiquitin ligase, UBR1, concentration. Inclusion of a tetR repressible promoter also permitted control of gene dosage via doxycycline concentration. The cell viability of mutant strains containing either the Condensin degron gene alone or the Condensin degron gene combined with Topoisomerase-2 degron gene was ascertained. Furthermore, using microtubule inhibitors (Thiabendazole and Benomyl), the sensitivity of cell growth to mitotic spindle disruption was analysed under conditions of differing levels of mutant degron gene expression.

RESULTS

Synergistic inhibition was displayed upon partial disruption of both Topoisomerase-2 and Condensin gene expression, in comparison to partial inhibition of one or the other of the two genes. Furthermore, although the sensitivity of mutants is potentially increased to Thiabendazole, this is not seen with Benomyl. Therefore, results assessing whether microtubule inhibitors exacerbate this synergistic effect are inconsistent and

require further investigation.

CONCLUSION

This evidence of synergism, when targeting Condensin and Topoisomerase-2 in concert, provides a basis for assessing therapeutic potential in human cells, which could increase the efficacy of clinically used Topoisomerase inhibitors. These studies provide valuable complementary insights to the ongoing clinical research programme that will ultimately aid the development of novel topoisomerase-based chemotherapeutic regimes.

PS 145

Protamine and cholera toxin alter the expression of tight junction proteins in Ca?o-2 cells

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AIM

The aim of our work was the investigation of effects of protamine and cholera toxin on the expression and localization of tight junction proteins claudin-1,-3 and occludin in cultured CaCo-2 cell monolayers.

INTRODUCTION

Tight junctions (TJ) divide apical and basolateral domains of plasma membrane and contribute to the paracellular barrier functions. It is well known that protamine, a polycation arginine-rich protein, and cholera toxin have the contrary effects on the "leaky" epithelial cells and tissues [1, 2]. Protamine significantly increases the transepithelial electrical resistance (TER) of epithelium, whereas cholera toxin decreases it. However the effects of these agents on the TJ proteins of "leaky" epithelia are poorly investigated.

METHODS

All the experiments were performed on human epithelial cell line CaCo-2, which is one of the most widely used models of "leaky" intestinal epithelium. Protamine (100 mcg/ml, 30 min) and cholera toxin (1 mcg/ml, 4 h) were added on the apical surface of the CaCo-2 cells on the 14th day of cultivation. The control cells received the same volume of Krebs-Ringer without protamine or cholera toxin. To determine whether the cell monolayers were confluent until that time we used a transmission electrone microscopy. To analyze the expression and localization of TJ proteins we used methods of fluorescense microscopy and confocal laser scanning microscopy, using indirect immunostaining of TJ proteins and measuring the fluorescence intensity of labelled TJ proteins.

RESULTS

We showed that protamine induced the significant increase of expression of tightening TJ protein claudin-3 ($p<0.01$) by 45% in comparison with control, but not claudin-1 or occludin. Cholera toxin effects were quietly different. It caused the significant decrease of expression of tightening TJ protein claudin-1 ($p<0.05$), but not occludin or claudin-3. The localization of TJ proteins after treatment with protamine or cholera toxin was the same as under the control conditions.

CONCLUSION

In conclusion, protamine and cholera toxin induce alterations in the epithelial barrier function of CaCo-2 monolayers that involve the TJ proteins. The results of protamine and cholera toxin action on CaCo-2 cell monolayers make these agents a perfect tool for regulation of tight junctions. Our results suggest that components of chyme may take a part in regulation of paracellular permeability of intestinal epithelium.

PS 81

Multiple microarray comparison in breast cancer: differential genes expressed between T1 and T2 with/without lymph node invasion

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AIM

Assessment of genes differentially expressed between T1N0/T1N1 and T2N0/T2N1 in breast cancer.

INTRODUCTION

Breast cancer is the most frequent non skin malignancy in women and it is known that a woman who lives 90 years has one chance in eight in developing this pathology. A TNM staging is used, as in many other cancers, in order to better clinically classify and assess the breast cancer. We gave special attention to the transition from "in situ" carcinoma to invasive carcinoma, due to the relevance of successfully assessing different gene expression between these two stages. In recent years, microarray analysis of gene expression patterns has provided a way to improve the diagnosis, pathogenesis and risk stratification of many cancers by exploring thousands of genes simultaneously.

METHODS

A total of 1188 microarray were collected from GEO database and grouped according to the TNM classification as: T1N0 (n= 298), T1N1 (n= 100), T2N0 (n= 522), T2N1 (n= 268). All CEL files were independently preprocessed using RMA normalization. Only the common array probes between different platforms were considered in further processing. The gene expression differentiation analysis was performed using bayesian model with limma in R-package with previous log2 transformed and re-normalization using quartile normalization method. The final proves selection include an adjusted p-value < 0.05 and a fold rate > 2, also all selected proves were annotated to correspondent genes identification (entrez gene id and gene name). The p-value was adjusted for multiple comparisons using false discovery rate (FDR) method. The identified genes were mapped into a home-compiled protein-protein interaction network for further pathways and GO enrichment analysis.

RESULTS

Significant differences were found in more than 50 genes between T1N0 and T1N1 groups but no significant differences were found between T2N0 and T2N1. The enrichment analysis revealed significant cell cycle, cancer and chronic myeloid leukemia metabolic pathways even when other are also involved. The GO biological process showed a statistical significant enrichment in regulation of transcription from RNA polymerase II promoter and cell cycle processes. However, a statistical significant enrichment of negative regulation of macromolecule metabolic process and negative regulation of gene expression processes was also found.

CONCLUSION

As expected, due to the activation of mitotic pathways needed for the tumor to proliferate, statistical significant enrichment was found in transcription from RNA polymerase II promoter and cell cycle biological processes. Moreover, the statistical significance observed in cell cycle and pathways in cancer pathways were also as expected. However, we stated a very significant cross-way between breast cancer and chronic myeloid leukemia pathway. Also, a negative regulation of macromolecule metabolic process and negative regulation of gene expression processes was also observed. Further metabolic and biological analysis is needed in order to better understand these results.

PS 184

Effects of ciglitazone, ppar? Agonist, on prostate cancer cell proliferation and invasion

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AIM

The aim of this study was to investigate whether the proliferation and invasion capacity of LnCap cells are affected by PPAR? ligand ciglitazone (CGZ) and if, after the treatment, the expression of genes involved in solid tumor development , cell adhesion, proliferation, migration, invasion and angiogenesis is altered.

INTRODUCTION

Regarding to World Health Organization, prostate cancer is the second leading cause of cancer death in men, with no treatment for the hormone refractory forms.

Peroxisome proliferator-activated receptor ? (PPAR?) agonist has been proposed as potential anti-neoplastic agent, but its role remains controversial. While some reports demonstrated anti-proliferative role of PPAR? ligands in cellular and animal models, other documented that activation of PPAR? can induce cell growth and tumor proliferation.

METHODS

LnCap cells were grown at optimal culture conditions and were treated with 1-10 µM CGZ for 24, 48 and 72 hours. The cell proliferation was evaluated using Burker counting chamber and the survival was detected through Trypan Blue staining. Cell invasion was evaluated by Matrigel Invasion Assay. Finally, we investigated gene expression by Real Time PCR in 1 µM CGZ treated cells.

RESULTS

Control cells are 310 000, 340 000 and 360 000 at 24, 48 and 72 h counting. Cells treated with 1 and 10 µM CGZ are 290 000, 270 000, 280 000 and 300 000, 280 000, 250 000, respectively, at 24, 48 and 72 h counting. The dead cell rate is very low for controls (9, 9 and 14% at 24, 48 and 72 h), increments at 1 µM CGZ treatment (20, 30, 26% at 24, 48, 72 h) and is extremely high at 10 µM (11, 77, 84% at 24, 48, 72h). Related to controls, the invasion assay showed that after 24 h treatment with 1 and 10 µM CGZ, the number of invading LnCap cells increased. Finally, the gene expression analysis identified no significant measurement differences between control and treated cells regarding Thrombospondin-1, Thrombospondin-2, Angiopoietin-2, Leptin and VEGF.

CONCLUSION

Our results show that CGZ induces cell cycle arrest in the first 24 h by stopping proliferation, but doesn't induce cell death. After 48 h, the percentage of apoptosis increases depending on the dose and duration of treatment. Interestingly, invasive capacity of treated LnCap cells is increased during the cell cycle arrest. Unfortunately, none of the analyzed tumor development factors were found responsible for these effects, although VEGF showed a slight increment by 1.2 fold in treated cells with 1 µM CGZ. Considering our data, we support that CGZ is an effective apoptotic agent, but at submolar concentration it increments LnCap invasive potential.

PS 325

Is BRAF activation in PTC a modulator of tumour microenvironment?

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AIM

We aimed to use the prevalence of mutations in BRAF in a series of metastatic PTCs to search for an association between the presence of mutations in primary tumours and corresponding metastases, and to clarify if BRAF mutation may be related to any clinicopathological features of the cases.

INTRODUCTION

Papillary thyroid cancer (PTC) is the most common carcinoma of the thyroid representing 85 to 90% of the tumours of this gland. It is relatively indolent and highly curable despite presenting a considerable recurrence rate after initial treatment (20% at 10 years and 30% at 30 years of follow-up).

The V600E activating mutation in the BRAF gene is the most frequent somatic mutation in thyroid cancers, mainly in PTC (40-45%) and anaplastic carcinoma (20-40%). The association between the presence of BRAFV600E mutation and poor prognostics factors, such as male gender, older age, lymph node metastases, recurrence and aggressive phenotype remains controversial.

METHODS

The study was made by analysing 258 samples from 154 patients. In 104 patients primary tumour and corresponding lymph node metastases were analysed, in 46 patients only the primary tumour was studied and in the remaining 4 patients only the metastases. DNA was extracted from microdissected paraffin-embedded samples. Genomic DNA was amplified for BRAF and NRAS by PCR and then subject to automated sequencing. The clinicopathological evaluation was carried out by two pathologists. Statistical analysis was performed using the SPSS Statistics version 20.

RESULTS

BRAFV600E mutation was found in 47% of 148 primary PTCs and 42% of 100 metastases. It was observed that 30% of the cases showed discordance regarding the BRAF status (28 cases): 18% (17 cases) presented BRAFV600E in primary tumour and were BRAF WT in lymph node metastases, whereas 12% (11 cases) were BRAF WT in primary PTC and BRAFV600E in the respective metastases.

The BRAFV600E in primary tumours was associated with: older age (p=0.007), conventional variant of PTC (p=0.016), papillary pattern (p=0.002), presence of oncocytic cells (p=0.023), abundance of stroma (p=0.021) and absence of vascular invasion (p=0.002).

The BRAFV600E in lymph node metastases was associated with: older age (p=0.019), papillary pattern (p=0.015) and absence of vascular invasion (p=0.003).

CONCLUSION

The frequency of BRAFV600E in this series of metastatic PTCs is similar to those observed in series of PTC without lymph node metastases, thus indirectly indicating that BRAFV600E does not appear to carry a guarded prognosis. The absence of association of BRAF mutation with poor prognosis clinicopathological factors does not also support a more aggressive behaviour in BRAFV600E mutated PTC. Fitting with this conclusion vascular invasion was less frequent in cases with BRAFV600E than cases WT for BRAF.

In 30% of the cases we observed differences in the molecular profile of the primary tumours and corresponding metastases. We think that this variability could be explained by the intratumoral heterogeneity.

Finally the cases with BRAFV600E were more frequently associated with the abundance of stroma. This result suggests that BRAFV600E activating mutation may play a role in modifying the tumour microenvironment. Further studies are ongoing to test this hypothesis.

PS 333

Influence of porins OmpF and OmpC in the influx of Sparfloxacin

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AIM

This work aims to realize the importance of specific porins of gram-negative bacteria in the influx of a particular antibiotic (sparfloxacin) and its copper complexes.

INTRODUCTION

The use of antibiotics to treat bacterial infections is nowadays fairly common, but the increasing misuse of these drugs leads to the development of resistance by bacteria and renders treatments as ineffective. So, it is essential to understand how antibiotics act within the microorganisms and how they can be more efficient and less prone to their mechanisms of resistance. In this particular case, will be analysed two porins. Porins are channel proteins present in the outer membrane of gram-negative bacteria, which facilitate the passage of solute into the microorganism. The OmpC and OmpF are the most abundant porins in E. coli.

METHODS

In this study, minimal inhibitory concentrations (MIC) were determined for different strains of E. coli: JF568 (OmpC+ OmpF+), JF701 (OmpC- OmpF+) and JF703 (OmpC+ OmpF-). The antibiotic used was sparfloxacin (spar), a third generation fluoroquinolone, as well as copper-complexed variants binary copper(II):sparfloxacin2 and ternary copper(II):sparfloxacin:phenantroline.

RESULTS

Values of MICs(μM) with sparfloxacin and its copper complexes:

Sparfloxacin

JF568: 0.062

JF701: 0.062

JF703: 0.117

Cu(II):spar(1:2)

JF568: 0.016

JF701: 0.008

JF703: 0.124

Cu(II):spar:phen(1:1:1)

JF568: 0.062

JF701: 0.062

JF703: 0.124

CONCLUSION

OmpF has a great contribution to the influx of sparfloxacin and its complexes into the cell – the absence of this porin in E. coli JF703 leads to a significant increase in the MIC; the same does not happen, however, in the absence of OmpC in the JF701 strain. Also, looking at the results for the binary complex, we can see that the values of MIC are generally lower than those of free sparfloxacin; in fact, it is important to realize that at pH=7.4 and at these concentrations almost all the complex is dissociated and the effect we perceive is that of twice the free antibiotic. Therefore, comparing the two porins, it is shown that OmpF seems to have a higher impact on the susceptibility of E. coli to sparfloxacin.

PS 334

Characterization of Selectin Ligands in Dendritic Cells

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AIM

To identify the function and contribution of known adhesion molecules for interactions with endothelial selectins.

INTRODUCTION

In 2011, Ralph Steinman received the Nobel Prize for his discovery of dendritic cells (DCs), which allowed the creation of the first therapeutic anti-cancer cell-based vaccine. Steinman, who had been diagnosed with pancreatic cancer, tested the DC vaccines on himself and extended his survival. However, few days prior to receiving the award, Steinman died.

The limited efficacy of monocyte-derived-DC (mo-DC) -based vaccines is primarily due to the reduced mo-DC migratory capacity. One undefined aspect is the initial binding of mo-DCs to endothelial cells and vascular selectins.

Our previous data revealed that Sialyl Lewis x (sLex) is required for maximal binding of mo-DCs to activated endothelial cells under static conditions. Removal of sialic acid abrogated mo-DC cell tethering to immobilized, purified P-, L- or E-selectin under flow. The requirement of sLex -dependent binding of mo-DC to vascular selectins was further validated by using sLex free sugar and anti-sLex antibody, which significantly suppressed mo-DC-selectin binding. P-selectin glycoprotein ligand-1 (PSGL-1) was required for mo-DC binding to both P- and L-selectin, but it is dispensable for functional E-selectin recognition[1]. In fact, it is still unknown which are the ligands necessary for mo-DC binding to E-selectin .

Therefore in this study we are investigating the role of the E-selectin binding carbohydrate determinant, sLex, and candidate adhesion proteins, such as CD44, in E-selectin-dependent DC binding.

METHODS

We obtained human mo-DCs isolated from healthy donors (using anti-CD14 antibody magnetic beads) and cultured with GM-CSF and IL-4 cytokines, as we described [2, 3]. Mouse DCs were genetrated from bone marrow cells (BMDCs) collected from mice's tibias and femurs as we published previously [2]. CD44 and sLex expression was evaluated by Western blot and Flow Cytometry. The expression of E-selectin ligands, including HCELL, is being determined using E-selectin-Ig chimera (E-Ig) strategy. Unspecific binding is being evaluated in the presence of 20 mM EDTA which abrogates all selectin-mediated binding.

RESULTS

The analysis of flow cytometry and western blot data obtained showed that human mo-DCs and mice BMDCs strongly express CD44 and that the expression of sLex is dependent of DCs maturation state. The identification of E-selectin ligands through E-Ig staining in mo-DCs and BMDCs is being optimized so that in the future we can increase the expression of these molecules (e.g enzimatically or metabolically) and therefore improve DC migratory capacity.

CONCLUSION

In the future we intend to perform a combination of parallel plate flow chamber studies to characterize the role of these molecules in mediating rolling under shear. It is anticipated that the results of these studies will provide fundamental insights that will guide the development of novel therapeutic strategies using DCs to treat advanced cancer and metastatic lesions.

PS 328

The dengue virus capsid protein inhibitor peptide, pep14-23, converts to alpha-helical structure upon binding to negative lipids

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AIM

To study the conformation of the N-terminus disordered region of dengue virus capsid protein and of the pep14-23 peptide (based on that region) upon interaction and eventual binding to lipids.

INTRODUCTION

Dengue virus (DENV) causes a mosquito-borne disease affecting millions of people that is spreading to temperate regions including North America and Europe and for which no effective treatment available. [1] DENV infects the host liver, leading to the increase in the number and size of lipid droplets (LDs). LDs are intracellular organelles crucial for viral formation through their interaction with dengue virus capsid protein (DENVC). [2, 3] The host group previously studied DENVC-LDs interaction, identifying a conserved segment of DENVC disordered N-terminus involved in the binding, which was used to design the pep14-23 peptide, a novel inhibitor of this critical interaction. [4]

METHODS

We used bioinformatics tools (I-TASSER and Chimera), combined with circular dichroism (CD) and zeta-potential analysis, to determine the structural parameters and the tendency of DENVC and pep14-23 to interact and bind lipid vesicles.

RESULTS

Bioinformatics analysis suggests that the Flavivirus capsid protein N-terminus region, roughly corresponding to pep14-23, has a high alpha-helical tendency and is likely to interact with lipid systems. CD analysis supports this since pep14-23 undergoes a structural conversion to alpha-helix conformation in the presence of negative phospholipids. Zeta-potential further shows that the peptide binds negative lipid vesicles, with the binding simultaneous with the structural conversion.

CONCLUSION

The mechanism of pep14-23 inhibition of DENVC binding to LDs may involve its initial binding to negative phospholipids and the conversion to an alpha-helical peptide. This will contribute to the improvement of future pep14-23 based treatments for DENV and similar Flavivirus infections.

PS 352

Mechanotransduction in cardiac stem cells: role of YAP/TAZ in the cellular response to the microenvironment

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AIM

Investigate the regulation of YAP/TAZ trafficking in cardiac stem/progenitor cells (CPC) and dissect the molecular pathways involved in the mechanotransduction in these cells.

INTRODUCTION

Cardiac diseases represent the first cause of mortality in industrialized countries. Despite being promising to treat such pathologies, stem cell-based therapies have met modest success likely due to the limited knowledge on how the cells interact with the surrounding microenvironment. Cell fate determination results from the concerted integration of multiple parameters: biochemical (cytokines, hormones), physico-chemical (charge, pH) and mechano-structural (extracellular matrix composition, nanotopology and stiffness), factors of the microenvironment in which stem cells reside. Therefore, the improvement of tissue engineering strategies requires the identification of key molecular pathways and regulators that may be harnessed to favor cardiac regeneration. This experimental work addresses this complex problem by studying the role of two transcriptional co-activators, YAP and TAZ, whose activity is known to influence cell fate in different stem cell populations. The activity and regulation of YAP/TAZ in the maintenance and differentiation of a murine Sca-1+ CPC cell-line were investigated as an effect of substrate mechano-physical properties selective modification.

METHODS

The activity of YAP/TAZ was estimated considering their shuttling activity to the nucleus by confocal immunofluorescence and/or Western blot. The direct interaction of YAP/TAZ with putative partners (GATA-4, Tbx-5 transcription factors and vinculin, paxilin adhesion proteins) was assessed by immunofluorescence. The relevance of YAP/TAZ in cell adhesion was studied by Alamar Blue assay. The dependency of YAP/TAZ activity on pathways involved in cell mechanotransduction was investigated by using inhibitors such as cytochalasin D and blebbistatin. The effect of cellular constraint on YAP/TAZ expression was assessed by micropatterned surfaces with different adhesion areas. Wild type and YAP/TAZ-/- CPC were challenged with synthetic substrates displaying stiffness values in the Pa, KPa and MPa ranges, including temperature-responsive polymers.

RESULTS

YAP/TAZ localization is influenced by cell confluence, its nuclear translocation being inhibited by cell tension and related to cytoskeleton integrity, as shown by inhibitors' treatment. YAP/TAZ activity was shown to be related to substrate stiffness, its expression being mostly cytoplasmic in cells grown on soft substrates

(Pa range), while nuclear on stiffer surfaces (kPa, MPa values). YAP/TAZ involvement in cell adhesion was mostly required on stiff surfaces, while its inhibition was found to impair CPC migration and endothelial differentiation on soft matrices. Dynamic changes in scaffold stiffness and nanotopography modulated YAP/TAZ localization and activity. A correlation between YAP/TAZ localization and the activation of a transcription factor involved in cardiac lineage commitment (Tbx-5) was demonstrated by single cell analysis on micropatterned surfaces.

CONCLUSION

YAP/TAZ are mechanical sensors involved in CPC sensing of the microenvironment and respond to conditions such as cell confluence, tension, constraint and changes in the stiffness and nanotopography of the surface. Interestingly, the sensitivity of CPCs to substrate stiffness is different from other stem cell populations. YAP/TAZ activity seems to be related with cardiac gene expression, as demonstrated by their interaction with cardiac transcription factors. Thus, such factors are likely involved in several stem cell processes such as adhesion and differentiation. As such, future stem cell-based therapies targeting the heart and relying on bioengineered scaffolds should contemplate YAP/TAZ as modulators of CPC fate, by triggering appropriate cellular responses towards the desired therapeutical outcome.

PS 307

S119 Phosphorylation is Key to Prrxl1 Function

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AIM

Our aim is to characterize Prrxl1 phosphorylation sites and the impact of these aminoacid residues on Prrxl1 function.

INTRODUCTION

Prrxl1 is a paired-like homeodomain transcription factor essential for the connectivity and survival of nociceptive neurons in the mouse embryo dorsal root ganglion and spinal cord, as Prrxl1 null mutant mice show altered patterning of nociceptive afferent projections to the dorsal SC, neuronal loss, reduced nociception and failure to thrive (1-2). Prrxl1 displays a multiple band pattern on western-blots (3) which is abrogated by incubation with a phosphatase, suggesting that Prrxl1 is phosphorylated.

METHODS

Prrxl1 was analysed by 2D electrophoresis and by mass spectrometry. Constructs corresponding to mutated versions of identified Prrxl1 phospho-sites were generated by site-directed mutagenesis and analysed by western blotting. The transcriptional and DNA-binding activities of Prrxl1 mutants were determined by luciferase-reporter and DNA pull-down assays.

RESULTS

Prrxl1 band pattern analysis by 2D electrophoresis suggests the existence of at least eight phosphorylation sites. Mass spectrometry analysis was performed, revealing two of those sites: Serine 119 (S119) and Serine 238 (S238). S238A mutagenesis has no apparent impact on Prrxl1 transcriptional activity. S119 residue is highly conserved across species and its substitution to alanine induced a loss of Prrxl1 transcriptional activity, with no impairment of Prrxl1 dimerization or DNA-binding.

CONCLUSION

The phosphorylation of S119 is crucial for Prrxl1 function. Studies to identify the molecular mechanisms underlying the role of this phosphorylation along nociceptive system development are currently underway.

PS 359

Genetic polymorphism of autosomal STR loci in the population of Vojvodina

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AIM

The aim is to analyze genetic polymorphism of the autosomal STR loci in the population of Vojvodina and to evaluate the possibility of their use in forensic analysis.

INTRODUCTION

The analysis of microsatellite loci (Short tandem repeats – STR), located in the non-coding regions of the deoxiribonucleid acid molecule, is a method of choice in analysis in the field of medical criminalistics, disputed kinship testing and human identification. The necessity of standardization and comparation of the results of analysis between different laboratories resulted in forming of CODIS – Combined DNA Index System - database, that includes data about genetic polymorphism of 13 STR loci - CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, which represent the base of most of the commercial kits.

METHODS

The research was conducted on the results of DNA analysis of 597 non-related adults living on the territory of Vojvodina. The database was made using the standard analysis for the isolation of nuclear DNA with Chelex-100 reagens i Proteinase K, and amplification with the polymerase chain in combination with AmpFISTR Identifiler identification kit. The detection of amplification products was performed with capillary electrophoresis. The statistical parameters were calculated, and the forensic were obtained using the PowerStats 1.2 software package, Promega Madison, WI. The Student's t – test was used for the statistical analysis.

RESULTS

The highest polymorphism rate was shown on loci D2S1338 (PIC = 0.86) and D18S51 (PIC = 0.86), and the lowest on TPOX (PIC = 0.55). All of the observed loci showed PD>0.85 and percentage of heterozygosity >70%, except for the locus TPOX. There is no statistically significant difference between the observed and expected heterozygosity values.

CONCLUSION

The research results are in concordance with the Hardy – Weinberg's law, while alleIs show high polymorphism rate, which makes them suitable for the use in the fields of medical criminalistics, disputed kinship testing and human identification on the territory of Vojvodina.

PS 370

The behaviour of metalloproteinases-2, -8 and -9 in MGUS and Multiple Myeloma

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AIM

In the present study, we aimed to explore the role of MMPs, namely MMP-2, MMP-8 and MMP-9, in the pathogenesis of MGUS and progression to MM. We also pretended to correlate this results with clinical/ laboratory data and prognostic factors.

INTRODUCTION

Multiple myeloma (MM) is a B-cell neoplasia characterized by the proliferation of malignant plasma cells in the bone marrow, increased angiogenesis, and the development of osteolytic bone disease. The first pathogenic step is a premalignant monoclonal gamopathy of undetermined significance (MGUS). In the progression from MGUS to MM, complex genetic and/or epigentic events occur in the neoplastic plasma cell (PC), and in the bone marrow (BM) microenvironment, including induction of angiogenesis, suppression of cell-mediated immunity, and development of paracrine signalling loops involving interleukin-6, insulin-like growth factor 1, interferon ? and vascular endothelial growth factor. Furthermore, although tumour progression is observed mainly within the bone marrow during the early stages of the disease, extramedullary spreading occurs during the terminal stage of the disease and malignant cells can be detected in peripheral blood of many patients.

Matrix metalloproteinases (MMPs) are a family of structurally and functionally related proteinases characterized by the ability to degrade the extracellular matrix (ECM). Based on their substrate specificity and domain structure, MMPs were divided into subgroups. One of these subgroups is represented by gelatinases, which degrade gelatine and several types of collagen, like gelatinase A (MMP-2) and gelatinase B (MMP-9). MMPs are known to play a role in cell growth, invasion, angiogenesis, metastasis, and bone degradation, all important events in the pathogenesis of cancer. However, the role of MMPs in the development of MGUS and progression to MM is poorly understood.

METHODS

A total of newly diagnosed 17 MGUS patients and 13 MM patients and 2 healthy individuals (controls) were included in this study. Expression of MMP-2, MMP-8 and MMP-9 was assessed on bone marrow plasma cells

(PC) by flow cytometry using a four-color staining assay. The average MGUS patients' age is 70.59 years (39-89 years) and for MM is 76.85 years (68-86 years). According with gender, MGUS patients' are 65% female and 35% male and MM patients` are 77% female and 23% male. The monoclonal protein in MGUS patients is 35% IgA, 59% IgG and 6% IgM, while in MM patients is 45% IgA and 55% IgG. According to the International Staging System (ISS) , 65% of MGUS patients are in stage I and 35% in stage II. On the other hand, 8% of MM patients are in stage I, 23% in stage II and 69% in stage III.

RESULTS

Our preliminary study shows that MGUS and MM PC patients have higher MMP expression levels compared with controls. On the other hand, MM patients show higher percentage of PC expressing MMP-9 when compared to MGUS patients. However, in CD19+/CD138+ PC the intracellular MMP expression levels are higher in MGUS patients when compared to MM, especially MMP9. Besides that, in malignant PC (CD19-/CD138+) these differences are mainly observed in MMP-9. When analyzed both PC patient population in MGUS and MM, we observed that CD19+/CD138+ PC have higher MMP intracellular expression levels and percentage of cells expressing MMP, when compared with PC CD19-/CD138+, especially MMP-2 and MMP-9. Besides that all MM patients are positive for at least two MMPs. On the other hand, in MM patients in stage II and III, we observe an increase in MMP-9 expression compared with patients in stage I. Besides that, in MGUS IgA patients an increase in MMP9 expression was observed when compared with MM IgA patients.

CONCLUSION

Our findings suggest that PC MMP expression may be correlated with transition of MGUS to MM, promoting extramedullary spreading and disease evolution. Since MM remains incurable, confirmation of these results may contribute to a better understanding of MM biology and can lead to new therapeutic approaches.

PS 340

Wnt/B-catenin, Notch and Hedgehog pathways as a target in ALL treatment

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AIM

The main goal of this study was to evaluate the therapeutic potential of Wnt/B-catenin, Notch and Hedgehog inhibitors, respectively IWR-1, gamma-secretase inhibitor XXII (GSI) and GDC-0449, alone and in combination in an ALL cell line.

INTRODUCTION

Conserved embryonic signaling pathways such as Hedgehog (Hh), Wingless (Wnt) and Notch, critical for stem cell self-renewal and differentiation in hematopoiesis, have been implicated in the pathogenesis of several hematological malignancies.

Acute lymphoblastic leukemia (ALL) is characterized by the abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow and lymphoid tissues, which can develop from the aberrant activation of the Wnt/?-catenin, Notch and Hedgehog signaling pathways. On account of that, these pathways may constitute new potential candidate targets for ALL therapy.

METHODS

To evaluate the effect of these signaling pathways inhibitors on cell viability, we use an ALL cell line, the CEM cells, submitted to different concentrations of the inhibitors during 24 to 72h in monotherapy and in association. The IC50 (half maximal inhibitory concentration), was determining using the blue trypan assay. The cell death was assessed by optical microscopy (with May-Grunwald staining) and by flow cytometry, (using Propidium Iodide/Annexin V staining, measuring BAX and BCL-2 levels, and monitoring the mitochondrial membrane polarization). Besides these assays, were also evaluated , by flow cyitometry, the cell cycle and some proteins related with cell cycle regulation, as p53 and Cyclin D1.

RESULTS

The results observed showed that, in CEM cells, GSI, IWR-1 and GDC-0449 induced a cytostatic and cytotoxic effects. These inhibitors suppressed cell growth and induced a decrease in cell viability in a time- and dose-dependent manner, when administrated alone or in combination with each other. The half maximal inhibitory concentration (IC50) of GSI, IWR-1 and GDC-0449 was 50 µM, 30 µM and 150 µM, respectively, after 24h of treatment. These compounds induce cell death mainly by apoptosis, that may be related with

the observed increase in caspases levels and decrease in mitochondrial membrane potential and BAX/BCL-2 levels. We could also observe that in the presence of these pathways inhibitors, p53/cyclin D1 and BAX/Bcl-2 levels were diminished,thus inducing cell cycle arrest.

CONCLUSION

In conclusion, our results suggest that GSI, IWR-1 and GDC 0449 are potential new targeted therapies that could be efficient in ALL treatment.

PS 363

Influence of hydrogen peroxide and menadione in DNA methylation status – An in vitro study in Acute Lymphoblastic Leukemia cells

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AIM

In this work, the main objective is to evaluate the role of oxidative stress by studying the effect of hydrogen peroxide (H2O2) and menadione, a superoxide anion (O2•-) donor, in cell death and methylation status in Acute Lymphoblastic Leukemia.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) originates from the malignant transformation of lymphocyte progenitor cells of the B- and T-cell lineages. However, most of the known large scale genetic aberrations in ALL are not alone sufficient to induce the disease, suggesting that there are other genetic or epigenetic alterations that act in leukemic transformation. Increasingly evidence shows that oxidative stress and reactive oxygen species (ROS) are involved in carcinogenesis, since they can cause DNA damage. In addition, one of the mechanisms involved in apoptotic cell death and abnormal proliferation may be mediated by oxygen free radicals. So, depending on cellular concentration, ROS have a dual effect, they induce cancer cell proliferation and/or death, being this last one used as a cancer therapeutic approach. Moreover, it is now clear that epigenetic mechanisms as well as genetic changes are imortant in the development of cancer. Many well established tumor suppressor genes have been shown to be inactivated predominantly by promoter hypermethylation and many of the genes linked to leukemia development have themselves been shown to be epigenetic regulators, such as the histone methyltransferase MLL. Same studies suggest that oxidative DNA damage can affect patterns of DNA methylation leading to aberrant gene expression and possibly contributing to the development of malignancy.

METHODS

For this purpose, we used a well established ALL cell line, the CEM cells. To evaluate the effect of oxidative stress on cell viability and growth, CEM cells were treated in the absence and presence of different concentrations of hydrogen peroxide and menadione and were analyzed by the trypan blue assay. The intracellular levels of H2O2, O2•- and the non-enzimatic antioxidant defense GSH were determined by flow cytometry (FC), using the probes DCFH-DA, DHE and Orange Mercury, respectively. Cell death and mitochondrial potential were also evaluated by FC using the Annexin V and Propidium Iodide double staining and JC-1 probe, respectively. We also analyzed apoptotic proteins expression levels, namely BAX, BCL-2, FAS, FAS ligand and caspases, by flow cytometry. Cell cycle was also analyzed by FC using Propidium Iodide/RNase staining. Global DNA

methylation and hydroxymethylation were analyzed by ELISA using commercial kits.

RESULTS

Our results show that hydrogen peroxide and menadione induce a decrease in viability cell in a dose and time dependent manner. In fact, we observe that the half maximal inhibitory concentration (IC50) of H2O2 and menadione in CEM is 25 uM and 7.5 uM, respectively, after 24 hours of exposure. The results obtained by FC show that these compounds induce cell death mainly by late apoptosis/necrosis, and are in agreement with the observed decrease in mitochondrial membrane potential, increase in caspases levels and with the pre-G1 peak observed in cell cycle analysis. The ratios of BAX/BCL-2 and FAS/FAS ligand were also increased in cells treated with H2O2 and menadione, respectively. Besides that, these effects may be mediated by OS as we observe an increase in ROS levels and a decrease in GSH. These compounds also induce S phase arrest, suggesting DNA damage, probably also related to ROS increase. Finally, we also observed an increase in 5-hydroxymethylcytosine (5hmC) levels in cell treated with 25 μM H2O2, fact that can be a result of 5-methylcytosine oxidation via oxidative damage, and consequently induction of global hypomethylation. In contrary, in cells treated with 7.5 μM of menadione 5hmC levels decrease.

CONCLUSION

In conclusion, our results suggest that, despite the influence of H2O2 in cell death, oxidative stress levels can lead to changes in DNA methylation status.

PS 335

Anti-oxidant supplementation and reproductive outcome in aged female mice

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AIM

In the present work it was aimed to determine whether diet anti-oxidant supplementation of aged female mice before and during pregnancy might improve reproductive ability.

INTRODUCTION

In mammals advanced maternal age is known to be a risk factor for loss of reproductive capacity. Although age-related changes in the ovary account for most of the loss, aging also affects the function of the uterus. As a consequence, both maternal and progeny outcome is affected by maternal age [1]. An imbalance in the redox status with enhanced reactive oxygen species production or reduced scavenging has been suggested to play a role in the development of pregnancy complications.

METHODS

Experiments were performed according to the Portuguese law on animal welfare and according to the guidelines issued by Federation of European Laboratory Animal Science Associations (FELASA). Mice had free access to tap water and standard mice chow. Uterine samples of non-parous female C57BL/6J mice aged 11–15 weeks or 43-45 weeks were obtained and protein carbonylation was determined in the uterine epithelium by fluorescent immunohistochemistry techniques. Non-parous female C57BL/6J mice aged 8–12 weeks or 40-42 weeks were matted with male C57BL/6J mice aged 3–4 months. Reproductive outcome was evaluated by counting the number of viable fetus and reabsorption sites. Anti-oxidants were administered to aged females in the drinking water prior to and during pregnancy. Aged females were treated with a SOD mimetic (TEMPOL, 1 mM) or a NOX inhibitor (apocynin, 5 mM) and reproductive outcome was re-evaluated. Results are presented as mean ± standard error mean.

RESULTS

The histological appearance of the uterus in the aged female mice was different from the young mice. Uteri of aged female mice were hypertrophied and contained cysts. Moreover, protein carbonylation was increased in the uterus of aged mice.

Pregnant aged females showed an age-related decreased in the number of viable fetuses [young females 6.0 ± 1.2 (n=9) and aged females 2.0 ± 0.4 (n=6), P=0.03 (Student's t test)]. This decrease was accompanied by early and late fetal loss and reabsorption.

Aged anti-oxidant treated female mice showed no differences in water intake when compared to aged controls. Anti-oxidant treatment increased the number of fetuses from aged female mice [control 2.0 ± 0.4 (n=9), TEMPOL 3.5 ± 0.9 (n=4) and apocynin 4.2 ± 0.6 (n=5), P=0.05 (ANOVA)].

CONCLUSION

The increase in age-related uterine protein carbonylation indicates a local imbalance in redox homeostasis that is accompanied by impaired reproduction. Anti-oxidant treatment ameliorated aged female reproduction outcome.

PS 298

TRAIL and Survivin inhibitors – The therapeutic role in overcome relapse in Acute Lymphoblastic Leukemia

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AIM

In this work, the main objective is to evaluate the therapeutic efficacy of a recombinant human TRAIL (rhTRAIL) and survivin inhibitors, namely gambogic acid (GA) and silibinin (SLB), alone and in combination with conventional chemotherapeutic agents, in Acute Lymphoblastic Leukemia (ALL), namely in relapse.

INTRODUCTION

ALL represents the most common malignancy affecting children and the major cause of mortality from hematopoietic malignancies in adults. A substantial number of these patients relapse or become resistant to chemotherapy, requiring the development of alternative treatment strategies. The cytokine tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL/Apo-2L) can activate apoptosis pathway by binding to its two agonistic cell surface death receptors 4 (DR4) and/or 5 (DR5), with low toxicity in normal cells. Nevertheless, in some cases treatment with TRAIL alone may not be sufficient for an effective response. Take into account, GA and SLB, have given encouraging results to restore TRAIL sensitivity for up-regulation of DR4 and/or DR5, down-regulation of anti-apoptotic proteins or facilitation of TRAIL receptor aggregation.

METHODS

For this purpose we maintained in culture two ALL cell lines, the CEM (obtained from a patient at disease presentation) and MOLT-4 cells (established from cells taken from a patient at relapse), and tested the effect of different concentrations of rhTRAIL, GA and SLB in monotherapy, in association with each other and with conventional drugs (Doxorubicin or Vincristine). Cell viability was assessed by the trypan blue assay and cell death by Optical Microscopy (May-Grunwald staining) and flow cytometry (FC) using the Annexin V and Propidium Iodide double staining. TRAIL, TRAIL-Receptors and survivin levels were evaluated by FC using monoclonal antibodies.

RESULTS

Our results show that all the tested drugs have an antiproliferative and cytotoxic effects in a dose, time and

cell type dependent manner. In CEM cells treated with rhTRAIL we observed an IC50, at 72h of exposure, of 500ng/mL, which is not reached in MOLT-4, with higher doses and time exposition. On the other hand, in both cell lines treated with GA and SLB, the IC50 is reached at 24h. However, in CEM cells it is approximately 400nM for GA and 100uM for SLB, while in MOLT-4 cells the IC50 for GA is 750nM and about 50uM for SLB. Moreover, in both cell lines, when we administrated these compounds at low concentration daily, during 72h, we noted an increased in cell death comparatively with the equivalent dose in a single administration. Furthermore, in MOLT-4 cells when we combined rhTRAIL with conventional agents and/or with GA or SLB we observed a synergist cytotoxic effect. However, in CEM cells this effect is more patent with SLB association. The results obtained by FC and morphological analyses show that the cell death occurs mainly by apoptosis. The different therapeutic efficacy of rhTRAIL in both cell lines may also be correlated with the ratio between TRAIL pro and anti-apoptotic receptors, which are higher in CEM than in MOLT-4, in contrast with survivin levels.

CONCLUSION

Our study suggests that rhTRAIL and survivin inhibitors, in monotherapy or in combination, may constitute a new potential therapeutic approach to overcome the failure and relapse in ALL treatment.

PS 365

Genetic Cancer susceptibility in Down Syndrome – A preliminary study

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AIM

The aims of this study are to analyze the prevalence of polymorphic variants in chromosome 21 genes involved in folate metabolism and the methylation pattern of p16, DAPK, p15 and MGMT genes, in order to identify its role in the development of cancer/leukemia in Down Syndrome patients.

INTRODUCTION

Down Syndrome (DS) or trisomy 21 is the most common constitutional aneuploidy with an incidence of 1 in 700 births. Children with DS have an increase 10- to 20-fold risk for leukemia than children without this syndrome, in particular acute megakaryoblastic leukemia. In contrast, the risk of developing solid tumors is lower in DS children.

On the other hand, folic acid plays a key role in the maintenance of genomic stability, DNA methylation of several genes, particularly those involved in cell cycle regulation and DNA repair enzymes (p16, DAPK, p15 and MGMT, respectively). Folic acid metabolism may be influenced by genetic polymorphism of Folate Carrier Transporter (RFC), Methyl Tetrahydrofolate Reductase (MTHFR) and cystathionine beta-synthase (CBS) enzymes, playing an important role in susceptibility to aneuploidy and to the development of early events in carcinogenesis.

METHODS

The analysis of polymorphic variants CBS 844ins68 and T833C (co-segregate in cis), SOD1 A251G, RFC1 A80G and MTHFR A1298C by PCR-RFLP assay in 31 fibroblasts samples of DS children and 30 perypheral blood samples healthy controls (CTL). Methylation pattern of p16, DAPK, p15 and MGMT genes were analyzed in bisulfite converted DNA by MSP assay.

RESULTS

Our results show a slight decrease in wt 844ins68 CBS allelic frequency in DS (81%), compared to CTL (84%). In CTL, about 3% of 844ins68 homozygous, 13% of 844ins68 heterozygous and 84% without an insertion were observed for CBS gene genotypic frequency, while in DS it was 0%, 19% and 81% respectively.

We also observed that wild type allelic frequency of SOD1, RFC1 and MTHFR polymorphisms (A allele) were similar in CTL and DS (SOD1: 92%/90%; RFC1: 52%/50%; MTHFR: 70%/69%, respectively for controls and DS). Similar results were observed in SOD1 and MTHFR genotype analysis (SOD1: 83%/80% AA, 17%/ 20%

AG and 0% GG genotypes; MTHFR: 13%/10% AA, 33%/42% AC and 53%/48% CC genotypes, respectively of controls and DS). Besides that, RFC1 AA genotype was decrease in DS (19%) compared to CTL (27%). The genotypic frequencies observed did not show deviation from Hardy-Weinberg equilibrium, except in controls on CBS gene.

The strength of association between polymorphisms and DS risk was assessed by odds ratio (OR) with the corresponding 95% confidence interval (CI95%). No significant relation was observed between these polymorphisms and DS. However, CBS wt 844ins68 [OR=0.833 (0.2248-3.089)], SOD1 AA [OR=0,8333 (0.2248-3.089)] and RFC1 AA genotypes [OR=0,6600 (0.1980-2.200)] could have a protective effect. On the other hand, CBS var. 844ins68 [OR=1.560 (0.3927-6.198)], SOD1 AG genotype [OR=1.20 (0.3237-4.448)] and MTHFR AC [OR=1.444 (0.5095-4.095)] genotypes might be a risk factor. Furthermore, DNA methylation analysis reveals that p16, DAPK, p15 and MGMT genes were all unmethylated in children with DS.

CONCLUSION

Besides, no statistical association was detected between these polymorphisms or the methylation status in children with DS, the increase in patients and controls samples could contribute to a better risk analysis of the influence of these polymorphisms in DS development.

Book of Abstracts

Physiology & Immunology

PS 181

The influence of repeated immobilization stress on the histological features of the thymus in mice

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AIM

The aim of this study was to examine the influence of repeated immobilization stress on the histological features of the thymus.

INTRODUCTION

The animal model is widely represented in our studies of the pathogenesis of stress and its characteristics. It is well known that stress affects the immune system and the changes can be observed on the thymus.

METHODS

The research included 15 mice separated into two groups: the control group (5 mice) and the experimental group (2x5 mice). Experimental group was divided into two smaller groups. The first group has been put under stress for 10 consecutive days, and the second group for 20 days. Four nonadjacent parts of the thymus were photographed by the microscope and then we determined volume fraction of thymic cortex and medulla in ImageJ computer program.

RESULTS

The thymus in the control mice group had a specific structure, composed of two lobes covering 2/3 of the anterior side of heart, while the stunted lobes in the experimental mice group were located just in corona cordis. Significant reduction in thickness of the cortical thymic part was noticed in the experimental group which were influenced by stress for 20 days after stereological and statistical analyses of the histological cortical and medullary sections in the control and the experimental group.

CONCLUSION

(Grossly) thymic involution in mice stressed for 10 and 20 days and the reduction in thickness of the cortical thymic part was noticed in the experimental group stressed for 20 days.

PS 227

Karyometric analysis of goblet cells in patients with bronchial asthma and allergic rhinitis

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AIM

The study objective was to quantify and compare nasal epithelium goblet cells nuclei characteristics in patients with bronchial asthma and allergic rhinitis.

INTRODUCTION

The most frequent allergic diseases are respiratory allergic diseases: asthma and allergic rhinitis as the two most important clinical entities. Difficulties in their diagnostic differentiation are due to similarity in their pathophysiology, clinical manifestations, and therapy.

METHODS

The nasal mucosa cytology specimens were obtained by nasal smear from 20 asthma patients and 14 patients with rhinitis. All samples were stained by hematoxylin-eosin method and examined using an Olympus BX50 microscope. Visual fields were randomly selected and digitalized. The following parameters were analysed: nuclear area, longest and shortest nuclear axis, ratio of longest axis to shortest axis, nuclear perimeter, nuclear roundness, nuclear density, and area profile on polygonal plane.

RESULTS

The cytology samples showed a large number of goblet cells, neutrophils, a few plasma cells and eosinophils. All examined parameters showed significant differences between analysed groups. Average nuclear area of globet cells, longest and shortest axis lenghts, nuclear perimeter, nuclear density and area-polygon were larger; while longest axis to shortest axis ratio and nuclear roundness were smaller in patients with rhinitis allergica.

CONCLUSION

Our study showed that goblet cells karyometric parameters differ among patients with asthma and allergic rhinitis, which might be ascribed to the difference in activity of these cells.

Karyometric examination of goblet cells could help in cases of diagnostic uncertainty between these diseases.

PS 217

Overactive bladder: on the role of ATP release from the urothelium triggered by UDP-sensitive P2Y6 receptors

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AIM

In this study, we investigated the role of UDP on the micturition reflex triggered by bladder distension (intravesical saline infusion 2.4 ml/h) in urethane-anaesthetized male Wistar rats (300?450 g).

INTRODUCTION

Overactive bladder (OAB) is a complex clinical syndrome based on self-reported symptoms of urgency with or without incontinence, daytime frequency and nocturia. There is a considerable bulk of evidence showing that ATP released from the urothelium has a prominent role in the regulation of urinary bladder function, and in the pathophysiology of detrusor overactivity. ATP-sensitive P2X1 receptors are found on the detrusor muscle, while P2X3 receptors localize on afferent nerve fibers. The hypothesis that uridine nucleotides, UTP and/or UDP, may also act in autocrine/paracrine pathway has only recently gained experimental support. Metabotropic P2Y2 recognizes both ATP and UTP, while P2Y4 and P2Y6 receptors were recently identified as UTP- and UDP-selective receptors in humans, respectively.

METHODS

We compared in vivo cystometric assays with in vitro myographic recordings; UDP (100 µM) was applied either through a cannula inserted in the bladder dome or directly to the bathing solution outside the bladder. Since, the effects of adenine and uridine nucleotides may be cut-short by rapid extracellular inactivation via ectoNTPDases we examined the contractile effect of UDP on bladder strips extracted from ecto-NTPDase 1 deficient mice.

RESULTS

Intravesical UDP (100 µM) increased the voiding frequency (VF) by 45±8% (n=12), without affecting the amplitude (A) and the duration (?t) of bladder contractions. This effect was significantly ($P<0.05$) attenuated by intravesical MRS2578 (100 nM, a P2Y6 selective antagonist). Intravenous perfusion with A317491 (100 nM, a selective P2X3 antagonist) prevented UDP-induced increases in the VF. UDP was unable to cause bladder contractions in vitro. Contractile responses to UDP were only evidenced in bladder strips from ecto-NTPDase1 knockout mice. Inactivation of UDP in the bladder wall was confirmed by enzymatic histochemistry and immunofluorescence confocal microscopy.

CONCLUSION

Data indicate that UDP-mediated increases in the voiding frequency depend on urothelium and requires intact nervous circuitry. Expression of P2Y6 receptors in the urothelium and P2X3 receptors in suburothelial nerve fibres were confirmed by immunocytochemistry. We, therefore, hypothesized that UDP excitation results from stimulation of urothelial P2Y6 receptors, which may trigger the release of ATP leading to the activation of P2X3 receptors on suburothelial sensory nerve fibers.

PS 168

Title: everolimus use in association with cyclosporin in renal transplant receptors: 5 years results.

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UNIFESP

AIM

To evaluate the safety and tolerability of EVR use, combined with CSA, during the first 5 years of aftercare of renal transplant receptors.

INTRODUCTION

The combination of everolimus (EVR) and cyclosporin (CSA) is synergetic and efficient in the prevention of acute rejection after renal transplant. The use of reduced concentrations of CSA in combination with concentrations of EVR between 3-8 ng/ml is efficient in the prevention of acute rejection and preserves renal function. However, adverse events related with the use of EVR may determine its withdrawn from the treatment.

METHODS

we analyzed retrospectively data from 110 receptors of renal transplant which received initial immunosuppression with EVR, CSA and prednisone aft between 03/31/1999 and 08/30/2007.

RESULTS

mean age was 40 years, being 65% men, 67% white, 6% diabetics and 70% were recipients of kidneys from living donors. The mean period of follow up was 6 years (2,7 to 11 years). The cumulative incidence of acute rejection confirmed by biopsy was 25%. The renal function remained stable from the 1st (1,60 mg/dL) to the 5th year of the transplant (1,67 mg/dL). By the end of the 5th year, patient's and graft's survival were 93% and 75%, respectively. The incidence of adverse events related to the use of EVR were 88,2%. Among these events it was observed: 80,6% of dyslipidemia, 32,0% of anemia, 17,6% of diabetes mellitus, 14,5% lymphocele, 9,1% operatory wound dehiscence, 3,6% urinary fistula and 1,9% lymphorrhea. During the evaluated period of follow up, a total of 36 patients (33%) discontinued treatment with the EVR. The interruption of both the EVR and CSA occurred in 17 (47%) patients (8 by dysfunction, 5 by lack of efficiency, 2 by severe infection and 2 for a hemolytic-uremic syndrome). The interruption of just EVR was indicated in 19 (53%) patients (3 due to graft's chronic dysfunction, 2 due to lack of efficacy, 5 due to dyslipidemia, 2 due to severe infections, 3 due to segmental and focal glomeruloesclerosis, 1 due to proteinuria and 3 due to other reasons).

CONCLUSION

this analysis of 110 kidney transplant recipients shows that association of everolimus and cyclosporin is efficient, safe and tolerable, been the discontinuation of the initial regimen during 5 years of follow up indicated in 33% of patients.

PS 245

The effects of red-wine polyphenolic compounds in experimentally induced allergic asthma

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AIM

The aim of asthma pharmacotherapy is to find the new sources of drugs able to modulate smooth muscle hyperreactivity and the degree of airways inflammation. Some epidemiologic and experimental studies suggested that the polyphenolic compounds might reduce the occurrence of asthma symptoms.

INTRODUCTION

Our experimental work was aimed at the influence of red-wine polyphenolic compounds (Provinol®) on defence airway reflexes (cough, bronchoconstriction) and on inflammation during experimentally induced allergic asthma. We studied the effects of Provinol, and combinations of Provinol with clinically used antiasthmatics (Budesonide and Theophylline).

METHODS

We utilized the model of guinea pig airway hyperreactivity induced by 21 days allergen (ovalbumin -OVA) administration, during which the experimental animals were treated by Provinol® (20 mg/kg/day p.o.), Budesonide (1 mM by inhalation), Theophylline (10 mg/kg/day i.p.) or by half dose combinations of them. The airway smooth muscle reactivity in vivo was evaluated by specific airway resistance (sRaw) to nebulized histamine (10-6 mol.l-1). The cough in guinea pigs, induced by citric acid aerosol (10-3 mol.l-1) was measured by in vivo method in double chamber body-plethysmograph. The tracheal smooth muscle reactivity to bronchoconstrictor - histamine (10-8-10-3 mol.l-1) was examined by in vitro method. Bronchoalveolar lavage fluid (BALF) levels of IL-4, IL-5 (using ELISA) and expression of nitric oxide synthases (NOS) from lung homogenate (using Western blot) were utilized as parameters of anti-inflammatory effect of Provinol®.

RESULTS

Administration of Provinol® caused significant decrease of sRaw after histamine nebulization and the decline in tracheal smooth muscle contraction amplitude to this mediator. It also led to significant decrease of parameters of chemically induced cough reflex. The bronchodilatory and antitussive effects of Provinol® were comparable to Theophylline and Budesonide. The half-dose combinations Provinol®+Theophylline and Provinol®+Budesonide exerted bronchodilatory and antitussive effects, exceeding the activity of these substance used in monotherapy. Antiinflammatory effect of Provinol® was demonstrated by IL-4, IL-5 and eosinophil account decrease. Provinol® increased the expression of constitutive form of NOS.

CONCLUSION

In conclusion, we can summarize the most important findings of our experiments: The polyphenolic compound Provinol® posseses efficient antiasthmatic activity. Provinol® had bronchodilatory, antitussive effect, suppressed asthmatic inflammation of the airways. Furthermore Provinol® amplified the bronchodilatory and antitussive effect of Budesonide and Theophylline. Provinol®'s antiasthmatic effect is probably partially mediated through the metabolism of NO.

PS 281

The influence of long-term administration of crac channels antagonist on experimentally induced allergic asthma in guinea pigs

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AIM

The aim of presented work is to evaluate the influence of long-term therapy by CRAC antagonist on airways hyperreactivity, pathological cough and degree of inflammation in condition of experimentally induced allergic asthma.

INTRODUCTION

The role of CRAC channels in secretory functions of mast cells, T cells and eosinophils is described in details. Previously, we demonstrated both, their role in contraction of airways smooth muscle cells (ASM) and pathophysiology of experimentally induced allergic asthma in guinea pigs test system.

The aim of presented work is to evaluate the influence of long-term therapy by CRAC antagonist on airways hyperreactivity, pathological cough and degree of inflammation in condition of experimentally induced allergic asthma.

METHODS

Allergic inflammation of the airways was induced by repetitive exposure of guinea pigs to ovalbumine. The selective antagonist of CRAC channels (3- fluoropyridine-4-carboxylic acid) was administered intraperitoneally in the dose 1.5 mg/kg b.w during 14 days. The following methods were used for assessment of long-term administration of CRAC antagonists and positive control drugs codeine and salbutamol:

1. Evaluation of specific airways resistance (sRaw), in vivo and contractile response of isolated ASM strips, in vitro;

2. Citric acid-induced cough reflex;

3. Measurement of exhaled NO levels (ENO);

4. Assessment of NO-synthase isophorms levels;

5. Immunohistochemical staining methods evaluating the mast cells infiltration of tracheal

and pulmonary tissue sections.

RESULTS

Long- term application of CRAC antagonist resulted in significant cough suppression, bronchodilatory effect in vivo and inhibited ASM contractility in vitro conditions exceeded activity of control drugs codeine and salbutamol. The measured levels of ENO and the results of immunohistochemical analysis confirmed anti-inflammatory effect of CRAC antagonist.

CONCLUSION

The results confirmed role of CRAC in pathophysiology and symptoms of experimental asthma model and due to dual bronchodilatory and anti-inflammatory activities could extend possibilities or design new strategy in asthma treatment in future.

PS 183

Role of adenosine in the proliferation and differentiation of fibroblasts of the rat subcutaneous connective tissue

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AIM

In this study, we aimed at characterizing the extracellular enzymatic pathways responsible for adenosine formation from the catabolism of ATP, as well as to evaluate the expression and function of adenosine receptors in the proliferation and type I collagen synthesis by fibroblast cell cultures from the rat subcutaneous connective tissue.

INTRODUCTION

Increased connective tissue stiffness due to fibrosis, as well as extracellular nucleotides/nucleosides, may play a role in the pathogenesis of intractable chronic pain. Fibroblasts, the predominant cell of the connective tissue, release huge amounts of ATP in response to stressful conditions. Depending on its extracellular catabolism, fibrosis may be influenced by ATP and/or its metabolites through the activation of nucleotide-sensitive P2 and/or adenosine (P1) receptors.

METHODS

Fibroblasts were isolated from the subcutaneous connective tissue of adult Wistar rats. The catabolism of ATP, ADP, AMP and adenosine in fibroblast cell cultures was evaluated through quantification of adenine nucleotides and nucleosides by high-performance liquid chromatography (with UV detection). The expression of E-NTPDases1, 2 and 3, as well as of ecto-5'-nucleotidase, was evaluated by immunocytochemistry. The role of subtype selective adenosine receptor agonists in the proliferation and type I collagen synthesis was assessed by MTT and Sirius Red assays, respectively.

RESULTS

ATP was hydrolyzed (t1/2=12.5 minutes) to ADP, adenosine and inosine. AMP was quickly dephosphorylated into adenosine (t1/2=3 minutes), whereas ADP (t1/2=68 minutes) and adenosine (t1/2=29 minutes) tend to accumulate in fibroblast cell cultures. Cells exhibited positive imunoreactivity for NTPDase2, NTPDase3 and ecto-5'nucleotidase, but no significant labelling was found for NTPDase1. Incubation of cell cultures with selective A1 and A3 receptor agonists increased fibroblast cells proliferation. Activation of A1 receptors also favored type I collagen synthesis, an effect that may be counteracted by co-localized inhibitory A2A receptors.

CONCLUSION

Although preliminary, data suggest that ATP release by stressed fibroblasts may be sequentially dephosphorylated via an enzymatic cascade involving NTPDases2, -3 and ecto-5'-nucleotidase leading to adenosine accumulation. Activation of subtype selective adenosine receptors may influence connective tissue remodelling and fibrosis. In this regard, activation of high-affinity A1 receptors might have a pro-fibrotic effect, which may be partially counteracted by inhibition of collagen synthesis via A2A receptors. Thus, targeting the purinergic pathways underlying fibrosis might be useful to design novel therapeutic strategies to tackle musculo-skeletal chronic pain.

PS 291

Influence of sympathectomy on autonomic nervous system in patients with hyperhidrosis

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AIM

The aim of this study was to quantify the activity of the autonomic nervous system in patients with primary hyperhidrosis before and after sympathectomy.

INTRODUCTION

Hyperhidrosis is excessive sweating that exceeds the needs of normal thermoregulation. Interruption of the sympathetic chain by thoracoscopic sympathectomy is an effective treatment for essential hyperhidrosis. Considering these facts, it is reasonable to postulate that sympathectomy affects the autonomic nervous system. The quantitative marker of autonomic function is based on the heart rate variability (HRV).

METHODS

In this study 22 female and 18 male subjects were included. Measures of Heart Rate Variability were collected one day before and one month after the surgery.

RESULTS

There are significant gender differences regarding the time domain markers before and after sympathectomy. No gender differences were detected analyzing frequency domain variables. The influence of sympathectomy on HRV variables was statisticaly significant in the group of female participants. The increase of time domain variables and high frequency spectral ranges (lnHF and HFn) represent increased parasymphatetic tone, while the low frequency band (LFn) after the surgery showed decreased sympathetic tone. No change was detected comparing time and frequency domain variables before and after sympathectomy in group of the male participants.

CONCLUSION

There are sex differences in time domain markers of HRV both before and after surgery. Sympathectomy has significantly influenced the quantitative markers of HRV in a group of female subjects, in terms of increase parasympathetic tone and decrease sympathetic tone after surgery.

PS 243

Autoimmunity in Selective IgA Deficiency

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AIM

The aim of this study is to investigate the prevalence of autoimmune disorders in the Iranian population of selective IgA deficiency (SIgAD) patients and to evaluate the probable associations between the autoimmunity with clinical and immunological findings in these patients.

INTRODUCTION

SIgAD is the most common primary antibody deficiency which its incidence varies from 1:396 to 1:15,000 in different regions. This disorder is defined as a serum level of IgA lower than 0.07 g/L with normal serum levels of IgM and IgG in an individual older than 4 years of age while other causes of immunodeficiency have been ruled out. Although more than half of the cases with SIgAD are asymptomatic, recurrent infections in the respiratory and gastrointestinal tracts, autoimmune diseases and allergy are the most common manifestations in symptomatic patients.

METHODS

Fifty-seven patients (37 males, 20 females) with confirmed diagnosis of SIgAD who were referred to our center from 1984 to 2011 were enrolled in this study. Demographic data, clinical and laboratory features, as well as the history of recurrent and chronic infections, autoimmunity or other complications were recorded. SIgAD patients were divided into 2 groups according to the presence or absence of autoimmunity (G1 and G2 respectively).

RESULTS

Patients were followed for a total of 297 patient years with a mean follow-up of 5.3 years per patient. The mean (SD) age of patients was 13.7 (4.5). The most common clinical condition among patients was pneumonia (33patients, 57.9 %), followed by allergy occurring in 32 subjects (56%). The major features of the allergy diseases were atopic dermatitis, asthma, and allergic rhinoconjunctivitis. Two patients (3.5%) evolved to common variable immunodeficiency during follow-up period. Autoimmune disorders were documented in 17 cases (29%, 9 males and 8 females); thyroiditis and vitiligo were the most common manifestations with 3 subjects for each disorder (17.6%). Consanguinity was observed in 8 out of 17 patients in G1 and 11 out of 40 patients in G2 which there was no significant difference between two groups (47.1% vs. 27.5%; P= 0.15). Serum level of IgM in G1 was significantly higher than G2 (P=0.01) but WBC in G1 was significantly lower than G2 (P= 0.03). Ten patients (17.5%) showed autoimmune disorders in their other family members while positive family history of autoimmunity had no significant difference among G1 and G2 (P=0.9).

CONCLUSION

Autoimmune disorders along with infections and allergic diseases appear to be among the most important clinical manifestations of SIgAD patients. Prevalence of autoimmunity in this study was 29% which agrees with the previous reports of autoimmune disorders in SIgAD patients .

PS 233

A new cardiovascular bioimplant: development of mitral valve based on a textile tubular structure

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AIM

In the attempt to overcome the limitations of the commercially available prostheses for mitral valve replacement, a new bioimplant was developed: a textile reinforced tissue engineered mitral valve based on fibrin gel as cell carrier.

INTRODUCTION

Mitral valve (MV) regurgitation and prolapse are common diseases that require valve replacement in severe clinical cases. Current mechanical and biological prostheses present key limitations such as the need for a long term anticoagulation and failure by calcification respectively. Moreover, they are mostly designed to be implanted in the aortic position without taking into account the specific hemodynamics of the native MV. Here we present the development of a tissue engineered MV by injection moulding of fibin gel as cell carrier and a textile structure as co-scaffold. The valve is based on a tubular design which recapitulates the key elements of the native MV: the leaflets and the chordae tendineae.

METHODS

The mould for the MV was designed with a 3D CAD software and realized by rapid prototyping technology. The valves were produced by polymerizing a fibrinogen solution with calcium chloride, thrombin and vascular cells. A warp-knitted tubular textile structure was simultaneously embedded in the resulting fibrin gel. Different cell sources were explored: ovine umbilical cord artery, vein and carotid artery. The valves were cultivated in dynamic conditions for 21 days in a custom made bioreactor. Routine histology and immunohistochemical staining were performed. The collagen content was assessed by hydroxyproline assay.

RESULTS

The polymerization of the fibrin gel resulted in a MV consisting of two asymmetrical leaflets and two chordae tendineae. Analysis of the valve by means of high speed video revealed optimal coaptation and no prolapse. The mechanical conditioning resulted in remarkable tissue development. Immunohistochemical images showed abundant collagen fibers aligned along the stress lines and significant deposition of elastin. Collagen synthesis was more pronounced for cells with higher contractile activity as indicated by the presence of alpha smooth muscle actin.

CONCLUSION

We report here for the first time the development of a tissue engineered MV. The high collagen synthesis, the fiber orientation and the presence of elastin are promising results for the achievement of optimal mechanical properties. To this end, the textile reinforcement is of crucial importance. The tubular design of the valve resulted in optimal functionality. Collagen synthesis is directly correlated with the contractile activity of the cells and the consequent retraction of the developed tissue. This is one of the major drawbacks of heart valve tissue engineering resulting in valvular insufficiency. The presented tubular design allows to compensate for this by moulding an initial longer tubular structure. Ongoing research focuses on the integration of a commercial mitral valve ring to facilitate the implantation of the valve in preclinical studies and on the optimization of the conditioning protocol.

PS 219

Abnormalities in lymphocyte populations in autoimune diseases – A preliminary study

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AIM

To evaluate the role of human lymphocytes subpopulations and the expression of FOXP3, NF-kB, APRIL and BAFF in patients with autoimmune diseases (AID).

INTRODUCTION

For a long time, scientists have studied the delicate balance in which the immune system acts. It has been proposed that when certain types of immune cells proliferate, they can cause inflammation. However, these immune cells often do not withdraw, but rather generate an enduring chronic inflammation, stimulating cell proliferation and possibly immune-mediated organ damage, thus relating to auto-immune diseases.

Human lymphocytes are classified in three main populations: T lymphocytes (TL), B lymphocytes (BL) and natural killer (NK) cells. TL can be subdivided in different populations: helper T (HTL) and cytotoxic T (CTL) lymphocytes. Besides that, there are several molecules and cells involved in the cellular and humeral immunity, such as interleukin 17 (IL-17), interleukin 21 (IL-21), APRIL, BAFF and TACI lingands, NF-kB, FOXP3 and regulatory T cells (Treg). Abnormalities in lymphocyte populations and in these molecules have been documented in patients with the referred diseases. The role of these abnormalities is not clarified.

METHODS

We studied a total of 21 patients diagnosed with AID and 3 healthy donors (HD). Using flow cytometry, we determined in peripheral blood (PB) the major lymphocyte subpopulations: total number of lymphocytes (CD3+), BL (CD19+) and NK cells (CD3-/CD56+) as well as HTL (CD3+/CD4+), CTL (CD3+/CD8+) and Treg (CD4+/CD25high/CD127low). We also quantified and analysed the expression of IL-17; IL-21; NF-kB; FOX-P3; BAFF; BAFF-R; APRIL and TACI by flow cytometry.

RESULTS

Our results show that there are several differences on cells population when comparing HD to AID patients.

These discrepancies are most noted in the BL population, with a decrease of BL in patients with autoimmune diseases, and an increase of TL subpopulations and in the total T cell population. Moreover, our results show a decrease in the percentage of Treg cells in patients with AID. Besides that, our preliminary results show, in AID patients, a decrease in the percentage of cells and in the expression levels of IL-21, NF-kB, FOX-P3, BAFF, BAFF-R, TACI and APRIL, compared with controls. However, AID patients had an increase in the percentage of cells and in the expression levels of IL-17.

CONCLUSION

Since autoimmune diseases evolution may be associated with several immune alterations, altered regulatory pathways and abnormal lymphocyte populations, the prognosis and therapeutic success of these diseases might be influenced by these factors. More studies are needed to confirm our results.

PS 356

Survivin Role in Pulmonary Arterial Hypertension

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AIM

To investigate right ventricular (RV) expression of survivin and smac/DIABLO throughout the hemodynamic and morphometric progression of monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH).

To characterize the effects of the survivin antagonist terameprocol in pulmonary artery smooth muscle cells (PASMC).

INTRODUCTION

PASMC play a pivotal role in the vascular remodeling distinctive of PAH by acquiring a hyperproliferative and apoptosis-resistant phenotype (1).

Survivin is the smallest member of the “inhibitor of apoptosis” (IAP) protein family and exhibits pro-proliferative and anti-apoptotic properties. It is expressed in PASMC of patients and rats with PAH, but it is absent in pulmonary arteries from control subjects (2). Smac/DIABLO is a mitochondrial protein that interacts with survivin and inhibits its functions, thus promoting apoptosis. Gene therapy targeting survivin reverted PAH in in a rat model and improved survival (2). However, pharmacological modulation of survivin in PAH would probably be more promptly translated into clinical care than gene therapy. In this setting, terameprocol is a pharmacological agent that suppresses survivin gene expression and is currently being studied as an anti-cancer therapy (3).

Recent studies also point out a crucial role of survivin in cardiac remodeling in the setting of heart failure (4). Additionally, cardiomyocyte apoptosis is recognized as a major feature of right heart failure. However, RV survivin expression in the setting of PAH remains uninvestigated.

METHODS

Adult male Wistar rats received a subcutaneous injection of MCT (60 mg/Kg) or equal volume of vehicle. On days 1, 3, 7, 14 and 21 after injection (n=7-12 per group per time-point), RV pressures were measured, heart and lungs were weighted and RV and lung samples were collected for histological analysis. Survivin and smac/DIABLO expression in the RV was determined by immunohistochemistry and western blotting. In a different protocol, a primary culture of PASMC isolated from sham and MCT-treated rats (day 21) was established and the effects of terameprocol (concentrations: 0; 0.1; 1; 10; 20 and 50 ?M) in cell proliferation and apoptosis were evaluated by BrdU and TUNEL assays, respectively.

RESULTS

Immunohistochemistry and western blotting demonstrated a significant increase in RV survivin expression in the MCT groups since day 7, when compared with the SHAM groups. Smac/DIABLO followed an inverse expression pattern with a significant decrease in the MCT groups identified since day 7. This time-point also corresponded to the first evidence of RV hypertrophy in MCT-treated rats, as evaluated by cardiomyocyte cross-sectional area. Interestingly, survivin overexpression preceded hemodynamic manifestations of the disease, which only started at D14 with significant increases in RV peak systolic pressure and absolute values of dP/dtmax and dP/dtmin.

Terameprocol significantly inhibited proliferation and induced apoptosis of PASMC from sham and pulmonary hypertensive rats in a dose-dependent manner. The pattern of proliferation and apoptosis did not differ significantly between SHAM and MCT groups.

CONCLUSION

Our results demonstrate that survivin upregulation and smac/DIABLO downregulation in the RV precede hemodynamic manifestations of PAH and pair RV hypertrophy, strongly suggesting a role in cardiac remodeling. Terameprocol halted cell proliferation and induced apoptosis of PASMC from both sham and pulmonary hypertensive rats. These findings suggest that targeting survivin in PAH could have dual beneficial effects by reversing pulmonary vascular remodeling and myocardial hypertrophy.

PS 372

Diastolic adaptive response to acute myocardial stretch - Role of Protein Kinase G

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AIM

Evaluate the role of protein kinase G (PKG) and associated signaling pathways in the diastolic adaptive response to acute myocardial stretch under basal and ischemic conditions.

INTRODUCTION

Acute myocardial stretch induces an adaptive response both at systolic and diastolic levels. The mechanisms responsible for diastolic adaptation remain largely unknown.

METHODS

Rabbit papillary muscles (0.2Hz, 30ºC) were acutely stretched from 92% to 100% of Lmax in a modified Krebs-Ringer solution (A) under basal conditions and in the presence of (B) Rp-8-Br-PET-cGMPS (an inhibitor of PKG, 10-6M, n=7), (C) L-nitro-arginine (an inhibitor of nitric oxide synthase (NOS), 10-5, n=8) and (D) A-71915 (a natriuretic peptide receptor-A (NRPA) antagonist, 10-6M, n=7). Group E was stretched during ischemia and other protocols were performed in the ischemic setting in the presence of (F) 8-Bromo-cGMP (an agonist of PKG, 10-5M, n=7) and (G) Natriuretic Peptide B (BNP, 10-6, n=7). Immediate and delayed responses to muscle stretch were evaluated. Results are presented as mean±standard error of mean ($P<0.05$).

RESULTS

Under basal conditions (group A), after immediate increase in myocardial passive tension (PT) induced by acute myocardial stretch there was a significant and time-dependent decrease in PT of 46.2±1.8% in the 15 minutes following stretch. The presence of an inhibitor of PKG (group B) attenuated the decrease in PT to 26,3±1,1%. The inhibitor of NOS (group C) had no significant effects on PT variation (decrease of 41,1±2,7%), whereas the NPRA antagonist (group D) significantly attenuated the PT decrease to 39,4±2,2%. In the ischemic group (group E), diastolic response to acute stretch was completely abolished throughout the 15 minutes of ischemia (increase in PT of 3,5±8,1%). The presence of an agonist of PKG (group F) promoted a decrease in PT of 20,6±3,2% after stretch during ischemia. On the contrary, in the presence of BNP (group G) the diastolic adaptation to acute stretch remained abolished throughout the ischemic period (decrease in PT of 5,6±6,7%).

CONCLUSION

PKG signaling pathway seems to be central in the diastolic response to acute stretch. This response is independent of NOS activity. BNP-NPRA pathway appears to be involved in this adaptation under basal conditions and the natriuretic peptide resistance may contribute to the abolishment of the diastolic adaptation under ischemic conditions. The modulation of PKG pathways represent an important therapeutic target in settings of dysfunctional diastolic response to stretch. Furthermore, the comprehension of its

alterations under ischemic conditions may have important therapeutic implications.

PS 388

Neuregulin attenuates pulmonary endothelial dysfunction in pulmonary hypertension

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AIM

This study aims to evaluate the effects of NRG-1 chronic treatment on pulmonary endothelial dysfunction in an animal model of pulmonary arterial hypertension (PAH).

INTRODUCTION

Neuregulin-1 (NRG-1) is an epidermal growth factor (EGF)-related protein with cardioprotective and cardioregenerative properties. To date, no studies have determined the effects of NRG-1 in pulmonary vasculature, in health or disease.

METHODS

Male Wistar rats (180-200g) randomly received monocrotaline (MCT, 60mg/Kg, sc) or vehicle. After 14 days, animals from these groups were randomly assigned to receive treatment with either NRG-1 (40ug/Kg/day, ip) or vehicle. The study resulted in 3 groups: control (n=8); MCT (n=8); MCT+NRG (n=5). 21 to 24 days after MCT administration animals were anesthetized, heart and lungs were excised en bloc and pulmonary arterial rings were isolated and mounted in a myograph. Endothelial function was determined by a dose-response curve to acetylcholine in phenylephrine pre-contracted rings. After the experimental protocol arterial rings were stored in formalin (10%) for histological analysis. Only significant results are presented (mean ± SEM, $p<0.05$).

RESULTS

MCT animals presented PAH associated with endothelial dysfunction, has shown by a decreased relaxation, mediated by acetylcholine in phenylephrine pre-contracted rings, when compared to the CTRL group (35±2% vs control 86±2%). Treated animals, MCT+NRG, presented a significant improvement in endothelial function (48±3%). Histological analysis revealed vascular remodeling in arterial rings of MCT animals when compared with the CTRL group as shown by an increase in tunica media thickness (53.24±1.84mm vs 31.33±0.83mm), tunica media area (104.50±7.48mm2 vs 67.85±3.93mm2) and the ratio tunica media area/lumen area (41.23±1.48% vs 31.97±2.99%). Animals from the MCT+NRG group presented a significant decrease in vascular remodeling as shown by improvements in all parameters analysed (34.26±0.91 mm, 75.64±5.10 mm2 and 29.56±2.46 %, respectively).

CONCLUSION

NRG-1 chronic treatment significantly reduced the severity of endothelial dysfunction and vascular

remodeling in rats with induced PAH. These results show that NRG-1 system has a crucial role in vascular function, specifically in PAH, proving to be a potential therapeutic target in this condition.

Book of Abstracts

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PS 49

Characteristics of cerebral hemodynamics, metabolism and functional condition at mental work at students with various motivation

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AIM

Evaluation of correlation between possible changes of functional condition, brain hemodynamics, metabolism at rest and in purposeful activity and different effectiveness of result achievement in students with various motivation structure.

INTRODUCTION

Influence of motivational excitation of hypothalamic centers on brain structures was widely investigated on animals. Now there are few similar researches concerning humans. And the main question is what orientation of motivation (achievement or avoiding) will mobilize an organism for achievements, convalescencec Why does one patient recover, but the second not in two identical clinical casesc It depends much on skills of the doctor, medicine technologies in medical process, but what role does patient havec And can ´the thirst for life[a] be a leading curing factorc

METHODS

Research was involved 40 students of RyazSMU (15 women,25 men, the mean age 21,4). We used mental activity model as behavioral model. Mental activity has been designed in using Quantitative relations test. The motivation orientation (expressiveness of motives of achievement and avoiding) has been evaluated with multifactorial psychodiagnostic motivational test. Changes in organism functioning have been evaluated with metabolography, rheoencephalography, method of the heart rhythm mathematical analysis. Data are expressed as median and range. Data were analized by Wilcoxon T-criteria, Mann-Whitney U test, Spearman correlation, Multiple regression(Statistica 6.0). A p-value <0.05 was considered statistically significant.

RESULTS

We arranged students in two groups 20 successful and 20 unsuccessful students according to the data of Quantitative relations test. We obtained significant prevalence of achievement motives in successful group and prevalence of avoiding motives in unsuccessful group. We also obtained strong correlation between achievement motives and high-frequency component of heart rhythm spectrum (reflects activation of an autonomic contour of regulation; predisposing to transition to rest condition) in successful students, between avoiding motives and very low frequency component of heart rhythm spectrum (reflects activation of sympathetic nervous system; testifies to stress) in unsuccessful students, correlation between achievement motives and change level of metabolic expenses, between achievement motives and change of change of blood filling speed of the brain in successful students.

CONCLUSION

We obtained strong dependence between prevalence of achievement motives and changes of brain hemodynamic and metabolism reflecting optimization of activity. Decrease of activity effectiveness has been noticed in students with prevalence of avoiding motives.

PS 80

Gamma knife treatment of growing vestibular schwannoma in Norway: A prospective study

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AIM

Gamma knife radiosurgery (GKRS) has been increasingly used in the treatment of vestibular schwannoma (VS). Very few studies relate tumor control and post-treatment growth rates to pre-treatment growth rates.

INTRODUCTION

Vestibular schwannoma (VS) is a rare, benign tumor of the eight cranial nerve. Historically these were surgically removed, but tumors are now detected when they are smaller and alternative treatments like conservative management and gamma knife radiosurgery (GKRS) are growing in popularity. GKRS delivers radiation to the tumor, in theory causing the arrest of tumor growth, but in practice there is insufficient evidence. The majority of VS patients in Norway come to our center for diagnosis and treatment. Since 2000, all 413 of these patients have been included in our prospective database - thus providing us with a unique possibility to investigate VS growth dynamics.

METHODS

We prospectively included 45 consecutive VS patients who were initially conservatively managed and then received GKRS between 2000-2007 because of demonstrated tumor growth. Pre- and post-treatment tumor volumes were estimated. Patients underwent audiograms, reported complaints and responded to the SF-36 questionnaire on each visit.

RESULTS

Volume doubling times before and after treatment were 1.36 years (95% confidence interval 1.14 to 1.68) and -13.1 years (-111.0 to -6.94) respectively. Tumor control, defined as a post-GKRS growth rate c0, was achieved in 71.1%, with highest odds for tumor control among older patients and with larger tumors. The five-year retreatment-free survival rate was 93.9% (76.5-98.5). None of the clinical endpoints investigated showed statistically significant changes post-GKRS, but improvement was seen in a few SF-36 parameters.

CONCLUSION

GKRS alters the natural course of the tumor by reducing growth. Mathematical models yield poorer tumor control rates than those found by clinical assessment. Symptoms were unaffected by treatment, but quality of life was improved.

PS 60

The relationship between symptoms of hyperactivity and problems with peer relationships among schoolchildren in Sao Paulo

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AIM

The objective of this study was to evaluate the relationship of psychopathology related to hyperactivity to social maladjustment assessed by the SDQ.

INTRODUCTION

Social relationships begin to be established during childhood and are important determinants of quality of life. Children with hyperactive symptomspresent several dysfunctional behaviors that are likely to produce negative impact in their relationships with parents, teachers and peers. Studies suggest that children with ADHD symptoms have an increased risk of social dysfunction that persists until adulthood.Thus, the objective of this study was to evaluate the relationship between hyperactivity as a dimensional trait presentin the population and peer problems in school-age children ascertained from public schools.

METHODS

We ascertainedchildren 6 to 12 years-old from 35 public schools of São Paulo City, Brazil. The primary caretaker of the child completed the Strengths and Difficulties Questionnaire (SDQ) , which evaluates appropriate behaviors (capacities) and inadequate (difficulties) of children and adolescents. The questionnaire has 25 items and five different subscales: Emotional Symptoms, Conduct Problems, Hyperactivity problems, Peer Relationship Problems and Pro-Social Behavior. We investigated the correlation between hyperactivity symptomsandpeer relationship problems, controlling for age, sex and the presence of any DSM-IV mental disorder.. . Subsequently, we compared the proportion of children with high (10th percentile) versus low (90th percentile) levels of hyperactive symptoms who have relationship problems. Significance level was set at 0.05.

RESULTS

The sample comprised 1257 children, 54,8%were male. Mean age was 9.49 (SD 1.8) years. Mothers were the informants in 88.7% of cases. Correlations between hyperactivity and social problems wasr = 0,38($p = 0.01$). Children with highlevels of hyperactive symptoms were approximately three times more likely OR=3,35; IC95%=2,20 - 5,12; $p<0,001$)to present social problems than children withlow levels of hyperactivity.

CONCLUSION

Hyperactive symptoms as assessed by the SDQ are associated to social problems in school-age children from the community, independently of age, gender or any DSM-IV co-morbid disorders. Therefore, children with

hyperactivity may represent an important risk group for social problems.

PS 134

The Effect of Active Amyloid-beta Immunisation on Neuronal Morphology in Alzheimer's Disease

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AIM

To assess the effects of immunisation on the neuronal morphology and changes in axonal protein in relation to the pathological changes previously observed.

INTRODUCTION

Alzheimer's disease (AD) pathology is characterised by accumulation of Amyloid-beta (Abeta) and hyperphosphorylated tau (phospho-tau) proteins. The amyloid cascade hypothesis puts Abeta aggregation at the heart of AD pathogenesis, triggering phospho-tau accumulation and synaptic dysfunction leading to neuronal loss. The extracellular and intracellular protein accumulations occupy valuable functional space and are thought to disrupt neuronal morphology. Clinical trial of Abeta immunisation resulted in the removal of Abeta and phospho-tau in neuronal processes. However, there was no clinical improvement in the patients, which may be due to an inability of immunisation in targeting phospho-tau in neuronal cell bodies (tangles).

METHODS

Formalin fixed tissue from 11 AD patients immunised with Abeta42 (iAD - AN1792, Elan Pharmaceutical) and 28 unimmunised AD patients (cAD - South West Dementia Brain Bank) were immunostained for Neurofilament Protein and quantification was performed to obtain NFP load (%) and NFP ratio. Using slides previously stained for phospho-tau (AT8), phospho-tau load per dystrophic neurite (AT8/DN) was quantified. Data were correlated with previously findings obtained on Ac42 and phospho-tau.

RESULTS

There was no significant difference in NFP load between both groups (cAD=3.611, iAD=3.742, P=0.983) but a significant decrease in NFP ratio in iAD cases was observed (cAD=1.429, iAD=1.278, P<0.0001), indicating a normalisation of neurite morphology. There was a significant reduction in AT8/DN after immunisation (cAD=0.249, iAD=0.105, P<0.001), showing a reduction of phospho-tau in neuronal structures. In cAD, correlations observed were: (i) of NFP ratio with NFP load (r=0.513, P=0.005) and with Abeta42 load (r=-0.661, P<0.001); (ii) of AT8/DN with tau load (r=0.483, P=0.009) and number of tangles (r=0.491, P=0.008). In iAD, two correlations observed: AT8/DN with NFP ratio (r=-0.795, P=0.003) and AT8/DN with Abeta42 load (r=0.636, P=0.048).

CONCLUSION

Our study shows that Abeta-immunisation can influence neurite trajectory even in an old brain, but this was not directly related to Abeta42 clearance. Therefore other key factors may be involved in disrupting neuronal

morphology including activated microglia, oligomeric Abeta and reactive oxygen species. The reduction of phospho-tau in DN confirms previous reports that Abeta removal reduces tau burden and gives validation to the amyloid cascade hypothesis. This study confirms that targeting Abeta42 can impact on phospho-tau and axonal processes; however, this is yet to yield any significant results clinically.

PS 172

The Reliability of Functional Magnetic Resonance Imaging for Presurgical Broca's Area Mapping

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AIM

The aim of this study was to evaluate the accuracy of presurgical fMRI for localizing cortical language areas by comparing fMRI data to the results from intraoperative DES mapping.

INTRODUCTION

The development of different techniques for functional brain mapping has made possible total neurosurgical resection of lesions located in close proximity to important cortical areas. Direct electrical stimulation (DES) mapping is currently considered the gold standard of functional cortical and subcortical mapping. Functional magnetic resonance imaging (fMRI) offers an opportunity for non-invasive preoperative mapping which reliability needs to be tested.

METHODS

We studied 14 patients(5 men and 9 women), operated on in the Department of Neurosurgery, University Hospital "Sv. Ivan Rilski", Sofia during the period 2009-2011. The mean age was 40 years and 6 months. All of the patients underwent presurgical fMRI(1,5T) and awake intraoperative DES mapping(5-12 mA). There were 13 right-handed and 1 left-handed patients. All patients had a lesion in the frontal or parasylvian region of their dominant brain hemisphere. The language task during the preoperative fMRI and during the awake phase of surgery was visual object naming. Broca's area was defined as the area of speech arrest during DES. We used standardized brain models to assess the degree of overlapping between the activated areas on fMRI and the intraoperative findings.

RESULTS

Results obtained from the comparison between fMRI and DES mapping were distributed between 4 groups according to the degree of their concordance: group 1(100% correlation), group 2(50-99% overlapping of the activated cortical areas), group 3(less than 50% overlapping) and group 4(no correlation at all). There were 2 patients in group 1, 3 patients in group 2, 6 patients in group 3 and 3 patients in group 4. Total lesion excision was accomplished in 10 patients. Resection was subtotal for the other 4 patients.

CONCLUSION

Our study showed moderate accuracy of fMRI with single task of object naming for presurgical localization of Broca's area. Insufficient correlation between fMRI and DES results was observed. Therefore fMRI with the single task of object naming is difficult to be considered as a reliable alternative of DES for preoperative

language mapping. Introduction of more tasks and the development of fMRI methodology could improve fMRI mapping accuracy.

PS 202

The frequency and type of disorders of higher cortical functions during aura in migraine with aura

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AIM

The aim of this study was to evaluate the frequency and type of transient disorders of speech, praxia, gnosia and memory that occurs as part of the visual and/or sensory aura in patients with migraine.

INTRODUCTION

Migraine is a primary headache with an estimated annual prevalence of 11% of adult population. Aura occurs in 20-30% of migraine patients and usually precedes the headache. Typical symptoms of aura are completely transient homonymous visual symptoms with positive or negative phenomena, less one-sided positive sensory symptoms (tingling, pricking) or negative phenomena (numbness), or transient disorders of speech. Descriptions of aura obtained from patients suggests that in addition to the primary cortical areas that affected, also are affected secondary and tertiary cortical parts. Large parts of the temporal, parietal and occipital lobes are polymodal associative areas that integrate sensory informations of different modalities which is the basis of higher cortical functions (HCF).

METHODS

The study group included 89 patients who were diagnosed with migraine with aura at the Department of Neurology, Clinical Center of Serbia during 2005-2011. The diagnosis was based on the criteria of The International Classification of Headache Disorders. For purposes of this study was designed a special questionnaire. The questionnaire was filled by 60 patients. Based on the results patients were divided into two main groups. Group I was consisted of patients who had at least one symptom of HCF disorder, while Group II consisted of patients who did not have any symptoms of HCF disorders. These two groups were compared with each other according to demographic characteristics and characteristics of the aura. With further analysis, Group I was divided into subgroups: Subgroup Ia (patients with only visual aura) and Subgroup Ib (patients who had a visual and/or sensory aura). Also, we were specially analyzed patients who had dysphasia associated with one or more HCF disorders. To assess the significance of differences were used chi-square test and t-test for two independent samples. Significant difference was considered to be one in which the $p<0.05$. To assess a correlation between duration of aura and number of disorders of HCF we were used Spearman`s test.

RESULTS

Of the 60 patients analyzed, 39 (65.13%) reported at least one HCF disorder and these patients were classified as Group I. There was no statistically significant difference between Group I and Group II regarding sex, age at the time of examination, age at the time of the start of migraine with aura, years of education and frequency of attacks of migraine with aura per year. Aura lasted significantly longer in Group I (28.51±16.39 min.

vs. 19.76±11.23 min, $p<0.05$). The most common disorder of HCF was dysphasia - motor type (82.05%), followed by nomination disorder (30.74%) and transient amnesia (23.08%). Motor dysphasia (96.30% vs. 50.00%, $p=0.000$) and anomie (40.74% vs. 8.33%, $p=0.008$) were significantly more prevalent in the Subgroup Ib, compared with patients in Subgroup Ia. Patients in Subgroups Ib had a longer time duration of aura compared with patients in Subgroup Ia. Also, we were established a positive correlation between increase in time duration of aura and increase of number of HCF disorders.

CONCLUSION

The results clearly indicate that HCF disorders were significantly more common than is considered, as well as the length of duration of the aura affects the appearance of HCF disorders.

PS 237

Protein deprivation but not food restriction leads to major changes in some populations of interneurons of the dentate gyrus of

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AIM

Does protein deprivation or food restriction lead to major changes in some populations of interneurons of the dentate gyrus of the adult ratc

INTRODUCTION

Several studies have demonstrated the vulnerability of the adult hippocampal formation to malnutrition. In this study, we have compared the effects of two models of dietary deprivation, food restriction and protein malnutrition, in the number of neurons of dentate gyrus and parvalbumin and calretinin interneuronal subpopulation related to the control of calcium homeostasis and fine tuning of the hippocampal circuits.

METHODS

Two month-old rats were randomly assigned to a control group, a food-deprived group (40% caloric restriction) and a low-protein diet group (8% casein). After 8 months of treatment the rats were perfused with adequate fixatives and using stereological methodology, the total number of granule and hilar cells as well as the volumes of the regions of the dentate gyrus were quantified. The density area of parvalbumin and calretinin interneurons of the dentate gyrus labeled using immunocytochemical methodology were estimated.

RESULTS

It was found that total number of granule and hilar cells was reduced in protein-malnourished rats. The volumes of the hilus, molecular and granular layer were similar in all groups studied. Parvalbumin-immunoreactive interneurons located in the hilus were increased in protein-deprived rats, when compared to the other groups. However, the number of interneurons labeled for calretinin was decreased in the hilus of the protein malnourished animals. Our data also shows that food deprivation does not affect the number of neurons or the parvalbumin and calretinin interneurons of the adult dentate gyrus.

CONCLUSION

These results support the view that protein deprivation may provoke a disturbance in calcium homeostasis leading to neuronal death. The up-regulation of parvalbumin-immunoreactive cells may reflect a protective mechanism to counteract the calcium overload and protect the remaining neurons of the dentate gyrus contrasting with the vulnerability of calretinin interneurons. These data may justify some learning and memory impairments described in protein-deprived animals. The contrast between the results of food-restriction and protein deprivation should be further analyzed.

PS 244

Neuroplastin expression in fetal hippocampal tissue

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AIM

The aim of this study was to analyze developmental pattern of neuroplastin immunoreactivity in human fetal hippocampal tissue.

INTRODUCTION

The hippocampus is a three-layered cortical structure located in the medial temporal lobe of the brain that receives afferent input from cortical regions processing a range of sensory modalities. Neurodevelopmental studies of human hippocampus showed formation of first synapses during early fetal period, mostly within 15. gestational week (g.w.). It has been evidenced that large and diverse family of cell adhesion molecules is involved in complex regulation of neural development and plasticity. Neuroplastin, a cell adhesion transmembrane glycoprotein is expressed mostly within postsynaptic densities where it is involved in intercellular interactions.

METHODS

Materials used in this study were formaline-fixed paraffine-embedded fetal human hippocampal sections, in gestational age of 12.-40. g.w. Neuroplastin expression was analyzed immunohistochemically using primary anti-neuroplastin antibody. Following diaminobenzidine visualization, the sections were analyzed using light microscope Olympus AX70 Provis and neuroplastin immunoreactivity was additionally quantified by ImageJ program (NIH).

RESULTS

In our study, neuroplastin immunoreactivity in human hippocampal tissue was not observed earlier than mid-fetal and late-fetal period. Gradually higher neuroplastin immunoreactivity was observed with increasing gestational age. Data from other studies on human brain transcriptome showed that neuroplastin gene expression increases from early fetal period and reaches a peak expression during late fetal and neonatal period. Our data on neuroplastin protein expression in human hippocampus is in accordance with published human brain transcriptomic analysis.

CONCLUSION

We conclude that increasing neuroplastin expression in hippocampus during brain development coincides with formation of hippocampal synapses and suggest that neuroplastin is one of the molecules involved in regulation of synaptic plasticity.

PS 249

Hippocampal expression of neuroplastin in alzheimer's disease

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AIM

The aim of this study was to analyze the expression of neuroplastin in human hippocampal tissue affected by Alzheimer's neurodegeneration in comparison with age- and gender-matched controls.

INTRODUCTION

Neuroplastin, a cell surface glycoprotein found in two splice isoforms (np65, np55), is a member of immunoglobulin superfamily of cell adhesion molecules. Association of brain-specific neuroplastin isoform, np65, with developmental processes such as neuronal migration, neurite outgrowth and synaptogenesis has been found in rodent brain. Although distribution of np65 in adult human brain has been described, there are no data on np65 expression during aging and neurodegeneration. Alzheimer's disease (AD) is characterized by up-regulated expression of plasticity molecules, particularly in hippocampus and entorhinal cortex, reflecting compensatory reorganization of remaining cellular structures.

METHODS

For that purpose, paraffin-embedded sections of AD and control hippocampal tissue were analyzed by immunohistochemistry, using primary anti-neuroplastin antibody. In addition, homogenates of hippocampal tissue samples are being used for Western blot analysis and RNA isolation, in order to determine neuroplastin expression at both transcriptomic and proteomic level.

RESULTS

Results on tissue distribution of neuroplastin immunoreactivity confirmed its extracellular localization in both control and AD hippocampal sections. The overall intensity of neuroplastin immunoreactivity was higher in AD than in control hippocampi, and was most notably expressed in neuronal population of dentate gyrus inner molecular layer. Interestingly, intracellular localisation of neuroplastin was detected in AD hippocampi, mostly in subiculum, which may indicate altered posttranslational modification and trafficking of neuroplastin molecule.

CONCLUSION

We conclude that neuroplastin may serve as a plasticity marker and that detected increased expression of neuroplastin immunoreactivity in AD hippocampal tissue is related to neuronal remodelling and plasticity reactivation occurring in neurodegeneration.

PS 206

Interactable in intervention for children with autism spectrum disorder (ASD)

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AIM

This project intends to offer a new complementary form of intervention for children with ASD, both in the therapeutic and the pedagogical context. In particular, it intends to enhance different capabilities such as interactivity, figure-image-object relationship and overall intellect.

INTRODUCTION

Interactable is a system which employs the capabilities of a Microsoft Kinect to create a cause-effect link between the handling of specific objects (eg: coloured cubes) and some chosen reaction. Since image processing is frequently one of the strong points of children with ASD, who also are keen on predictable and repetitive scenarios, it was found that allowing them to manipulate simple objects with attractive colours and drawings would be a good way to facilitate the intervention process. On top of that, music is also a common interest to these children and is often used as a means of therapy, being thus considered that a musical reaction to the objects' handling would be most suitable.

METHODS

To test the system in an intervention context, cubes representing musical instruments were employed. There were four cubes each of which with a different instrument painted on its face: djembe, guitar, maracas and piano. These particular instruments were chosen due to their familiarity and appeal to this children. Putting a cube on the ground would then automatically start playing a melody of the corresponding instrument. Changing the cube's face pointing up would change the melody, but not the instrument. Moving the cube along the surface would affect the volume of the song. In this setup two trials were made with a musical therapist: a two-years-old child with autistic disorder and a twelve-years-old with Asperger syndrome.

RESULTS

Both children showed interested in the system, and the musical therapist recognised that it made the beginning of the session smoother. The youngest child, while not being able to fully grasp the system's capabilities, revealed itself capable of establishing the association between the djembe painted on the cube and the instrument itself. The older one managed a more thorough understanding of the system, trying to combine different instruments in order to obtain a desired musical response. However, the children's attention was frequently drawn to the camera itself, distracting them from the session's purpose.

CONCLUSION

It was considered that the system is capable of improving the impact of musical therapy with children with ASD, in the sense that it allows them to have a more active role in the intervention. This may increase the children's levels of interactivity. Moreover, the system can be used outside of the scope of musical therapy for pedagogical purposes. Adapting the cubes' themes to the children's interests, it can be used to capture their attention and work on often intervened areas such as notion of quantity, of colour, or shape, overall knowledge (eg: on animals or means of transports) and the ability to count.

PS 280

Correlating clinical presentation and neuroradiological findings in patients with neurological presentation of Wilson's disease

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AIM

The purpose of the study was to evaluate the main clinical presentation at the time of the disease onset, to detect the brain abnormalities on magnetic resonance imaging (MRI) examination and to correlate the radiological features and clinical findings.

INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disease of copper metabolism, initially presenting with a wide spectrum of nonspecific signs and symptoms. Unfortunately, it is therefore usually not included in the differential diagnosis of patients with neurologic, psychiatric or hepatic disorders and the diagnosis and treatment are often delayed.

METHODS

Thirty seven patients with clinically proven WD underwent brain 1.5-T MRI and the following pulse sequences were used: (a) sagittal gradient echo (GE) T1-weighted (T1W) [repetition time (TR) / echo time (TE) / flip angle (FE) 266/6/80, 5-mm slice thickness with 0.1 interslice gap, 250mm field of view (FOV) and 192x256 matrix], (b) axial turbo spin echo (TSE) - double echo (T2W) [TR/TE - 3300/19-96, 6mm slice thickness with 0.1 interslice gap, 230mm FOV and 192x256 matrix]. Correlation with clinical findings was performed. The Fisher exact test was used for statistical analyses.

RESULTS

The diagnosis of WD was established in 24 men and 13 women. The age at the disease onset was between 12-41, mean 28. Pseudoparkinsonian, cerebellar and dystonic symptoms were evident in 45%, 70% and 35% of patients, respectively. Brain abnormalities were found on MRI study in 95% of patients. The lesions were 100% symmetric in putamina, caudate nuclei, midbrain, pontine tegmentum and cerebellum. Putamen was affected in 78% of patients, but in 100% of those with dystonic clinical presentation. Correlation between putaminal affection and dystonia was statistically significant (p=0.035).

CONCLUSION

MRI is very sensitive diagnostic modality to detect abnormalities in patients with WD. Symmetry of the basal ganglia or brainstem abnormalities in young adults are typical for this disorder. Early recognition of WD is crucial in order to prevent irreversible changes in the brain parenchyma.

PS 254

Effect of anti-psychotic drugs on l-dopa uptake in a dopaminergic-like neuronal cell line

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AIM

To evaluate the influence of different anti-psychotics on L-DOPA transport in a cell line (SH-SY5Y) that is used as an in vitro model of dopaminergic neurons.

INTRODUCTION

The typical anti-psychotics have been associated with extrapiramidal signs and symptoms (EPS) and tardive dyskinesia more than the atypical ones, although mechanisms involved are poorly understood. The main targets of anti-psychotics in the brain are the dopamine D2 receptors, binding with varying affinities on nondopaminergic sites, such as c1-adrenoreceptors, which partially explain some side effects. c2-adrenoceptors type, are one of the main presynaptic inhibitory regulators of noradrenaline and dopamine release. Recent studies have shown that activation of c2 adrenoceptors decreases L-DOPA uptake in cells and blockage of the receptor increases tissue levels without producing significant changes in L-DOPA synthesis(Vieira-Coelho, MA, et al.).

METHODS

SH-SY5Y cells were differentiated into dopaminergic like neurons by a sequential treatment with retinoic acid (10 µM 3 days) and phorbol ester (80 nM 3 days). Differentiated cells were incubated with different concentrations of L-DOPA (2.5-2500cM), in the presence and absence of anti-psychotic drugs (10cM) that were present in the medium 30 min before L-DOPA. L-DOPA levels were evaluated by high performance liquid chromatography with electrochemical detection. Results are presented as arithmetic mean ± standard error mean.

RESULTS

L-DOPA uptake was concentration (2.5-2500 µM) dependent for CT [KM (µM) =1417±458; Vmax (nmol/mg protein/6 min) = 286±42], olanzepine [KM (µM) = 1309±501; Vmax (nmol/mg protein/6 min)= 279±48], chlorpromazine [KM (µM) = 1515±736; Vmax (nmol/mg protein/6 min)= 215±49], quetiapine [KM (µM) = 1406±386; Vmax (nmol/mg protein/6 min)= 389.5±45.71], haloperidol [KM (µM) = 1205±577; Vmax (nmol/mg protein/6 min)= 275±54), clozapine [KM (µM) = 2726±1764; Vmax (nmol/mg protein/6 min)= 366±140), ziprasidone [KM (µM) = 1710±576 and a Vmax (nmol/mg protein/6 min)= 289±50] and risperidone [KM (µM) = 1578±814 and a Vmax (nmol/mg protein/6 min)= 333±83]. L-DOPA uptake was significantly higher in quetiapine incubated cells, and significantly lower for chlorpromazine incubated cells. The other anti-psychotics did not alter significantly L-DOPA uptake.

CONCLUSION

The significant rise in the L-DOPA accumulation triggered by quetiapine might be partially explained by its attach to c-adrenoreceptors in general, although no significant effects are seen in the presence of clozapine, which seems to share the same targets with the previous one. The decrease in L-DOPA uptake caused by chlorpromazine may help explain the EPS effects, which characterizes typical anti-psychotics. Further studies are necessary to understand the mechanism underlying these results.

PS 302

Possible Role of Cholinergic Deficits in Hipocampal Epileptogenesis

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AIM

The aim of this study is to investigate the role of cholinergic system in epileptogenesis.

INTRODUCTION

Temporal lobe epilepsy (TLE) is one of the most common types of seizure disorders in humans. It can be accompanied by a neuron loss in the hippocampal formation (HF) and reorganization of the temporal lobe neuronal circuits. TLE is frequently treatment-resistant. Although the exact etiology of TLE is unknown, there is strong evidence that it may be related to the low efficacy of hippocampal intrinsic inhibitory mechanisms, which, in turn, renders neurons hyperexcitable and able to produce spontaneous discharges. Therefore, identification of the factors capable of reducing the efficacy of hippocampal inhibitory processes might help unravel possible causes of epilepsy. In prior works, it has been shown that the levels of neuropeptide Y, one of the most potent inhibitory neuromediators, are decreased in epileptic HF and that its synthesis and expression are dependent on the ascending cholinergic afferents. Thus, we hypothesized that cholinergic activity is also reduced under these conditions, which might partly explain hippocampal disinhibition in epilepsy.

METHODS

We induced status epilepticus in one group of rats (SE), whereas another group served as control. Two months later, all rats from SE group showed spontaneous recurrent seizures. The animals were perfused and their brains were post-fixed and cut into 40-µm coronal sections. The sections were systematically sampled along the entire septotemporal axis of the HF, yielding 10 sections per animal, and were processed for immunocytochemistry with a primary antibody directed against the vesicular acetylcholine transporter protein (VAChT). From each section, 2 images of the dentate gyrus hilus were taken at 2 different focal planes. The number of VAChT-positive fiber varicosities was counted within a rectangular area (140s100 µm) located in the center of the dentate hilus using the ImageJ image analysis software. The surface area of each of the varicosities was also measured.

RESULTS

We found that, in both groups, the majority of the varicosities fall within the range 0.1-3.0 µm. Small varicosities (0.1-0.2 µm) were the most numerous. There were more varicosities in control rats than in SE rats, which was confirmed by the statistical analysis ($P<0.05$ for the size range 0.2-0.6 µm). Secondly, the overall surface area of the varicosities for each size range was calculated by multiplying a given surface area by the number of varicosities that fall into that range. The data were again presented in the form of a histogram as a function of the surface area. Inspection of the plots has shown that small to medium-sized varicosities (0.2-0.7 µm) have major contribution to VAChT content in the dentate hilus, suggesting their particular importance in cholinergic innervation of this brain region. However, the overall surface area of the

varicosities in this size range was dramatically reduced in SE rats vs. controls. This effect was highly significant ($P<0.00001$). We further calculated the area under the curve for both groups, using the trapezoidal rule of integration, and found that the overall area of VAChT-positive hilar varicosities (0.0-3.0 µm size range) was reduced by 35% in epileptic rats when compared to control rats.

CONCLUSION

This study provides evidence for the cholinergic hypothesis of epileptogenesis.

PS 296

Psychiatrist's personality towards psychosis/ mood disorder. Do different diagnoses influence patients' expectationsc

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AIM

To verify the hypothesis about the differences in the Image of Ideal Psychiatrist[IIP] between adult patients with different diagnoses.

INTRODUCTION

Psychiatrists present a variety of personality traits. So far, studies showed that psychiatric patients pay attention to doctor's qualifications, appearance and form of emotional contact. It is proved that psychopathological symptoms have an impact on patient's needs so that patient expectations are correlated with diagnosis. Researchers emphasize that psychiatrist's personality may be an effective and cheap medication, if it meets the patient's needs.

METHODS

50 consecutive patients from the Psychotic Disorders Unit[Group S] and 50 from the Affective Disorders Unit[Group A] participated in the study. Patients met ICD-10 criteria for major depression, anxiety-depressive disorder, organic mood disorder, bipolar disorder in Group A and schizophrenia and schizoaffective disorder in Group S

The Adjective Check List [ACL] was used to assess patients' expectations towards the ideal psychiatrist [IIP]. Specific software was used to convert ACL results to personality profiles (24 scales). The data was analyzed with the Mann-Whitney U test.

RESULTS

The Mann-Whitney U test revealed statistically significant differences between groups A and S in 3 of the 24 scales: (1) Seeking change($p=0.009$), (2) Lability($p=0,006$), (3) Negative terms($p=0.003$). On these scales, Group S received higher scores than Group A.

CONCLUSION

The findings showed that there are differences in the expectations of patients with psychotic disorders and patients with affective disorders. The results may indicate that patients in group S appreciate discretion, independence, spontaneity and unconventionality as psychiatrist personality traits more than patients in group A. A higher score in Negative terms suggests that more individuals in group S prefer impulsive, cynical, rebellious persons as psychiatrists than in group A.

Understanding patients' expectations gives possibility of modifying the behavior of doctors, which in turn can improve establishing rapport with patients and compliance.

PS 306

Green tea catechins prevent detrimental changes in the dendritic trees of aging hippocampal neurons - a Golgi study

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AIM

To verify if green tea catechins preserve the structure of hippocampal CA1 dendritic trees during aging.

INTRODUCTION

Aging is associated with oxidative stress-related biochemical changes that cause progressive cellular damage. The hippocampal formation is vulnerable to these degenerative events resulting in deleterious morphological modifications in neuronal organelles, lipofuscin accumulation and alterations of the dendritic trees accompanied by behavioral impairments. Catechins, present in beverages such as green tea, display strong anti-oxidant and anti-inflammatory properties that we have previously found to protect the hippocampal formation from oxidative stress damage and behavioral deterioration. In the present work, we intended to analyze the effects of the prolonged consumption of green tea and a green tea extract rich in catechins on the structure of the dendritic trees of hippocampal CA1 pyramidal cells.

METHODS

Male Wistar rats aged 12 months were treated with green tea (n=4) or a catechin-rich extract (n=5) until the 19 months of age. These groups of animals were compared with control groups aged 19 months (n=4). Following anesthesia the animals were perfused and the Golgi impregnation of the hippocampal formations was performed according to the method of Steensas. Ten CA1 pyramidal cells were sampled and the apical and basal dendritic trees were drawn, with the aid of camera lucida. The dendritic segments were allocated according to their different length distributions, i.e. terminal and intermediate segments. The length of each type of segment was measured and the total dendritic length was calculated as total sum of the length of all dendritic segments. It was also calculated the mean segment length of the terminal and intermediate segments. The number of each type of segments was counted as well as the total number of segments per cell.

RESULTS

The results of morphometric analysis of the apical and basal dendritic trees of CA1 pyramidal cells of rats that consumed green tea extract presented a higher total dendritic length when compared to age-matched controls. The total number of segments of these rats was also higher when compared to controls. The rats that consumed green tea presented also some favorable changes in the structure of the dendritic tree when compared to controls but some of the metric parameters evaluated did not reach statistical significance.

CONCLUSION

Our results demonstrate that the consumption of green tea catechins protects the basal and apical dendritic arborizations of CA1 pyramidal cells from aging-related declines. These changes can probably partially justify the repercussions on behaviour performance of old animals and can be related to the neuroprotection provided by the catechins in rats that consumed green tea or green tea extract.

PS 303

The OTH improves neurologic outcome in patients with acute incomplete spinal cord injuryc

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AIM

Contribute to the knowledge of the epidemiology of acute traumatic spinal cord injury in the Autonomous Region of Madeira, evaluate the neurologic outcome in those patients treated with and without HBO therapy; identify markers of good prognosis (clinical, functional and neuroimaging) and evaluate de complications during HBO therapy.

INTRODUCTION

SCI is a disabling condition, with great impact on patients quality of life. In the United States, the annual incidence of SCI is estimated to by about 40 cases per million population or approximately 12,000 new cases each year not including those who die at the scene of the accident. There are few data on SCI in Portugal. After the mechanical primary injury, a series of pathophysiological mechanisms that lead to degeneration and death of potentially viable neuropil occurs. Hypoxia/ischemia is considered the most important factor in tissue damage; Anatomical, bioquimical and phisiological studies showed, that blood flow and microvascular permeability is reduced after spinal cord contusion or compression. Many treatment modalities were investigated. Among these interventions, hyperbaric oxygen therapy (OTH) has been advocated to improve neurological outcome. Many experimental studies showed that the OTH improves motor function and recovery of the histological and bioquimical markers, culminating in a delay of motor neurons death and protection against ischemic injury.

We couldn't find any randomized controlled trials in de literature.

METHODS

A prospective randomized control study of traumatic spinal cord injury (SCI) treated with and without hyperbaric oxygen (HBO) therapy. Patients with acute, incomplete, traumatic cervical or thoracic spinal cord injury were selected. Patients with acute total section spinal cord injury or contraindications to perform HBO therapy (barautrauma) are excluded. Subjects participating in the study gave informed consent. The classification of spinal cord injury was performed using "standard neurological classification of spinal cord injury" of American Spinal Injury Association (ASIA scale). Randomization was obtind by coin method. HBO therapy was performed in Hyperbaric Medicine Centre of SESARAM EPE, in level 1 multiplace hyperbaric chamber. The HBO therapy protocol comprises 20 sessions: three HBO treatments in the first 24 hours, at 2.8 absolute atmospheric pressure (ATA) lasting 90 minutes and from the 4th treatment, a treatment/day, at 2.5 ATA, lasting 75 minutes. Patients who don`t improve with 20 sessions will proceed with complementary 10 sessions. The neurological status is assessed by ASIA scale, on the admission, after 10 and 20 sessions, at date of suspension of HBO therapy, and at 3, 6 months and 1 year after the injury. Evolution is also made by magnetic ressonance imaging and somatosensory evoked potentials. The complications (claustrophobia, barotrauma, seizures, pain, spasticity and disrreflexia) is also recorded.

RESULTS

Our data suggests that patients treated with HBO have an improvement at 30th day and after 20 sessions of therapy. These results appear to be independent of type of lesion.

CONCLUSION

This data supports the hypothesis that HBO is a valid treatment of SCI and gives a better outcome to these patients.

PS 309

Effect of long exposure to amyloid-c peptides in GABA release from hippocampal synaptosomes and its relevance for BDNF effects

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AIM

To evaluate how prolonged exposure to amyloid-c (Ac) influences the effect of brain-derived neurotrophic factor (BDNF) upon gamma-aminobutyric acid (GABA) release from rat hippocampal isolated nerve terminals. Whether calpains inhibition could revert an effect of Ac was also evaluated.

INTRODUCTION

The hippocampus is one of the most vulnerable brain regions in Alzheimer's disease (AD). AD is characterized by the accumulation of Ac peptide, but there is also a GABAergic dysfunction and a decrease on the levels of the neurotrophin, BDNF, in the brain of these patients. Recent work from the host lab demonstrated that Ac decreases the levels of the BDNF TrkB receptors in a mechanism dependent on calpains activation. Given that BDNF is able to inhibit GABA release from synaptosomes, we now aimed to know how prolonged exposure to Ac influences the effect of BDNF upon GABA release from rat hippocampal isolated nerve terminals and whether inhibition of calpains could reversean effect of Ac.

METHODS

Rat hippocampal slices were incubated for 3h with or without Ac (25cM), and when evaluating the effect of calpain inhibition, hippocampal slices were simultaneously incubated with both Ac and MDL28170 (20 μM), a calpain inhibitor. The synaptosomal fraction was obtained and incubated with [3H]GABA. Synaptosomes were layered over GF/C filters and superfused with artificial cerebrospinal fluid. At the 5th (S1) and 29th (S2) minutes, synaptosomes were stimulated with K+ (15mM). When testing for the effect of BDNF (30ng/ml), it was applied before the 2nd stimulation period. The eluent was collected for liquid scintillation counting, as were the filters at the end of each experiment. BDNF effects were calculated as changes in S2/S1 ratios under control conditions, Ac presence or Ac and MDL28170 presence. Ac effect in GABA release was calculated as changes in S2/S1 ratio as compared with controls in the same experiment. Data was analyzed as mean ± SEM for n experiments. The significance of the differences was calculated with one way ANOVA followed by Bonferroni correction for multiple comparisons. P-values inferior to 0.05 were considered statistically significant.

RESULTS

Ac treatment did not affect GABA release ($p>0.05$, $n=7$); however it abolished the BDNF inhibitory effect ($p<0.05$, $n=7$) , which suggests a functional modulation of BDNF effects by Ac in hippocampal slices. Inhibition of calpains (with MDL 28170) allows the recovery of the inhibitory effect BDNF, despite the presence of Ac ($p<0.05$, $n=4$).

CONCLUSION

These results strongly suggest that Ac impairs TrkB mediated actions in the hippocampus through a mechanism that involves calpain activitation.

PS 342

Experimental diabetes induced a decrease in the number of NPY neurons in the hippocampal formation of the Rat.

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AIM

The aim of this study is to evaluate the effects of diabetes induced by streptozocin (STZ) in the number of NPY interneurons of the hippocampal formation of young rats.

INTRODUCTION

Diabetes type 1 is a common disease that is frequently initiated in youth. Most of the complications of diabetes are related to the increase of the oxidative stress in the central nervous system which is very vulnerable to this type of aggression. The hippocampal formation is a limbic structure related to cognition and appears to be particularly vulnerable in diabetic. In fact, these subjects appear to have a higher risk of stroke, dementia, and cognitive decline. In this region of the limbic system, the hippocampal neurogenesis and the number of hilar neurons seems to be deficient in several experimental models of diabetes. In this study, we will evaluate the number of NPY-immunoreactive interneurons of the hippocampal formation. These neurons seem to be important in a variety of functions: affecting local blood flow, inhibiting glutamate release and have a role in long-term potentiation and seizures. Due to its importance, the NPY immunoreactive neurons of the hilus will be estimated in separate.

METHODS

Twelve-week old male Wistar rats (250-300 g) were divided into two groups: one control group of non-diabetic rats (N=4) and another group injected intraperitoneally with a STZ solution (N=4) to induce diabetes. The rats were killed 10 weeks after the STZ injection. After transcardiac perfusion NPY were identified by immunocytochemistry using a polyclonal antibody and the ABC technique in sections obtained by a vibratome. The areal density (number per unit area) was obtained in digital images and analyzed with the Image J software (National Institute of Mental Health). Means were compared using the Mann-Whitney U test.

RESULTS

Diabetes was confirmed by hyperglicemia three days after the injection of STZ. The rats treated with STZ presented a reduction of the number of NPY-immunoreactive neurons per unit area in the hippocampal formation and particularly in the hilus of the dentate gyrus.

CONCLUSION

The neurons that express NPY in the hippocampal formation are sensible to the metabolic alterations of diabetes explaining partially the cognitive deficits described in diabetic rodents. As these effects are associated with the increased oxidative stress in the brain it will be interesting to evaluate the action of several antioxidants in the prevention of the numerical reduction of these interneurons.

PS 275

LTP paradoxical increase upon ageing is due to NMDA receptor overactivation

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AIM

To study the mechanisms underlying the paradoxical Long-Term Potentiation (LTP) increase upon ageing.

INTRODUCTION

LTP is a form of synaptic plasticity being an electrophysiological correlate of the memory and learning processes. Previous studies, held at our laboratory, described an increase in LTP on aged subjects, which is paradoxical having in account that there is memory-performance deterioration upon ageing. Given that the elevation of postsynaptic calcium, essential for the induction of LTP in the CA1 area of the hippocampus, is mainly achieved via N-methyl-D-aspartate (NMDA) glutamatergic receptors, we investigated whether there was an overactivation of these receptors upon ageing, by blocking them with their uncompetitive antagonist: memantine (a drug widely used in the symptomatic treatment of Alzheimer's disease).

METHODS

The experiments were performed in hippocampal slices taken from male Wistar rats from three groups: young-adult, old-adult, and aged rats. Field excitatory postsynaptic potentials (fEPSP) were recorded extracellularly at the stratum radiatum of the CA1 area. Stimulation was delivered to the Shaffer collateral fibers. LTP was induced by a weak c-burst protocol. The effect of memantine was evaluated by comparing the LTP magnitude in slices pre-incubated with memantine, with control slices, in the three groups of rats.

RESULTS

As previously seen, we recorded an increase on LTP magnitude upon ageing. In the young-adult rat group we registered a $20 \pm 5\%$ (n=8) increase of fEPSP on the 46-60 minutes following LTP induction, whereas in the old-adult and aged rats groups we recorded a 43 ± 8 (n=4) and $68 \pm 10\%$ (n=5) increase, respectively.

The large LTP magnitude observed in hippocampal slices from old-adult and aged rats was significantly ($p<0.05$, students t-test) reduced by memantine 1 cM (to $20 \pm 4\%$, (n=5) and $29 \pm 4\%$, (n=7), respectively). On the contrary, on slices taken from young adult rats, memantine did not significantly change the LTP magnitude (n=7).

CONCLUSION

The results show that memantine decreases the augmented LTP found in elder rat hippocampal slices, whereas it does not change the LTP magnitude seen in hippocampal slices taken from younger rats, meaning that there is a NMDA receptor overactivation in "healthy" ageing and that this accounts majorly for the paradoxical LTP increase upon ageing.

Based on the fact that memantine preferentially blocks extrasynaptic - rather than synaptic - currents mediated by NMDA receptors in the same neuron and knowing that memantine decreases LTP magnitude to a further degree in aged rats, we hypothesized that there is an increase of extrasynaptic NMDA receptors activity upon ageing.

PS 192

Overexpression of adenosine A2A receptors in rats: effects on depression, locomotion and anxiety-like behavior

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AIM

To determine what the impact of adenosine A2AR receptor overexpression in rats on dopamine-related behavior, particularly depression, locomotion and anxiety-like behavior.

INTRODUCTION

Adenosine A2A receptors (A2AR) are activated by the neuromodulator adenosine, which influences the function of several neurotransmitters. The interaction with dopamine is one of the most intensively studied (1). Since there is uncertainty about the nature of this interaction (2, 3, 4) we aimed to determine what is the effect of A2AR overexpression on behaviors in which dopamine is involved, particularly depression, locomotion and anxiety-like behavior.

METHODS

Twelve 9 weeks-old male Sprague-Dawley rats overexpressing human A2AR in hippocampus, cortex and striatum under the control of CAMKII promoter (CAMKII-hA2AR) and twelve wild-types (WT) of the same strain and age were studied. The forced swimming test (FST), the sucrose preference test (SPT), the open-field test (OFT) and the elevated plus maze test (EPM) were performed to evaluate behavioral despair, anhedonia, locomotion and anxiety, respectively.

RESULTS

In the FST, CAMKII-hA2AR animals spent more time floating (Mann-Whitney test - medians [IQR] - WT: 2.6 [1.8-2.9] min, n=12; CAMKII-hA2AR: 3.1 [2.8-3.4] min, n=12; $p<0.05$) and less time swimming (Mann-Whitney test - medians [IQR] - WT: 1.8 [1.3-2.3] min, n=12; CAMKII-hA2AR: 1.4 [1.1-1.6] min, n=12; $p<0.05$). In the SPT, CAMKII-hA2AR rats presented a decreased sucrose preference at 44h (Mann-Whitney test - medians [IQR] - WT: 93 [91-94] %, n=8; CAMKII-hA2AR: 85 [4.3-91] %, n=7; $p<0.05$). They also covered higher distances in the OFT (unpaired t test with Welch's correction - mean±s.e.m. - WT: 2956±160 cm, n=12; CAMKII-hA2aAR: 3644±64 cm, n=12; $p<0.01$) and spent more time in the open arms of the EPM (Mann-Whitney test - medians [IQR] - WT: 2.4 [0-9.7] %, n=12; CAMKII-hA2AR: 10.35. [4.1-25.1] %, n=12; $p<0.05$) than the WT.

CONCLUSION

We conclude that CAMKII-hA2AR rats display depression-like behavior, increased locomotor activity and decreased anxiety, thus suggesting a direct modulation of dopaminergic pathways, which needs further detailing. These behavioral outcomes are different, even contradictory, from the ones resulting from administration of A2AR agonists, which suggest that chronic activation of A2AR regulates neuronal activity in a distinct manner that the acute one.

PS 358

Effects of chronic ethanol intake and withdrawal on the dorsal parvocellular division of the paraventricular nucleus

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AIM

This study aims to evaluate possible neurochemical changes due to long-term ethanol treatment and withdrawal in the dorsal parvocellular division of the hypothalamic paraventricular nucleus (PVNdp) of male and female rats.

INTRODUCTION

The medical consequences of alcoholism generally develop much more rapidly in women than in men. Women seem to be more vulnerable to brain damage and to the neurotoxic effects of alcohol than men. Likewise, there is evidence that chronic alcohol intake alters the activity of the hypothalamic-pituitary-adrenal (HPA) axis in a gender-specific way. Because there is clinical evidence of dissociation between adrenocorticotrophic hormone (ACTH) and glucocorticoid production, particularly in disease, we have addressed the possibility that the sex-related differences in the activity of the HPA axis observed during chronic ethanol consumption (CEC) and withdrawal (W) might also result from male and female differences in the autonomic regulation of the adrenal cortex. We will focus on the PVNdp because its neurons massively contribute for the autonomic-related descending projections that govern the adrenal cortex physiology.

METHODS

Male and female Wistar rats were maintained throughout the experiment under standard laboratory conditions. Solid diet and water were available ad libitum until rats were 2-month-old. At this age, rats were assigned to one of the next groups: Ethanol-treated: rats were given a 20% (v/v) ethanol solution as their only available liquid source for 6 months. Withdrawal: rats were treated with ethanol over 6 months and then smoothly switched to tap water for a further 2 months. Control: rats had free access to tap water. Food was freely available to all groups all over the experiment. Groups were composed of 6 males and 6 virgin females pooled at random stages of the estrous cycle. After perfusion, the right and left hypothalami were isolated and processed for immunocytochemistry. The total number of immunoreactive neurons for vasopressin (VP) in the PVNdp was estimated using unbiased stereological methods. Data were analyzed by using a 2-way analysis of variance with treatment and sex as the independent variables. Whenever significant results were found, Tukey HSD post hoc test was performed.

RESULTS

In males, there were no ethanol or withdrawal effects in the total number of VP neurons. However, in females the number of VP neurons was significantly reduced in ethanol-treated rats and withdrawal further aggravated this effect.

CONCLUSION

These results clearly show that effects of CEC and W on the neurochemistry of the PVNdp differ between male and female rats. In addition, they suggest that male-female differences in the autonomic innervation of adrenal cortex might also contribute to explain the deficient corticosterone production observed in females, but not in males during withdrawal from prolonged ethanol intake.

PS 128

Prevertebral vascular anatomy of the lumbar spine region in preoperative planning of anterior approach spine surgery

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AIM

The aim of our study was to assess anatomical variations of large vessels and their branches at the anterior surface of the lumbar spine for the purpose of planning a secure anterior approach surgery. We aimed to determine and quantify directly anatomical details which may limit an access to the lumbar spine during operation.

INTRODUCTION

In the surgical treatment of lumbar spine pathologies most approaches are posterior. However, in some situations like spine trauma or degenerative disc disease, the anterior approach may be necessary to perform an anterior lumbar interbody fusion.

The anterior approach is connected with vascular surgery elements due to the need of identification and manipulation of main vessels trunks running at the anterior surface of the spine.

METHODS

A group of 90 patients was analyzed retrospectively. Our exclusion criteria were: age above 75 years and presence of aorta aneurysms. The course of vessels in the L1 - S1 levels was assessed on the basis of computed tomography angiograms with 2D Multi-planar and Volume Rendered 3D reconstructions. Each reconstruction was evaluated and measured twice. We measured the diameters of vessels and their branches. Location, diversity of the levels of the vascular trunks' origins and variation of main arteries and veins' divisions were assessed. The differences in the position of the aortic bifurcation, its asymmetry and the position of the iliocava junction were investigated. We also assessed the width of the vascular windows defined as the 'free from vessels' area measured at the level of L5/S1 disc.

RESULTS

The median sacral artery was present in 72% of patients. The aortic bifurcation was located at the level of lower part of L4 vertebral body and fourth lumbar intervertebral disc in 65 % of cases. In 18,2 % of patients the course of aorta was significantly asymmetric.

The distance between the aortic bifurcation and the L5-S1 disc was assessed as between 8 and 34 mm. The iliocava junction was located at the level of L5-S1 disc in 10% and at the level of L4-L5 disc in 77,3% of cases. The localization can limit an access to the lumbar spine. The width of the vascular windows measured at the level of L5/S1 disc averaged 32,7 mm $\pm$ 12 mm.

CONCLUSION

Preoperative analysis of vascular imaging examinations allows for a precise qualification of patients for the anterior approach to the spine. There is a variation of the symmetry of aorta course, the level of aorta bifurcation and the level of iliocava junction in relation to the lumbar spine.

The anterior approach is often connected with manipulation of large vessels because the procedure should be safe for patients. Mobilisation of veins in operation field is usually more problematic and anatomical details play important role there. To avoid manipulation of inferior vena cava, the approach to the lumbar spine from the left after gentle mobilisation of aorta appears to be most secure for patients. A collaboration between vascular surgeons and neurosurgeons in the anterior approach operations is necessary.

PS 378

Age does not affect the total number, but induces hypertrophy of cholinergic neurons in the rat nucleus accumbens

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AIM

The present study was designed to investigate, by using stereological methods, whether there are age-related changes in the total number and somatic size of cholinergic neurons in the nucleus acuumbens (NAc) and, in the affirmative, if those changes can be reversed by nerve growth factor (NGF).

INTRODUCTION

The rat NAc is located in the rostroventral part of the striatum and is regarded as a functional interface between limbic and motor systems. About 90% of its neurons are densely spiny projection neurons, whereas the remaining are aspiny interneurons that produce either gamma-aminobutyric acid or acetylcholine. The cholinergic interneurons, which represent no more than 1.7% of the neuronal population of the striatum, are the only source of NAc cholinergic innervation. Despite the relatively small number of cholinergic neurons, the striatum possesses one of the highest contents of acetylcholine in the brain. Acetylcholine has been implicated in modulating functions that deteriorate with aging, such as the sleep-wake cycle, learning and memory. In particular, striatal cholinergic neurotransmission has been shown to be involved in age-associated cognitive impairment.

METHODS

The studies were carried out in a total of 15 male Wistar rats, 5 young (6 months) and 10 old (24 months). Half of the old rats were randomly allotted to NGF-treatment (n = 5). NGF-treated rats were intraventricularly infused with NGF during 12 days prior to sacrifice. At the end of the experimental period, animals were anesthetized and perfused. The brains were removed, coded and serially sectioned in the coronal plane. Sections used for cytochemical visualization of cholinergic neurons were selected at regular intervals of 160 cm (1 out of 4 sections) along the entire rostrocaudal extent of the NAc. The optical fractionator and the optical rotator were used to estimate the total number and somatic size of NAc cholinergic neurons, respectively.

RESULTS

The NAc of adult male rats contains, on average, 4426 cholinergic neurons and this number is altered neither by aging nor by NGF. Yet, the somatic size of NAc neurons is larger in old than in adult rats and significantly increases in response to NGF administration.

CONCLUSION

In contrast to what has been reported for other cholinergic populations, we found no differences in the number of cholinergic neurons in the NAc of aged rats. These results support the view that there is no widespread neuronal loss during aging and that the age-related loss of cholinergic neurons is selective, revealing the absence of an universal pattern across the entire brain cholinergic system. Concerning the somatic volume, we found that neuronal size is increased in old rats and that the infusion of NGF to old rats leads to an additional neuronal hypertrophy. Thus, our findings raise the possibility that, in the aged brain, degeneration of noncholinergic innervation of the NAc (e.g., the dopaminergic input) may induce hypertrophy of the NAc cholinergic neurons.

Book of Abstracts

Internal Medicine

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PS 82

Effects of Black Grape seed extract pre-treatment on tissue injury and oxidative stress after Acute Kidney Injury

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AIM

the aim of this study was to evaluate the effectiveness of Blackgrape seed Extract pre-treatment on tissue injury and oxidative stress after Acute Kidney Injury induced by Ischemia-Reperfusion.

INTRODUCTION

Renal Ischemia-reperfusion (I/R) Injury is characterized by two phases of sudden lack of blood perfusion to the Kidney resulting in Ischemia followed by a reperfusion phase which brings Reactive Oxygen Species (ROS) to the kidney tissue. I/R is a major cause for pre-renal (most common) type of Acute Kidney Injury (AKI) which means"inability of kidney to function properly in matter of hours to days."AKI complicates 5-7% of hospital admissions and its treatment is subject to a lot of debate. Pathophysiology of I/R is mostly due to inflammation and oxidative stress which damage renal tubules and glomeruli. Black Grape Seed Extract (GSE) is a known herbal drug used in traditional medicine and possesses a high degree of Radical Scavenging and anti-inflammatory potential for its Proanthocyanidine agent.

METHODS

In this Experimental study, 40 male Sparague-Dawly rats were assigned to 4 groups of Control, Sham, AKI and GSE. A week prior to a 30 minute surgical clamping of bilateral kidney vessels in AKI and GSE groups, the latter group Received GSE 50 mg/kg/day through gavage while the other groups received amount matched normal saline solution. After 24 hours of Reperfusion the right kidney was first removed and after snap freeze in liquid nitrogen, it was moved to the -70 °C freezer in order to measure its oxidative stress parameters (Malondialedehyde or MDA and Ferric Reducing/Antioxidant Power or FRAP). Then the left kidney was fixed in 10% formaldehyde to be studied histologically after being stained in hematoxylin-eosin. Results were analyzed using one-way ANOVA tests of Duncan and LSD for MDA and FRAP and non-parametric Kroskal-Wallis and Mann-Whitney tests for total histopathological score.$p<0.05$ was considered significant.

RESULTS

MDA level was significantly ($p<0.001$) lower in GSE group comparing with AKI(2.24 ± 0.86 vs. 3.77 ± 0.54) although it was still significantly higher than sham group while FRAP level was significantly($p<0.001$) higher in GSE comparing with AKI (1.21 ± 0.69 vs. 0.45 ± 0.15)and it reached the sham group ($p=0.534$) Total histopathlogical score in the GSE group was 18 which had significantly been decreased comparing with AKI(score 43) group ($P<0.001$).

CONCLUSION

Oral GSE pre-treatment can effectively decrease the oxidative stress in renal tissue following AKI. less oxidative stress was accompanied by decreased tissue injury which shows GSE can decrease the damage done by AKI via radical scavenging qualities of Proanthocyanidine. Renal functions should be checked for better judgment.

PS 103

The susceptibility to antifungal drugs of yeasts from Genus Candida isolated from human stool samples

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AIM

The aim of this study is to test Candida susceptibility to antimycotics, to determine most commonly found resistance markers and to compare the susceptibility of Candida albicans and non-albicans species (Candida spp).

INTRODUCTION

Fungi from the Genus Candida are opportunistic microorganisms which cause severe endogenous infections in immunocompromised patients. The resistance to antifungal drugs has increased in the past few decades.

METHODS

The experiment was performed at the Institute for Public Health of Vojvodina. It included 200 Candida isolates, originating from the human stool. Standard microbiological methods were used for cultivation and identification of yeasts and for differentiation of Candida albicans from other species from Genus Candida. In vitro susceptibility of all isolates to five antifungal agents was established by using commercial ATB FUNGUS 3 (bioMérieux, France). The results were interpreted by reading minimal inhibitory concentrations (MIC) for 5-flucytosine, amphotericin B, fluconazole, itraconazole and voriconazole. ?_ test was used for statistical processing.

RESULTS

Out of total of 200 isolates from genus Candida, resistance markers were found in 43% isolates. Resistance markers were found to 5-flucytosine (1% of isolates), fluconazole (5.5% of isolates), itraconazole (22% of isolates) and voriconazole (2.5% of isolates). Resistance to amphotericin B was not found. Azole-cross resistance was found in 2.5% isolates. MIC to all 3 azoles was increased at the same time in 20.5% of sensitive isolates. Multiresistance was found in 3.5% of the strains. The frequency of resistance markers was statistically higher in Candida spp. compared to Candida albicans. Out of total of 110 Candida albicans isolates, resistance markers were found in 3.64% isolates. Resistance to itraconazole was found in3.64% of Candida albicans. Out of total of 90 Candida spp. isolates, resistance markers were found in 43% of isolates. Resistance markers were found to 5- flucytosine (2.2% of Candida spp. isolates), fluconazole (11% of isolates), itraconazole (42% of isolates) and voriconazole (6% of isolates).

CONCLUSION

The presence of antimicrobial resistance and increased minimal inhibitory concentrations in Genus Candida show the importance of systematic monitoring of susceptibility to antifungal drugs of these yeasts.

PS 115

Physiotherapy interventions in Cystic Fibrosis: Physiotherapy Evidence Database

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AIM

To investigate the effectiveness of Physiotherapy treatment in patients with Cystic Fibrosis and contribute to the systematization of knowledge of this subject in order to promote a clinical practice according to scientific evidence.

INTRODUCTION

Cystic Fibrosis is an autosomal recessive condition (1) and multisystem, that by increasing the production of secretions, affects mainly lung and pancreas (2).

Despite discovery of the Cystic Fibrosis gene in 1989 (3), no curative treatment has yet been developed.

However, specialized medical care, pharmacological treatments, as well as other therapies, including physiotherapy, improve the quality of life of patients with Cystic Fibrosis (2).

To overcome the major problem of sputum retention, a variety of airway clearance techniques have been developed (15), all of which have a clear goal: to reduce disease progression by augmenting the normal mucociliary clearance mechanisms and to facilitate expectoration (16).

Modern physiotherapy in Cystic Fibrosis is a combination of inhalation therapy, airway clearance techniques, physical education/exercise and ongoing education about the disease and its treatment. According International Physiotherapy Group for Cystic Fibrosis (18), the role of the physiotherapist is, in co-operation with the patient and family, to tailor an individualized, reasonable, effective and efficient physiotherapy regimen. This should take into account all relevant physical and psychosocial factors.

METHODS

Systematic review in databases on PEDro, Pubmed/Medline and B-on to identify randomized controlled trials that evaluates various Physiotherapy interventions in Cystic Fibrosis, published between 2000 and 2010.

The research was conducted with the keywords Cystic Fibrosis, Physiotherapy and Randomized controlled trials, using logical operators (AND, OR).

To evaluate the methodological quality, we used the Physiotherapy Evidence Database scoring scale (PEDro) (19).

RESULTS

This review included 22 studies involving 1049 patients, with arithmetic mean methodology classification of 4.7 on the Physiotherapy Evidence Database scale. From the studies included in this review, 9 take into account the non-invasive positive pressure ventilation, 8 refer the use of ventilatory devices, 15 evaluated

the Physiotherapy with manual techniques application, and 4 evaluate the Physiotherapy through physical activity.

CONCLUSION

The evidence found in this systematic review suggest that Physiotherapy plays an fundamental role in the treatment of Cystic Fibrosis, namely through the use of manual techniques, ventilatory devices and with the aid of physical activity.

It should also be noted that we cannot say that one modality of physiotherapy is better than another, although no treatment modality not been proven more effective than another, does not mean that one form of physiotherapy intervention is not superior to another in a specific patient. In fact, the patient must have an active role to make physiotherapy more effective by collaborating with the physiotherapist during the treatment sessions. However, it is important to consider that motivation is the key to compliance with any procedure.

Limitations of this study relate to the fact that most investigations have a short duration and doesn´t check the long-term effects, as well as the methodological quality is reasonable (4.7 by 10 in the PEDro scale).

From the research carried, we suggest the relevance of new research about Physiotherapy treatments with long-term effects.

To improve the methodological quality of these studies, we propose that the patients allocation must be done by sealed opaque envelopes, the results cannot be evaluated by self-reported measures, as well as an analysis of "intention to treat" should be performed .

It is extremely important that new studies are conducted in order to demonstrate ever more and with better quality, the effectiveness of physiotherapy in this pathology, contributing to the systematization of knowledge of this subject to promote a clinical practice according to scientific evidence.

PS 204

Indications and Prevalence of Disorders in a Referral Clinic for Endoscopic Ultrasonography

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AIM

To report the indications and prevalence of lesions visualized by EUS, in a EUS center.

INTRODUCTION

Endoscopic ultrasonography (EUS) has a pivotal role in staging and diagnosis of different gastrointestinal (GI) disorders. Although this equipment is not readily available and not all gastroenterologists are trained in this technique, the importance and efficacy of this imaging modality is obvious for all gastroenterologists. We report the indications and prevalence of lesions visualized by EUS, in a EUS center.

METHODS

We evaluated the demographic characteristics, indications, and recorded EUS diagnosis of patients during a 5 year (2007-2011) period.

RESULTS

During the study period, 3198 patients (51.2% males) underwent EUS. Upper GI endosonography, within which, pancreatobiliary disorders followed by gastric cancer were the most common indications for patient referral. The rate of patient referral for EUS increased yearly.

CONCLUSION

EUS is an accurate method for the diagnosis of different GI abnormalities. The most common cause for patient referrals are benign pancreatobiliary disorders.

PS 224

Study of atopic dermatitis and giardiasis occurrence in children

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AIM

The objective of the research: to study the frequency of atopic dermatitis and giardiasis occurrence in children.

INTRODUCTION

Allergy is regarded as a disease of civilization. In the structure of children's allergic diseases atopic dermatitis holds the first place. Also one of the trends of development of pathogenic processes is the increase in infections, among which parasitic diseases are assuming greater importance. Intestinal parasites, which include Giardia, are one of the proven ethiopathogenetic factors affecting the course of dermatoses and leading to frequent recurrences, chronization of dermal processes (N.A. Gerasimova, L.I. Yurovskikh, M.M. Kokhan, 2011), since the products of the vital activity of helminths activate Ig E producing cells. (T.I. Avdiukhina, T.N. Konstantinova, M.N. Prokosheva, 2004).

METHODS

We have examined 173 children (within the period from 2007 to 2011) aged 3-17, who were undergoing treatment for atopic dermatitis in the Somatic Department of the 2nd Children's Clinical Hospital of Simferopol. Verification of diagnoses was carried out on the grounds of complaints, history taking, clinical and biochemical examination, urea breath testing.

RESULTS

Among the total number of the examined children 46 patients (27%) have been diagnosed with giardiasis. Clinical findings in children included not only typical symptoms of allergy but also skin manifestations inherent in giardiasis: paleness, cheilitis and angular cheilitis, hyperkeratosis follicularis punctata, creating the impression of goose flesh and 'grater' with localization on the extensor surfaces of arms, legs. The patients with associated giardiasis complained of the skin itch longer. In the result of long-term persistence of Giardia in the organism, accumulation of metabolic products, particularly due to the substances of protozoan life activity disintegration, the syndrome of chronic endointoxication is formed. Reflex and toxicoallergic action of parasites promotes the emergence of dyskinesia of gallbladder and sphincter apparatus (in 75% of patients) with the subsequent development of an inflammatory process of gastrointestinal tract (in 48% of patients), which increases the antigenic load on immune system. The SCORAD index in 22 children infected with Giardia corresponded to the moderate severity of atopic dermatitis, and in 18 patients severe form was confirmed. Giardia infection made the clinical findings of atopic dermatitis more serious and promoted a later effect of the conducted therapy.

CONCLUSION

The analysis held has shown that among the total number of the patients with atopic dermatitis in 27% of cases a helminthic infection – giardiasis was in evidence. The obtained data create the necessity of a more thorough examination of children for a pathology of gastrointestinal tract, particularly, an examination for helminths, and of a complex approach to the therapy of atopic dermatitis.

PS 136

Mortality and Morbidity in patients with X-linked agammaglobulinemia: a twenty-year study in Iran.

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AIM

To evaluate the Mortality and Morbidity rate and involved factors in survival of patients diagnosed with X-linked agammaglobulinemia (XLA).

INTRODUCTION

X-linked agammaglobulinemia (XLA) is a genetic disorder characterized by a defect in the generation of mature B cells, lack of antibodies, and susceptibility to recurrent bacterial infections. Understanding of the risk factors responsible for morbidity and mortality in these patients can help in a better management of this disorder. However, there is a lack of specific studies in the literature regarding the morbidity and mortality of XLA patients.

METHODS

In this study, we have registered the clinical data of patients diagnosed with XLA and referred to Children's Medical Center,Tehran,Iran, since 1990 and followed them up until 2010. At the time of diagnosis, a four-page questionnaire including complete medical information was filled for all patients. Follow-up information was gathered either by reviewing the patients' hospital records or visiting the patients and/or their guardians.

RESULTS

Among 41 patients, 11 patients (26.8%) have died during follow up period. The most common first manifestation was pneumonia. All of the complications before the initiation of treatment such as pnoumonia, otitis media and diarrhea, were reduced after receiving regular IVIG except sinusitis and conjunctivitis. Two out of 8 patients(25%) who had received polio vaccination, had developed flaccid paresis afterward. There were significant associations between Mortality and some immunological and clinical characteristics such as lymphocyte subsets(P=0.02) and consanguinity marriage(P=0.01).

CONCLUSION

Despite recent advances in the treatment of XLA, these patients still suffer from severe complications that leads to death. Mortality rate in our study was higher than other recent studies. Evaluating the clinical and some immunological characteristics may help physicians to develop the most efficient treatment strategies for patients with higher risk of Mortality and Morbidity.

PS 268

What is the 10-year risk of death from cardiovascular disease in patients with osteoporosis?

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AIM

Evaluation of the 10-year risk of death from CVD in pts with osteoporosis.

INTRODUCTION

Worldwide 200mln people suffer from osteoporosis(15-30% men in age over 50, 30-40% women after menopause). Osteoporosis coexists with coronary artery disease(CAD), hypertension(HA) and other cardiovascular diseases(CVD). Presence of common risk factors and inflammatory markers(CRP, IL6, TNFa) for osteoporosis and CVD suggest a similar pathophysiology.

METHODS

Approximately 50 pts from the Regional Centre of Menopause and Osteoporosis at WAM University Hospital in Lodz aged 50-90, consulted in terms of osteoporosis, divided into 2 groups: osteoporosis and without osteoporosis. The collected data: medical history, BMI, ECG; data from questionnaire: risk factors of osteoporosis, history of stroke, MI, CAD, HA, DM, family history of these diseases. DXA of the spine and/or neck of femur used to evaluate the BMD. TC and RR was measured.

RESULTS

The risk of fatal CVD evaluated with a Polish HeartScore(PHS) scale. Almost everyone (16/17) with osteoporosis have Heart Score equal/higher than 5%, while in pts without osteoporosis it was approximately half of them (9/16).

CONCLUSION

Higher risk of fatal CVD in osteoporosis pts than without osteoporosis, while there is equal number of cardiological diseases (Pearson correlation factor = 0,34). Pts with osteoporosis have "better" cardiological family history, although their risk of fatal CVD is higher acc. to PHS. Lower BMD in pts with osteoporosis correlates with higher risk of MI, CAD, HA, stroke, DM ((p=0,0045). osteoporosis correlates with increased level of TC. This may help identify groups of pts requiring wider evaluation and determination of further cardiologic treatment.

PS 252

The correlation between PPF, skin type and MED values in the population of Lodz.

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AIM

The aim of the project was to analyze PPF values in the population of Lodz and the relationship between PPF, skin phototype and MED.

INTRODUCTION

Commonly accepted is assessment of individual photosensitivity by determination of minimal erythema dose (MED). MED objectively describes a single individual response to the application of radiation of known wavelength (UVB, UVA, SSR). PPF (pigment protection factor) is objectively measured skin type.

METHODS

The research was conducted on the group of 130 volunteers: 58 males, 72 females (mean age 28,5 years old) with either II or III skin phototype defined by the Fitzpatrick Skin Phototype Classification. Phototesting of each volunteer was undertaken with an increasing dose series (UVB radiation) on six squares (1; 1 cm) on the back skin. The MED was defined as a perceptible erythema 24 hours later. Starting dose determined by history, physical examination and phototype ranged from 0,03-0,07 J/cm2. PPF was measured by a skin reflectance meter UV Optimize 555.

RESULTS

Results skin type according to Fitzpatrick was registered. Skin type 2 had 47.7% of participiants, skin type 3: 52.3%.

The data were statistically handled and the median values for MED were 0.15. The analysis showed thed median PPF values were 6.15.

The increase of PPF was positively correlated with MED.

CONCLUSION

Both MED and PPF objectively measure skin photosensitivity and they can be interchanged. The measurement of PPF is much easier and non-invasive.

PS 320

Monitoring RCT using CUSUM Can Save Lives

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AIM

The aim of this study is to test if CUSUM can be useful in the context of randomized controlled drug trial (RCT).

INTRODUCTION

Cumulative sum (CUSUM) is a statistical technique used in industry for quality control. Foresee Software custom made for clinical trials (http://jacob.puliyel.com/foresee/) was used. The raw dataset from the RCT of Estrogen for prostate cancer was reanalyzed.1,2 This trial was done to see which of 3 dose schedules 0.2 mg, 1 mg and 5 mg was beneficial.

METHODS

Using CUSUM we have done the analyses of dataset looking at survival compared to placebo. Death specifically from prostate cancer (failure of treatment) and death from cardiovascular causes (adverse effect of drug) were analysed separately. Sequential analysis was done at 6 time points, each time including all cases and placebos recruited up to that point. For purposes of this analysis we have telescoped time such that outcomes were assumed to follow-on after recruitment, before the next patient was recruited. Control lines were drawn using projections from placebo patients recruited up to that point, assuming the same event rate for the entire sample of placebo recipients. The accuracy of the projections in terms of changes in the control limits was also examined as more placebo data was acquired in subsequent analysis.

RESULTS

We found that 1 mg estrogen reduced all cause death where there little benefit with 0.2 mg or 5 mg. Comparing 1mg with 5 mg estrogen for cardiovascular deaths the 5 mg caused more deaths and the series crossed the lower control line after the 367th case was recruited. If this CUSUM had been in place while the trial was running, the 5mg trial could have been stopped at this point saving lives.

CONCLUSION

Our analyses of overall survival, and deaths from side effects would have permitted the trial of 5 mg oestrogen to be stopped and thus saved deaths from adverse effects. CUSUM is a useful tool to monitor ongoing drug trials. In the case of double blind studies, data monitoring boards with access to allocations of patients can perform the analysis.

PS 344

Predictors of arterial thrombosis in patients with antiphospholipid syndrome

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AIM

The objective of our study is to evaluate the prevalence of arterial thrombosis (AT) in patients with APS (primary or secondary), as well as the best predictors and their impact on the clinical and biological evolution.

INTRODUCTION

The antiphospholipid syndrome (APS) is defined by the presence of antiphospholipid antibodies (aPL) at 2 determinations 12 weeks apart along with arterial or venous thrombosis and/or recurrent spontaneous abortions.

METHODS

In this prospective analysis, we studied 85 patients with SAFL admitted between 2009 – 2011 at the Internal Medicine Department of Colentina Hospital Bucharest. The cases consisted of primary APS, secondary APS to systemic lupus erythematosus (SLE), secondary APS to other conditions. The diagnosis of APS was established according to Sidney criteria 2006. Our patients were tested for the usual aPL used in current practice: lupus anticoagulant, anticardiolipin, anti B2-glycoprotein I, but we also measured the level of IgM, IgG anti-phosphatidyl-serin (APYS) and anti-phosphatidyl-ethanolamine (APE).

RESULTS

In the studied population, 34 patients were diagnosed with primary APS, 34 patients with APS secondary to SLE, 17 patients with APS secondary to other conditions (rheumatoid arthritis, Sjogren's syndrome, Behcet's disease, polyarteritis nodosa) and 21 were controls. Arterial thrombosis was encountered in 26 pt (30.6%) from which 7 with primary APS and 15 with APS secondary to SLE. The mean age for the group with AT was 50.77 years±8.99, significantly higher than the mean age for the group without AT: 41.47 years±11.42, p=0.001. Also, the mean time from diagnosis in AT group 8.11±7.64, was significantly higher than in non-AT group 5.32±6.12, p<0.05. Some traditional risk factors for AT were found: dyslipidemia (OR=4.58; p=0.003), arterial hypertension (OR=4.09, p=0.004), waist/hip ratio >0.86/<0.86 (OR=3.06, p=0.02), mean value for HDL cholesterol was lower in AT group: 54.37±13.80 than 60.47±12.64, p=0.04, but also APE IgM antibodies (OR=3.42, p<0.05) and the mean value for APYS IgM antibodies (7,20±7,42 than 4,63±5,02, p<0,05) were significantly higher in AT group. Regarding the subgroup with primary APS, the correlations were the same as in the group with APS, in addition systolic arterial blood pressure was significantly higher in AT group (p=0.03), and the mean value for anti cardiolipin IgG antibodies (p=0.04). 69% of those with AT (18 out of 26) suffered a cerebrovascular accident (CVA). In addition with risk factors discovered above was also found the use of oral cortisone OR=3.34, p=0.03.

CONCLUSION

As expected, the analysis showed a higher incidence of AT in the subgroup with APS secondary to SLE confirming the literature data. The main manifestation of AT was CVA. The particularity of our study is that not the classical antibodies used for the diagnosis of SLA were found in the AT group but IgM anti-phosphatidyl-serin and anti-phosphatidyl-ethanolamine antibodies. This issue is relevant in clinical practice taking into account that the trend of the CI suggests that the association power will increase for a larger studied group and rise the question whether APYS and APE can be considered solely as a screening test for AT in APS patients.

PS 317

Prevalence of sleep disorders in first months after STEMI in patients with diabetes mellitus

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AIM

To assess the prevalence of sleep disorders in first months after ST-elevation myocardial infarction (STEMI) in patients with type 2 diabetes mellitus.

INTRODUCTION

Abnormal sleep duration is associated with many adverse effects on patients' health. Patients sleeping too much or too little are more likely to suffer from hypertension, coronary heart disease, stroke and obesity than the general population. Also diabetes mellitus, a highly prevalent disease, contributes negatively to patient's general risk profile by easing the development of ischaemic heart disease, stroke, chronic liver disease, nephropathy, peripheral neuropathy, dementia, and cancer. There is a need to establish whether presence of diabetes mellitus is associated with sleep disturbance and if their coexistence affects patients' long-term prognosis, including all-cause mortality.

METHODS

We enrolled in the study 379 consecutive patients hospitalised in a tertiary, high-volume cardiology department with a confirmed diagnosis of STEMI within 12 hours from the onset of symptoms. Presence of diabetes was established basing on confirmed diagnosis made previously by a qualified physician, taking insulin, oral hypoglycaemic agents or de novo diagnosis during hospitalization due to STEMI. Clinical data were retrieved by physical examination and medical record analysis. All patients were contacted via telephone and asked to give the habitual night sleep time in 3 months following discharge: How many hours do you sleep usually each night? Response: |_|_|. Basing on the answers, patients were divided into three groups: I - sleeping too little (<6 hours per night), II - sleeping normally (6-8 hours per night) and III - sleeping too much (>8 hours per night). Night sleep time over 8 and bellow 6 hours was considered sleep disorder.

RESULTS

The analysis covered 271 (71.5%) men and 108 (29.5%) women, aged 36 to 79 (mean age 59.38 ± 10.61). 317 (83.6%) patients slept within 6 - 8 hours range, while sleep disturbances were present in 62 (16.4%) patients. 36 (9.5%) patients slept less than 6 hours and 26 (6.9%) patients slept more than 8 hours per night. The Kaplan-Meier survival analysis showed 3-year mortality rate of 21% in patients with sleep disorders vs. 1.9% in patients with normal sleep time (HR 14.3; 95%CI 3.97-51.5; p<0.0001). Whole study population was divided into patients with (48 patients; 12.7%) and without diabetes (331 patients; 87.3%). Patients suffering from diabetes had higher body mass index (30.7 ± 5.0 vs. 27.0 ± 3.9; p<0.001), higher heart rate (80.4 ± 15.0 vs. 75.8 ± 15.5; p=0.05), and more often had hypertension (77.1% vs. 47.7%; p=0.0003) and dyslipidemia (45.8% vs. 22.4%; p=0.0009) compared to the patients without diabetes. Patients with diabetes had also more often sleep disturbances (27.1% vs. 14.8%; p=0.05).

CONCLUSION

Sleep disorders are highly prevalent in patients who underwent STEMI. Occurrence of sleep disorders is associated with poorer prognosis, including all-cause 3-year mortality. Patients with diabetes more often had sleep disorders compared to patients without.

PS 366

Development of resistance of Aspergillus fumigatus to clinical treatment- a side effect of agricultural antifungals?

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AIM

The purpose of this study is to unveil wether antifungals used in agriculture can cause resistance among clinical relevant pathogens like Aspergillus fumigatus.

INTRODUCTION

Aspergillus fumigatus is a saprotrophic mould that causes considerable losses in agriculture crop yields and it is known as an important opportunistic human pathogen among immunocompromised individuals, leading to a potentially lethal invasive infection. The continued use of fungicides became essential for effective agriculture disease control. Antifungals of classes similar to those used in human therapy started to be used massively, and resistance to agricultural antifungals also became problematic.

METHODS

Antifungal susceptibility testing of several isolates of Aspergillus fumigatus was performed according to the CLSI M38-A protocol. Afterwards, in vitro induction assays with susceptible Aspergillus fumigatus strains to both agricultural and clinical antifungals were performed with Prochloraz (PCZ), the most widely used antifungal in agriculture. Briefly, conidial suspensions were daily incubated at 35°C, 150 rpm with sub – inhibitory concentrations of PCZ. Minimal inhibitory concentrations (MIC) to PCZ were evaluated, every ten days. In order to assess the potential development of cross-resistance with clinical antifungals, whenever a two fold increase of PCZ MIC values was obtained, the MIC values of voriconazole (VOR) and posaconazole (POS) was determined. Along the induction process, microscopic and macroscopic observations were registered.

RESULTS

PCZ exposure lead to morphological colony changes: macroscopically the colonies turned white losing its pigmentation; microscopic examination revealed the absence of conidia. The MIC of PCZ increased, and cross-resistance to clinical azoles was observed.

CONCLUSION

The exposure of clinical relevant moulds to agricultural azoles is associated with the emergence of cross-resistance to clinical antifungals, which are crucial in hospital settings. PCZ exposure induced Aspergillus fumigatus morphological changes and it was evident increased MIC of PCZ as well as of clinical azoles. This change in the phenotype and in the resistance profile to antifungal agricultural may be correlated to a genotypic mutation that influences such phenotypic characteristics, a topic that will be addressed soon.

PS 351

Agricultural antifungal exposure results in cross resistance to clinical antifungals among in Candida spp – guilty or not guilty

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AIM

To expose clinical isolates of Candida albicans, C. glabrata and C. parapsilosis to prochloraz (PCZ), an agricultural antifungal, and to study its influence in the susceptibility profile to clinical antifungals.

INTRODUCTION

Resistance to antifungal drugs has increased during recent years. This resistance might be related to the hight amount of antifungals used in agriculture. Among antifungals used for crop protection, the azoles are one of the most used in European Union in agriculture but also in humans. Cross-resistance between azoles has already been described. The exposure to antifungals of classes similar to those used in the clinical setting may therefore result in a significant but yet undetermined impact in human health.

METHODS

We determined the Minimal Inhibitory Concentration (MIC) of PCZ for the species mentioned above, according to the Clinical Laboratory Standards Institute (CLSI) M27-A3 protocol. A single, randomly selected colony from a fresh 24h culture in Sabouraud agar medium was then incubated with sub-inhibitory concentrations of PCZ; the cultures were incubated at 35°C, 150 rpm. Every 24h, the culture was refreshed. At each sub-culture, a growth aliquot was stored at -70°C in 40% glycerol. MIC of PCZ was re-determined according to CLSI protocols once a week.

RESULTS

At day 40, strains became less susceptible to PCZ (MICs were at least four times higher) and resistant to fluconazole; C. albicans and C. glabrata also became resistant to caspofungin. MICs for posaconazole and voriconazole increased for all three species but not enough to reach a resistant profile.

CONCLUSION

Strains exposed to prochloraz became less susceptible to this drug. This exposure also caused cross-resistance with fluconazole, one of the most used azoles in the clinical setting, and with caspofungin, an antifungal of a different class.

These results show that continuous exposure to agricultural antifungal drugs can cause cross-resistance between agricultural and clinical antifungal drugs. As exposed environmental strains are often responsible for clinically relevant fungal infections, especially among immunocompromised patients, this information helps to explain why resistance to antifungal drugs has increased during the past decades and emphasizes the importance of new antifungal drugs, especially with different mechanisms of action.

As next steps, we will characterize at a molecular level the evolution of the development of cross-resistance to other drugs. Consequently, the acquired resistance mechanisms will be clarified.

PS 367

Cryptococcus neoformans: could antifungal resistance emerge in the wild or during therapy?

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AIM

To investigate whether it is possible for the yeast Cryptococcus neoformans to become resistant to clinical azoles through the continuous in vitro exposure to a clinical azole or an agricultural azole.

INTRODUCTION

Cryptococcus neoformans is a ubiquitous yeast , being harmless to most healthy individuals. However, under certain host conditions this yeast becomes pathogenic, being able to infect human hosts. The current treatment guidelines include an induction regimen based upon the combination of amphotericin B and 5-flucytosine, and a long consolidation and maintenance therapy with fluconazole. Other antifungals used to treat other fungal infections, such as echinocandins show a reduced efficacy. However, even if the signs of active infection are successfully eliminated, after stopping the maintenance regimen, recurrence is extremely common, especially with central nervous system infection. Interestingly, the isolates maintain their susceptibility to fluconazole throughout the treatment. It is important to clarify if the antifungal exposure is able to induce resistance. Our other concern is related to the possibility that the use of antifungals in the agricultural setting, very similar in chemical structure to the ones used in human therapy, might induce a cross resistance profile; thus, a patient could acquire a resistant or less susceptible strain.

METHODS

To address these problems we cultured repeatedly two Cryptococcus neoformans isolates in YPD broth with fluconazole (clinical azole) or prochloraz (agricultural azole). The concentrations used correspond to MIC's determined accordingly CLSI M27-A3 protocol, for both antifungals. At the twentieth day of culture, the MIC was again determined for the prochloraz incubated strains and the new MIC was used for the new culturing conditions. After ten days, a subculture was obtained and cultured without any antifungal. MIC's were obtained every 5 days for prochloraz, fluconazole, posaconazole and voriconazole.

RESULTS

The two strains cultured in the presence of fluconazole registered a major rise in the MIC to this antifungal and minor increases of the MIC's of other clinical azoles. The strains incubated with prochloraz registered an increase in the MIC's to this antifungal and also to fluconazole. By the day 40 of induction, one of the strains was already resistant to fluconazole. Interestingly, the strains incubated with prochloraz and then cultured in antifungal-free medium for ten days maintained the increased MIC values.

CONCLUSION

We demonstrated that the continuous exposure to fluconazole or prochloraz can make Cryptococcus neoformans isolates become less susceptible or even resistant to fluconazole. This could bring forth terrible implications for patients in need of treatment for this life-threatening infection. Additional research is being conducted in order to determine the mechanisms behind the decrease in susceptibility and resistance, and possible ways to block such profile.

PS 369

Contact screening in tuberculosis. Can we identify those with higher risk?

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AIM

To identify risk factors associated with a positive screening.

INTRODUCTION

Contact tracing is part of the tuberculosis (TB) elimination strategy. For its optimization, maximizing available resources, it is important to know which risk factors are associated with a positive screening.

METHODS

During 2011, contacts of patients with pulmonary TB (sputum, broncho-alveolar lavage or pleural fluid smear or culture positive), followed for screening in a TB reference centre, were questioned about their exposure to the index case. Positive screening was defined as active TB or latent infection. Contacts with exposure in open spaces, with incomplete characterization of exposure, unfinished screening or a past history of TB were excluded. A binomial logistic model was used to analyze the association between infection and potential risk factors of positive screening.

RESULTS

We observed 509 contacts of which 359 (153 men, median age: 32 years) were included in the analysis. 76 had a positive screening. Positive screening was associated with a positive sputum analysis of the index case (OR=2.62, 95%CI=1.33-5.14) and with coinhabitance (OR=3.42, 95%CI=1.66-7.07). Each additional year in age of the contact implied an increase in the odds for infection of 3% (OR=1.03, 95%CI=1.02-1.05) and each additional day of symptoms by the index case, previous to treatment, implied an increase in the odds for infection among his contacts of 1% (OR=1.01, 95%CI=1.00-1.02). No significant differences were found regarding size and ventilation of the exposure site.

CONCLUSION

This study shows that there is a significant increase in the risk of TB transmission to contacts for every day that the diagnosis of the index case is delayed. Increased risk was also shown for coinhabitants, contacts of older age and the presence of positive sputum smear or culture of the index case.

PS 350

Evolution of HIV infection in the portuguese population: epidemiological study of a Matosinhos Hospital cohort

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AIM

To describe the evolution of the characteristics of HIV positive patients, late diagnosis and associated risk factors.

INTRODUCTION

Portugal has a high prevalence of HIV, and the late diagnosis is a troubling reality.

The numbers of delayed diagnosis remain high. In Europe, the percentage of people with HIV who are diagnosed late varies from 18 to 43%.

The late diagnosis of infection means a high level of immunosuppression and more probability to presentation with opportunistic infections in addition to cause a reduction in life expectancy. Early detection is also reflected by a decrease in transmission by both the preventive action, or by a decrease in viral load result of therapy.

To achieve new progress in reducing mortality HIV-related, the numbers of delayed diagnosis must decrease. For that, it's important the identification of populations at risk.

METHODS

Retrospective, observational and not controlled study with patients evaluated in the Department of Infectious Diseases, Matosinhos Hospital, for HIV infection between January 2000 and October 2010. Were extracted for analysis the following data: gender, birth date, marital status, source of patients, the date of diagnosis of HIV infection, the transmission route, the count of CD4 + at diagnosis of HIV infection, the date of diagnosis of AIDS and AIDS-defining respective condition (if applied).

Delayed diagnosis was defined as presentation for care with a CD4 below 350 cells/µl or with an AIDS-defining event, regardless of the CD4 cell count.

A multivariate logistic regression was made for predictive variables of late diagnosis already defined and included gender, age, mode of transmission and diagnosis by hospitalization.

RESULTS

It was analyzed 445 patient data. During the analysis time, increased sexual transmission of infection and decreased among injecting drug users ($p<0.001$) were observed. Also, the diagnoses increased in individuals over 40 years ($p<0.001$) was also observed. 296 patients were diagnosed (65%) with features of delayed diagnosis. Risk factors associated with delayed diagnosis were: male gender (OR = 2.18, 95% CI, 1.64-3.74, $p = 0.001$), age over 40 years in men (OR = 2.12, 95% CI, 1:10 to 4:07, $p = 0.025$) and diagnosis during

hospitalization (OR = 3.62, 95% CI, 2.26-5.79, p<0.001).

CONCLUSION

Sexual transmission of HIV is increasing, against the injecting drug use. The high number of delayed diagnosis is a problem, especially among older men. It is urgent to increase efforts for the early diagnosis of infection.

PS 161

Anxiolytics use among medical students: a quantitative-qualitative view

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AIM

Objectives of this study were to investigate positive and negative correlations of anxiolytics use in population of medical students, to explore aims of stress relief and potential coping strategies.

INTRODUCTION

Medical students have excessive working obligations and full educational output. Benzodiazepines remain by far the most frequently used anxiolytics, but there are three main concernes about benzodiazepines use: side effects, possibility of physically and psychologically dependence or even addiction, rebound insomnia and withdrawal symptoms. In recent years, more evidences emerge about anxiolytics use by medical students, but insight in this problem is still lacking.

We hypothesize that medical students, due to their medical education workload, are population highly vulnerable and prone to stress and they might be at risk of prolonged anxiolytics use.

METHODS

Methodological design of this study includes quantitative and qualitative part (questionnaires and interviews). Using an anonymous self-reported questionnaire, the study surveyed a total of 323 undergraduate medical students at University of Sarajevo. The qualitative part of research was obtained through semi-structured interviews with 9 students who passed screening question (Use of sedation drugs) and signed agree to participate in the study. All interviews were face to face, and performed by previously agreed Standard of Operating Procedures (SOP). Data were tracked through hard paper copies, transcribed and coded, entered in SPSS software version 15, connected with quantitative sociodemographic data and evaluated statistically.

RESULTS

Positive responses on anxiolytics use were obtained for 12,4% students, more associated with female gender (78%). Mean age of consumers is 21 (SD±2.59). The most frequently used drugs are: benzodiazepines (Bromazepam, Diazepam) 53,8%, herbal medicinal products (Valerian) 35,9%, cannabis 7,7% and cocaine 3,6%. Concomitant use of anxiolytics and psychoactive drugs was found in 12,8% of consumers. 28% of students use drugs over 3 years. Data showed negative pearson correlation between grade and frequency of use (P=0.002), with significant rise of drugs use in 2nd (16,5%) and 4th (22,6%) grade of medical school. Reasons for anxiolytics consumption are: exam stress, stress-related conditions, insomnia, emotional problems and headache. 77,7% of students identified the high level of stress (4 and 5 on the scale 0-5) that makes them use drugs. Students described the effects of the drugs as following: relaxing, decrease of study concentration, sleep-inducing. Participants (100%) use drugs in their own arrangement. Majority (77,7%) think they should stop using the drugs because they are aware of the potential problem (“Benzodiazepines

cause dependence").

CONCLUSION

The study identified long-term benzodiazepines use (years) in contrary to any mentioned or published guidelines (2-4 weeks). Medical education and stress play a role in this phenomena. Students are aware of potential drug related hazard, but they can not help themselves. Data from our study actually rised the question: Do we educate our physicians in training to be doctors or to become patients?

PS 208

Does Sickle Cell Disease protect against Diabetes Mellitus: a cross-sectional study

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AIM

The co-existence of diabetes mellitus and sickle cell disease has been shown to be rare. We set out to establish the prevalence of diabetes mellitus in patients with sickle cell disease in a highly prevalent country.

INTRODUCTION

Bahrain has a high prevalence of both Diabetes Mellitus (DM) and Sickle Cell Disease (SCD); 15.3% and 2.1% respectively, ranking fifth in the world for DM. The co-existence of these two conditions in a population where both are highly prevalent has not been previously studied. This study aims to establish the prevalence of DM in patients with SCD in Bahrain.

METHODS

Cross sectional prevalence study in Bahrain. A random sample of 520 patients with SCD aged 18 years and over was taken from all SCD patients admitted to Salmaniya Medical Complex (SMC) between 2003 to 2010 (n=2204). Manual and computerized medical records were examined for the presence of DM.

RESULTS

376 patients were included with a mean age of 33.5 years (SD±11.2). 24/376 (6.4%) patients with SCD were determined to have DM. 32/376 (8.5%) patients had impaired glucose tolerance. The age/sex standardized prevalence of DM in SCD patients was 8.25%. There was no association between gender of the patients (?2=1.5, df=2, p=0.47) or number of admissions (?2=2.2, df=2, p=0.34) and the prevalence of DM.

CONCLUSION

The prevalence of DM in patients with SCD in Bahrain is high at 8.25% but lower than expected in this population (15.8%). There may be a protective effect of sickle cell disease towards developing diabetes. However, the impact of these two conditions on vascular disease suggests a need for screening and aggressive treatment of vascular risk factors in this population.

Book of Abstracts

Surgery

PS 61

Effect of anti-VEGF injections (Lucentis) on reading speed in wet-AMD patients.

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AIM

The objective of this study is to investigate the effectiveness of a new method, measuring the reading speed in wet-AMD patients treated with anti-VEGF injections.

INTRODUCTION

AMD (age-related macular degeneration), is the leading cause of blindness in individuals over the age of 55. Patients suffering from AMD are losing their reading capability .There is no cure for AMD but anti-VEGF treatments significantly minimize the vision loss over time.

METHODS

Routinely, anti-VEGF treated wet-AMD patients are evaluated by measuring the best corrected distant visual acuity (BCVA) via ETDRS chart and macular thickness via Optical Coherence Tomography (OCT). In addition to a full ophthalmological examination we measured the reading speed via Radner chart on 30 patients before and 3 months after treatment. The collected data were analyzed using a Paired T-test.

RESULTS

After the anti-VEGF treatment, BCVA increased from 59 to 67 letters, retinal thickness decreased from 362 µm to 225 µm. The reading speed (sentences/sec) improved from 16 seconds to 10 seconds per sentence, and the amount of words read per minute increased from 65 words/min to 101 w/min. The results were statistically significant ($p < 0.01$).

CONCLUSION

Significant improvement of reading speed was observed after Lucentis treatment in wet-AMD patients. Radner reading chart is an effective tool to follow the evolution, and the treatment-effect of wet-AMD patients.

PS 69

Femtosecond laser assisted cataract surgery

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AIM

To compare the use of FSL to the gold standard technique (phacoemulsification) in cataract surgery. In this poster I will, give a brief introduction of the ophthalmic uses of FSL, however my main theme will be a critical appraisal of its use in cataract surgery being the most common surgical procedure in the UK and worldwide.

INTRODUCTION

Modern cataract surgery went through various developments over the last 30 years. Starting with intra-capsular and extra-capsular cataract extraction; moving on to the use of phacoemulsification to emulsify the opaque lens via high frequency ultrasound. Phacoemulsification is the current gold standard technique and is generally considered a safe technique. A new alternative was made available with the creation of the femtosecond laser (FSL) which was licenced in the US back in 2000 and was then introduced worldwide over the next ten years. The use of FSL assisted cataract surgery appears to produce more precise and reproducible results with less surgeon related outcomes.

METHODS

Literature search.

RESULTS

The key advantages of FSL compared to phacoemulsification use are: a greater level of accuracy and reproducibility of the technique, less ultrasound energy use avoiding posterior capsular damage and thermal injuries, less corneal endothelial cell loss (advantageous in endothelial dystrophy cases), and easy correction intraoperatively of astigmatism. Comparatively phacoemulsification can produce unpredictable wound texture and capsulorhexis. However the main the disadvantages include cost, longer procedure time, inability to correct big astigmatism, and learning curve of mastering skill.

CONCLUSION

FSL assisted cataract surgery has recognisable advantages and a potential increase in its use in this most common surgical procedure once the cost is down and improvements on the speed and ease of use are made.

PS 97

Vascular Endothelial

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AIM

VEGF neovascularization research

INTRODUCTION

Neovascularization and edema in retinal disorders

Leading causes of blindness in the World

In people aged >50 years: age-related maculardegeneration (AMD)

In people aged <50 years:diabetic retinopathy

Major cause of blindness in both disorders is related to neovascularization and edema

VEGF is implicated in pathologic neovascularization

METHODS

Ocular vision,Fundoscopy,intraocular pressure,indirect and direct ophthalmoscopy

RESULTS

best results were seen after the first injection. Continuous monitoring of patients shown to reduce the effect of treatment after the initial 6 months

CONCLUSION

VEGF is an important target for therapeutic intervention.VEGF receptors inhibitors(Avastin)reduce the pathological neovascularization in patients with Age-Rated Macular Degeneration and diabetic retinopathy and delay its progression.

PS 117

Corneal thickness after phacoemulsification. Torsional technique proves to be less damaging for the cornea, compared to longitud

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AIM

To compare the longitudinal and torsional phacoemulsification technique and their influence on the cornea.

INTRODUCTION

Phacoemulsification has become a preferred method of cataract extraction over the years. The technique uses an ultrasonically driven tip to fragment the nucleus of the cataract and aspirate the lens. The tip can vibrate either longitudinally or torsionally.

These vibrations may damage the endothelial cells of the cornea, which leads to hydratation of the stroma, functional decompensation and consequent corneal oedema.

In cases of significant and persistent corneal oedema, keratopathy appears, which may compromise the functional results of the surgery. This is why it is important to use minimal ultrasound power and optimal phacotip vibrations in order to avoid these complications.

METHODS

The study involved 40 eyes of 40 patients, ranging in age from 55 to 65 years, with cataract, without any other eye pathology. Uncomplicated cataract extraction was performed in all patients. In the first group (20 eyes) the cataract extraction was done with longitudinal and in the second (20 eyes)– with torsional phacoemulsification using the Infinity Vision System. The surgical technique was the same in both groups. Central corneal thickness in all patients was measured, using non contact optic pachymeter, the day before surgery and in the first postoperative day.

RESULTS

An increase in the central corneal thickness to a different extent was found in all patients. In the patients' eyes, that had surgery with torsional phacoemulsification the increase was from 1 to 111 microns. (73,8 microns in average). In the eyes, done with longitudinal phaco the increase was between 21 and 197 microns. (95,5 microns in average).

CONCLUSION

The results of the study show, that in cataract extraction with phacoemulsification in the first postoperative day corneal thickness increases. In comparison to the longitudinal, torsional phacoemulsification is less damaging for the corneal endothelium. With the torsional movements of the tip less ultrasound energy is required for the fragmentation of the lens's nucleus. Torsional phacoemulsification is proven to minimize cell damage and lead to better anatomical and functional results.

PS 138

Frequency of precancerous lesions in patients with dyspepsia in northeast of Iran

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F

AIM

the aim of this study is to determine the Frequency of precancerous lesions in patients with dyspepsia in northeast of Iran

INTRODUCTION

Although gastric cancer mortality rates have been declining worldwide, its prevalence has been increasing in the last thirty years in Iran. Gastric cancer develops through reversible precancerous lesions (PLs). We are able to stop progression of gastric cancer rate, with early detection of these lesions. Know the frequency of PL and its probable relationships with risk factors of gastric cancer is an important factor to suggest an effective screening method for gastric cancer.

METHODS

To estimate the prevalence of gastric PLs, we had cross-sectional studied on 87 nontumoral patients (49.4% female & 50.6% male) who underwent upper gastrointestinal endoscopy for investigation of dyspepsia. After signing a written consent, History taking and physical examination was done by a physician. Following, tissue biopsies were taken from six locations of stomach, and checked by experienced pathologist. Results divided to chronic gastritis, atrophic gastritis, intestinal metaplasia and dysplasia, according to updated Sydney system and PATU system.

RESULTS

According to histopathological findings, there were 75.7% Chronic Gastritis, 1.4% Atrophic gastritis, 20.0% Intestinal Metaplasia and 2.9% Dysplasia. The prevalence of H. Pylori infection in our cases was 77.5% that was shown 41/53 in Chronic Gastritis, 0/1 in Atrophic gastritis, 13/14 in Intestinal Metaplasia, and 1/2 in Dysplasia respectively, but there were no significant differences were seen. Although the high frequency of opium abuse 38.4% and salt excess intake 34.7%, we did not found any significant differences in demographic data and risk factors among different PLs.

CONCLUSION

Our study shows that, the relatively common gastric lesion is chronic gastritis. The life style does not increase the risk of PLs progression, but It seems further research in Iran is needed to clarify different aspects of chains of events leading to cancer.

PS 149

Sensitivity of core biopsies in diagnosing pathohistological breast changes

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AIM

By comparing pathohistological findings obtained after CORE biopsy with definitive pathohistological findings obtained after open excisional biopsy, calculate the sensitivity of CORE biopsy.

INTRODUCTION

Breast cancers are second most frequent malignity in female population. Since there is no way they could be prevented, an early detection of changes in breasts plays a key role. Radiologically detected changes characterized as BI RADS 4 or 5 are considered to be suspective and undergo biopsy.

The golden standard in biopsying changes in breasts is open excisional biopsy, although in recent years a significant place has been reserved for less invasive biopsy methods, due to their numerous advantages.

METHODS

The research included 35 female patients who, due to the suspective breast change, in the period between July and November 2011 at the Institute of Oncology of Vojvodina, underwent CORE biopsy, after which surgical removal of the change was carried out.

RESULTS

In 34 female patients, pathohistological findings after CORE biopsy were identical with the definitive pathohistological diagnosis after the surgical removal of the change. In one patient the pathohistological findings after open surgical biopsy and CORE biopsy were different.

CONCLUSION

Through statistical processing of data, the CORE biopsy sensitivity of 97.14% was achieved, which puts this method into a group of highly sensitive ones.

PS 148

High incidence of subclinical acute rejection in low risk kidney transplant recipients on tacrolimus- based immunosuppressive re

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AIM

The incidence and impact of subclinical acute rejection (SCR) on renal graft function remains poorly understood, especially with recent calcineurin-sparing (CNI-sparing) immunosuppressive strategies. Thus, this study seeks to clarify these characteristics of renal graft function.

INTRODUCTION

This study analyzed the incidence of subclinical acute rejection in low risk recipients of first kidney transplant receiving initial immunosuppression with reduced tacrolimus (TAC) exposure, mycophenolate sodium (MPS) and prednisone (PRED) with planned conversion to sirolimus (SRL) at 3 months in a randomized fashion.

METHODS

149 protocol biopsies were performed at month 3 after transplant and the histological findings were evaluated according to Banff 2007 criteria.

RESULTS

Results: The mean age of this population was 44.7±13.1 years, 47.7% Caucasian, 68.5 % male and 55% recipients of kidney transplants from living donors. At 3 months mean TAC trough blood concentration was 5.9 ± 2.4 ng/ml, mean MPS dose was 1384 ± 191.1 mg/day, and mean PRED dose was 5.6 ± 3.0 mg/day. The incidence of subclinical acute rejection at 3 months was 8.7% (N=13/149, been 3 borderline changes; 5 IA; 1 2A and 1 2B and 3 suspected tubulointerstitial nephritis). No differences were observed in immunosuppressive drug dose or concentration, serum creatinine (SC, 1.31±0.526 vs. 1.43±0.418 mg/dl, p=0.422) or calculated creatinine clearance (cCrCl, 68.5±20.5 vs. 73.1±15.2 ml/min, p=0.452) in patients without or with subclinical acute rejection, respectively.

CONCLUSION

Conclusions: These results showed that in low risk kidney transplant recipients receiving CNI-sparing regimens, subclinical acute rejection and inflammation are frequent despite good and stable renal function and apparent adequate immunosuppression exposure. Surveillance biopsies may be necessary to monitor the efficacy of these regimens in the short and long-term follow up.

PS 212

Quadriceps tendon rupture: clinical findings, mechanism of injury and risk factors

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AIM

Our goal is to point out the main symptoms and mechanisms of this injury, as well as the most often systemic disorders that contribute to tendon rupturing.

INTRODUCTION

Quadriceps tendon rupture is an uncommon injury which requires early surgical treatment. Clinical findings include knee pain, inability to actively extend the knee, swelling, effusion and palpable gap above the patella. Risk factors for quadriceps tendon rupture are obesity, advanced age, diabetes mellitus, chronic renal failure, hyperparathyreoidism and rheumatic diseases.

METHODS

This research includes eighteen patients (17 are male). Average age at the time of injury was 53 (median-55,5 years). We have analyzed the following data: mechanism of injury, type of trauma, clinical findings, imaging diagnostic methods, time that has elapsed from injury until operation, risk factors, type of operation technique as well as potential early postoperative complications. Frequency was determined for categorical variables and quartiles were used to show the frequency of the numeric variable. In order to examine the relation between the type of trauma and coexistence of the risk factors, we used Fischer's exact test.

RESULTS

Most patients have injured due to a simple fall (n=7). The most common symptoms were knee pain and inability to actively extend the knee against gravity. Seven patients are diabetic, four of them suffer from chronic renal failure. Secondary hyperparathyreoidism is present in one case; in three cases patients were obese.

CONCLUSION

Systemic conditions defined as risk factors (diabetes mellitus, chronic renal failure, obesity) are present in most cases of quadriceps tendon rupture. Simple fall is the most common cause of injury. Coexisting risk factor contributes tendon rupturing due to minimal, inadequate trauma.

PS 248

Possibilities of ultrasound in the differentiation of choroidal melanomas

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AIM

In the present study We have tried to asses the correlation between echographic findings and histopathologic images in differentiating choroid melanomas

INTRODUCTION

Melanoma of the choroid is the most common primary intraocular malignant tumor and second frequency in the human body melanoma after his liver localization.It's origin is from melanocytes of choroid.The ability to predict the fate of patients with melanoma of the choroid is the ultimate goal and challenge for every ophthalmologist.

METHODS

Number of 59 eyes with choroid melanomas have been examinated in 5 years' period(02.2007-07.2011). The whole group consisted of 4 cell types:spindle A-type,spindle B-type,mixed A-B type and epythelloidal cell type.For examination we used ultrasound with 10 MHz and 20 MHz and A-scan for tissue differentiation. We used specific study protocol before enucleation-ophthalmoscopy,FA,MRI.Tumors,suspected for ciliary involvement,are excluded.The final ultrasound results were compared with the histological preparations.

RESULTS

The coincidence between sonographic data and histologically determined tumors was 89,8%.The most common melanoma of the total number of investigated cases was spindle A-type(33,9%).The correlation between recognized ultrasound and histological confirmed tumors is 86,4%.Between A- and B- type ,there is some histological differences, but so small that in practice, ultrasound can not distinguish the two versions.The convergation in epithelloidal cell types was 85,7%.The mixed type were often treated as one kind or another major.The correlation here was the smallest-73,3%.The total number of correctly recognized cases was 53 eyes(from 59) or 89,8% of all investigated.

CONCLUSION

Echography is useful tool in determinind melanoma type and predicting survival rate of patient.Important not only for prognosis but also for our therapeutic tactic is to know in advance what is the cell type of choroidal melanomas .In tumors with good prognosis have a longer period of time to choose an appropriate alternative method of treatment unless enucleation.Echography in this case is the most appropriate diagnostic method.

PS 247

Puncture laser revascularizative osteotrepanation in chronic atherosclerotic arterial ischemia of the lower extremities

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AIM

Assessment of puncture laser osteotrepanation as indirect revascularization method in complex treatment of patients with chronic ischemia of the lower extremities.

INTRODUCTION

Direct methods of vasoreconstrucion such as bypass surgery or prosthetics in cases of chronic arterial ischemia of the lower extremities are not always feasible, especially when small arteries are involved. Recently, methods of indirect revascularization, like tunneling of soft tissues, osteotrepanation or osteoperforation of the femur or tibia are more widely used.

METHODS

The study is based on 27 male patients (age 56,2±8,5) with III-IV degree of chronic arterial ischemia of the lower extremities. All the patients were heavy smokers, duration of the disease was 27,7±4,9 years. The length of painless pathway (intermittent claudication) lesser than 10 m was in 4 patients, 15-50 m – in 8 patients, 51-100 m – in 9 patients, greater then 100 m – in 6 patients. Nonhealing ulcers and necrotic changes of fingers was in 19 cases, throphic changes of the skin were in all the patients. Blood analyzes, angiography and Doppler USI of the vessels were performed. Atherosclerotic stenosis was diagnosed on the level of iliac or femoral artery in 5 patients, on popliteal artery – in 16, on anterior/posterior tibial arteries – in 6 patients. Puncture laser osteotrepanation of the tibia was made for all the patients in addition to the angioprotective and antibiotic therapy to increase angiogenesis (both sides in 18 patients, one side in 9 patients). Destruction of the bone tissue performed by a high energy laser irradiation (diode laser, wave length 940 nm, power 30W) throw a puncture needle. There were analyzed immediate (before 4 weeks), early (before 6 months) and later (after 1 year) results. Immediate results were based on the pain degree, color and temperature of the foot, oxygenation level of the soft tissues and ulcers healing dynamic. Early and later results assessed on the base of presence of trophic disorders, length of painless pathway (intermittent claudication), vocational rehabilitation degree and self-service possibility.

RESULTS

Immediate results was good in 21 cases, satisfy – in 4 patients and unsatisfied in 2 cases.

Pain disappearing or decreasing intensity within first two weeks was in 14 patients, on the 4 week – in 20 patients. After 2-3 days it was stated decreasing usage of analgetics. X-Ray showed linear zones of osteoporosis in places of osteotrepanation from a cortical layer to intramedullary canal. Positive dynamic of the blood rheology, skin temperature and soft tissues oxygenation levels were registered in all periods.

Puncture postoperative wounds healed by scab during 7-10 days. Early purulo-necrotic processes on distal parts of the lower extremities were regressed with complete epithelization in 16 (59,2%) patients. In 3 (1,1%) patients infection and gangrene were progressed and amputation was performed. In most of patients during a long-term observation period (from 6 months to 4 years) there were stated improvement of blood circulation, decreasing of painful sensation, prolongation of painless pathway. Recurrence of purulo-necrotic process was observed in 5 patients, which were undergone amputation of the lower extremity on different levels.

CONCLUSION

Laser osteotrepanation of the tibia is effective miniinvasive method of revascularization surgery which can be used in complex treatment of patients with chronic atherosclerotic ischemia of the lower extremities to decrease levels of ischemia and purulo-necrotic changes.

PS 324

Surgical revascularization prospective registry of patients with and without preceding percutaneous coronary intervention.

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AIM

The aim of the study was the comparison of short- and long-term results in patients who underwent previous PCI before being referred to elective CABG (post-PCI group) with those qualified directly to elective CABG (non-PCI group).

INTRODUCTION

Considerably different long-term results were revealed in latest studies comparing percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). Therefore fundamental effect on short and long-term results might have PCI performed in patients subsequently referred to elective CABG.

METHODS

155 patients with stable ischaemic heart disease who underwent elective CABG were gathered. The non-PCI group of patients comprised 43% (n=67) of overall registry population. Surgical risk, symptoms and coronary atherosclerosis extensiveness are the preoperative data that were compared. The study collected rates of intraoperative and early in-hospital complications. The control visits took place in 12- and 24-month periods of time and the follow-up was held in order to find significant adverse effects.

RESULTS

Mean age of post-PCI patients was significantly lower in comparison to non-PCI group (p=0,01). They were qualified to higher NYHA class (p=0,007), had lower left ventricle ejection fraction (LVEF, p=0,009), and less hemodynamically significant lesions in coronary arteries (Coronary Score, p=0,001). Considerably less distal anastomosis were performed (2,1 +/- 0,9 versus 2,7 +/- 0,9; p=0,002) in post-PCI patients. Trend towards higher risk of in-hospital adverse effects in the post-PCI group (2,4 +/-1,8% versus 1,9 +/-1,4%; p=0,08) was discovered. After 12- and 24-month period the risk of adverse effects occurrence in non-PCI patients was 5,9% and 1,5%, respectively. However, it was higher in post-PCI patients - 10,2% after 12 months and 6,8% after 24 months.

CONCLUSION

Registry data analysis revealed significant differences between these two groups in area of past myocardial infarction, extensiveness of coronary lesions, LVEF, and range of surgical management The lesser amount of surgically revascularized coronary arteries as well as initially worse function of the left ventricle in post-PCI group of patients may influence long-term results.

PS 661

Subcuticular Suture versus Staples for Skin Closure following a Caesarean Delivery: Critical Analysis

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AIM & INTRODUCTION

Caesarean delivery is considered to be one of the common surgical procedures carried out worldwide. Just as the rate of Caesarean deliveries is increasing, so are the complications of the surgery. This article will, therefore, evaluate which procedure--subcuticular suture or staples--has a higher risk of the patient developing wound complications when used for Pfannensteil skin incisions after a Caesarean delivery.

METHODS

Electronic databases (PubMed and MEDLINE) from 2000 to August 2011 were searched for prospective coherent studies and randomized controlled trials (RCT) comparing subcuticular sutures to staples after a Caesarean delivery. The main outcome searched for was whether or not there were wound complications, such as separation or infection. The secondary outcomes looked for were operating time, cosmetic and postoperative pain.

RESULTS

Four studies met the criteria: one prospective cohort study and four RCTs. Skin closure with staples (n= 778) was associated with twice the risk of separation or wound infection when compared with subcuticular sutures (n= 659). There was no significant statistical heterogeneity among studies. Subcuticular suture closure was associated with a longer time duration of the surgical procedure, but the two methods showed similar results with regard to cosmetic result and postoperative pain.

CONCLUSION

Closing the skin using staples is quicker to implement but is accompanied with a higher wound complication rate.