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The Sixth Young European Scientist Meeting

Special Supplement

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International Journal of Medical Students

The *International Journal of Medical Students* (IJMS), is a peer-reviewed open-access Journal, created to share the scientific production and experiences of medical students worldwide.

Cover photo

A perspective of the Auditorium of *Fundação Engenheiro António de Almeida* during talk of Prof. Dr. Reinhard Fässler (Max Planck Institute of Biochemistry, München, Germany).

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IJMS International Journal of Medical Students

The 6th Young European Scientist Meeting 2011

Delfim Duarte[1].

The *6th Young European Scientist Meeting - YES Meeting 2011* is an international conference build up by a group of students in Porto, Portugal.

YES Meeting website
www.yesmeeting.org

Since our first edition, in 2006, we have four main components that include students' presentations, world-class talks, interactive workshops and an interesting social program that uncovers the beautiful region of Porto.

We received a record number of registrations, and this year, for the first time, we had more than 400 students attending our meeting from more than 30 countries. We were thrilled to know that so many students see in *YES Meeting* an opportunity to present their research, to attend unique talks and to make friends from so many backgrounds. This is, in fact, the central point for *YES Meeting*: to give students a stage to present, to discuss, to learn, to discover and to surprise themselves. In line with our goal of supporting students' works, we formed a partnership with the *International Journal of Medical Students* (IJMS), to publish the abstracts presented during the meeting as a supplement of the first number of IJMS.

As part of the former *YES Meeting Organizing Committee* and as *Editor of the International Journal of Medical Students*, I am proud of such a collaboration between two organizations that promote students' research and science.

Figure 1. *Delfim Duarte, President of the 6th Young European Scientist Meeting 2011 at Opening.*

Another novelty of this year was the establishment of YES Meeting as an official partner of the *International Federation of Students Association – IFMSA*, one of the largest international students forums, representing more than 1.2 million medical students. This was, of course, a great honor for us and expresses the good work we have been doing along the years. It also brings responsibility and we hope to keep up to the expectations.

YES Meeting has also increased the participation of students from different scientific backgrounds, such as, *Medicine*, *Biology*, *Biochemistry*, *Engineering* and *Pharmacy*, just to name some. This is, again, very exciting for us and we believe that sharing knowledge is essential for scientific progress. In a time of financial and social stress like the one we are facing now, these discussions and learning processes can generate partnerships and join efforts that can make a difference.

Regarding the scientific program we had memorable talks in fields such as *Integrins*, *Bone Marrow*, *Brain-Machine Interface*, *Ageing*, *Cardiovascular Medicine* and *NOTES (Natural Orifice Translumenal Endoscopic Surgery)*. The intervening speakers gave an enlightening perspective on great scientific advances and included *Reinhard Fässler*, *Mikhail Lebedev*, *Rudi Westendorp*, *Mariela Jaskelioff*, *Mark Caulfield*, *Vickas Patel*, *Gero Hütter* and *Robert A. Montgomery*, among others. The workshops were highly anticipated and met the expectations with great opportunities for everyone to get "hands on" in many activities from surgical gestures to diagnostic medicine.

We also had first-class poster and oral presentations in 5 main areas: *Oncology & Molecular Biology*, *Neurosciences*, *Physiology & Immunology*, *Internal Medicine* and *Surgery*. The Abstracts are included in this Supplement.

We encourage you to keep up with your research interests and projects and to submit them to IJMS. We also invite you to visit *YES Meeting's webpage* at www.yesmeeting.org and to participate in future editions of this unforgettable scientific experience.

See photos of the YES Meeting on the next page.

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YES MEETING

Abstracts of the 6th Young European Scientist Meeting

INTERNAL MEDICINE *Session*

PS 2 Airway-invasive inoperable Thyroid Carcinoma: An Interventional Pulmonary approach to management in the Asian setting.

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Aim: To evaluate clinically and to treat the patients with airway invasive thyroid cancer using interventional pulmonary procedures. **Introduction:** Invasive thyroid cancer causing tracheal stenosis and obstruction is essentially managed by surgical methods. In patients who cannot be operated, or fail other methods, interventional pulmonary (IP) procedures offer reasonable options. There is scant data from the Asian and Indian setting in this regard. In this study, we report an IP approach to such patients with airway-invasive thyroid cancer, who failed or were not candidates for conventional options. **Methods:** 10 consecutive patients, over 3.2 years, with primary airway-invasive thyroid cancer referred for IP treatment were included. Clinical evaluation, CT imaging followed by bronchoscopy was done. Therapeutic modalities used included debulking using cauterization, balloon dilatation, and stenting. Thermoablation was done using APC and electrocautery. Periodic follow up was done, for recurrence. **Results:** 22 procedures were done in 10 patients (8 males and 2 females) with invasive thyroid disease, causing stridor as the main symptom. The mean age of the patients was 44.9 years (13 - 75 years). Rigid bronchoscopy was done in 80% and flexible bronchoscopy was used in 20%. Debulking and dilatation was done in all the patients, and silicone stenting (Dumon stent, Novatech Corp) was done in 5 patients. Airway patency was achieved in all patients (100% immediate success rate). The mean survival postprocedure was 23.1 months (2 - 38 months). There were no procedure related complications. One patient died 12 months post procedure due to non-stent related causes. In 1/10 patients, complete cure was achieved, and the stent was removed after 15 months. Another unique case was a 75 year male with papillary carcinoma, managed with recurrent (11) treatments over 3.2 years. **Conclusions:** IP procedures in invasive thyroid carcinoma offer good options to patients who have exhausted all conventional options, to maintain quality of life, and possible longevity. This is one of the first series in an Asian and Indian setting, demonstrating the utility of IP in invasive thyroid Ca. Other unique features in this series are the use of APC and electrocautery with outcomes at par with reports using lasers. Our study also shows a significant survival advantage with IP methods. In staging systems such as described by SHIN, › SHIN stage 2 survival is 1.5 years, while patients in our series (SHIN 4) had a mean survival of about 2 years.

PS 46 Evaluat ion of correlat ion between severity of dry eye syndrome and patients with diabetes mellitus.

Kashkouli M, Amani A.
Eye Research Center, Rasoul Akram Hospita l, Tehran University of Medical Sciences.

Aim: To assign the severity of dry eye syndrome in diabetic patients Introduction Diabetes is one of the most common systemic diseases. Dry eye syndrome is one of the eye involvements in diabetic patients. Evaluation of correlation between dry eye syndrome and diabetic retinopathy in patients with non insulin-dependent was the aim of this study. **Methods:** In this study two hundreds eyes from 100 diabetic patients (non insulin dependent) were selected and evaluated for dry eye syndrome. The patients then were divided into four group: Group 1: patients without diabetic retinopathy; Group 2: patients with non proliferative diabetic retinopathy; Group 3 : patients with proliferative retinopathy Group; 4: patients with proliferative diabetic retinopathy undergone PRP. All the patients were examined for Schirmer test, TBUT, staining the cornea with flourecein and then allocated in different group based on the severity of dry eye .Collected data were analyzed by X2 and Kendle- taue tests using SPSS software. **Results:** Prevalence of dry eye syndrome in patients in group 1 was 48% in group 2, 3 and 4 were 52%, 60% and 80% respectively. **Conclusions:** Dry eye syndrome is a complication of diabetes mellitus and its severity correlates with severity of diabetic retinopathy.

PS 52 ATTITUDES OF MEDICAL STUDENTS TOWARDS PSYCHIATRIC PATIENTS.

Vanja H.
Medical Faculty, University of Novi Sad.

Aim: To examine the attitudes towards psychiatric patients among first and fifth year medical students of Medical Faculty Novi Sad and to assess the differences in attitudes caused by duration of medical education. **Introduction:** Negative attitudes towards psychiatric patients are very spread in society. Among others, physicians also have the negative attitudes. **Methods:** Data were obtained using questionnaire which consisted of 27 questions. 127 first year and 74 fifth year medical students took part in the survey. We compared the attitudes between the two groups and also of fifth year medical students which have some family member who is treated for some psychiatric disorder, with those who do not have. **Results:** Fifth year students have generally more favourable attitudes. Greatest difference was in the attitudes in areas students met during their faculty education, such as therapy and psychiatric hospitals. The difference was less and statistically insignificant in social and personal attitudes. Statistically significant differences were found between the two groups of fifth year students. The group of students with family member treated for some psychiatric disorder had less negative attitudes. They also had more stances towards psychiatric disorders, and their responses were more uniform. **Conclusion:** Although there is a positive difference between first and fifth medical students' attitudes towards psychiatric patients there is a plenty of possible improvement in their education, in order to reduce stigma. Further research should be done on attitudes towards specific mental disorders.

PS 68 T2* magnetic resonance imaging of the liver in thalassemic patients in Iran.

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[3] Research Center of Iranian Blood Transfusion Organization.

Aim: To investigate the accuracy of T2*-weighted magnetic resonance imaging (MRI T2*) in the evaluation of iron overload in beta-thalassemia major patients. **Introduction:** Conventional treatment of beta-thalassemia major requires regular blood transfusions to maintain pretransfusion hemoglobin level above 90 g/L. A major drawback of this treatment is transfusion siderosis, which, in association with the increased intestinal iron absorption, apoptosis of the erythroid precursors and peripheral hemolysis, leads to inexorable iron accumulation in various organs such as the heart, liver and endocrine organs. The assessment of body iron is still dependent upon indirect measurements, such as levels of serum ferritin, as well as direct measurements of the liver iron content. Serum ferritin has been widely used as a surrogate marker but it represents only 1% of the total iron pool, and as an acute phase protein, it is not specific because the levels can be raised in inflammation (e.g. hepatitis) and

liver damage. Liver iron concentration measured by needle biopsy is the gold standard for evaluation of siderosis. However, it is an invasive technique which is not easily repeated and its accuracy is greatly affected by hepatic inflammation-fibrosis and uneven iron distribution. More recently, biomagnetic susceptometry and magnetic resonance imaging (MRI) have been validated for measuring iron overload, and these techniques have great merit in being noninvasive. Biomagnetic susceptometry is a non-invasive, well calibrated and validated method as a quantitative measurement technique, but it has limited clinical value because of its high cost and technical demands. MRI has been considered a potential method for assessing tissue iron overload, as iron accumulation in various organs causes a significant reduction in signal intensity stemming from a decrease in the T2 relaxation time. **Methods**: In this cross-sectional study, 210 patients with beta-thalassemia major having regular blood transfusions were consecutively enrolled. Serum ferritin levels were measured, and all patients underwent MRI T2* of the liver. Liver biopsy was performed in 53 patients at an interval of no longer than 3 mo after the MRIT2* in each patient. The amount of iron was assessed in both MRI T2* and liver biopsy specimens of each patient. **Results**: Patients' ages ranged from 8 to 54 years with a mean of 24.59 ± 8.5 years. Mean serum ferritin level was 1906 ± 1644 ng/mL. Liver biopsy showed a moderate negative correlation with liver MRI T2* (r = -0.573, P = 0.000) and a low positive correlation with ferritin level (r = 0.350, P = 0.001). Serum ferritin levels showed a moderate negative correlation with liver MRI T2* values (r = -0.586, P = 0.000). **Conclusions**: Our study suggests that MRI T2* is a non-invasive, safe and reliable method for detecting iron load in patients with iron overload.

PS 99 VITAMIN D RECEPTOR POLYMOR PHISM IN PORTUGUESE PATIENTS WITH SYSTEMIC LUPUS ERY THEMATOSUS.

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Aim: To determine whether VDR gene polymorphisms are related to the susceptibility to SLE, severity and activity of the disease in the Portuguese population. **Introduction**: Systemic Lupus Erythematosus (SLE) is an autoimmune disease with a large variation in clinical presentation - acute versus indolent - and associated syndromes: non severe pattern, cutaneous and articular versus nephritic, cerebral, etc. An explanation for this heterogeneity can be framed in the setting of the so called "mosaic of autoimmunity", in which genetic and environmental factors are generally acknowledged as playing a role in the onset of autoimmune disease. Genetic and environmental factors are generally acknowledged as playing a role in the onset of SLE. Vitamin D is a steroidal hormone that in addition to its role in calcium homeostasis, has been shown to possess immunomodulatory effects through the Vitamin D Receptor (VDR). Several studies have demonstrated a higher prevalence of vitamin D deficiency in SLE patients and a possible mechanism for its influence in the disease was already proposed (Figure 1). In humans, multiple polymorphisms of the VDR gene have been identified, some of them with functional impact. The relationship between SLE and polymorphisms in the VDR gene has been already subjected to scrutiny [1]. Further studies are necessary to better understand the risks and benefits of vitamin D supplementation in the prevention and treatment of autoimmune diseases and its real impact in daily clinical practice. **Methods**: A sample of 181 SLE patients (according to the ACR criteria) with Northern Portuguese descent, were studied and compared with 181 ethnically-matched controls (Control Population). SLE clinical and laboratory manifestations were also evaluated. Genotyping of the FokI polymorphism of the VDR gene was performed using TaqMan® allelic discrimination assay. Differences in frequencies were evaluated using the Chi-Square test and Fisher's exact test. Analyses were done with SPSS v.18 software. Significant levels were set at $p<0.05$ and confidence intervals (CI) were given at 95%. **Results**: No statistically significant differences, between patients and controls, for the VDR FokI genotype frequencies were found. However, we could identify an association with disease activity. The genotype ff of the FokI polymorphism was significantly less frequent in patients with SLEDAI i^34(n=79) than in patients with SLEDAI ‹4(n=74) (6.3% vs. 21.6%, corrected p [p(c)] = 0.009). **Conclusions**: We provide for the first data suggesting that the ff VDR FokI genotype reduces disease activity. Our findings are in agreement with previously published results that associated a more active immune system with the FF VDR genotype [2]. The discrepancies reported for this association, may be explained by the fact that VDR signalling, although contributing to the outcome of immune response, can be overruled by more potent immune pathways.

PS 129 Pscribe: a unique e-learning program to practice pharmacotherapy.

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Aim: E-learning is gaining importance in academics, especially in medical education1. In this study, we estimated the number of academic e-learning programs, later specified as medical and pharmacological e-learning programs. **Introduction**: Prescription errors are frequently made mistakes by physicians with potential serious health issues for the patient. It is estimated that up to 500 errors are made per 1000 patient hours. In order to prevent these mistakes, the University of Groningen has developed an e-learning program in collaboration with the University Medical Center Groningen to teach students to prescribe drugs; it is called Pscribe. This web-based e-learning program teaches proper prescribing in a case-oriented manner based on the World Health Organization 6-step (WHO 6-step). To estimate the potential benefits and use of Pscribe in medical education, we estimated the number of available medical e-learning programs. **Methods**: First, we estimated the total number of academic e-learning programs ([universities] x [estimated faculties/university] x [estimated programs/faculty]), medical e-learning programs ([medical schools] x [estimate programs/medical school]) and pharmacotherapy e-learning programs ([medical schools] / [medical courses]). We took many factors into account to make these estimates as accurate as possible. Second, we estimated the number of web-based e-learning programs that teach medical students prescribing through case-based exercises, like Pscribe. Criteria we used to match other programs to Pscribe are: inspiration by the WHO 6-step, possibility to create cases in a user-friendly way and datatracking; a function that might be useful for research purposes. **Results**: We estimate academic e-learning programs between 50.000-150.000 programs (8.547 x 6 x 1 or 3), medical e-learning between 20.000-40.000 programs (2.081 x 10 or 20) and pharmacotherapy e-learning between 1.000-2.500 programs (20.000 or 40.000 / 22). When we added the specified searching terms we found 3 other programs, i.e. Dynamisch Patiënt Simulator developed by the University of Leiden and the Leiden University Medical Center, The National Prescribing Curriculum developed by the University of South Australia and 'Signposts for good prescribing', a program developed by the British National Health Service. Although these programs have similar functions as are available in Pscribe, none of the programs had all these functions integrated in one program. Further, data-tracking was not available in the studied programs. **Conclusions**: Although several other e-learning programs are available to teach students how to prescribe drugs that might resemble Pscribe, we were not able to find another program that combines the different functions including the data-tracking function. Hence, we conclude that the pharmacotherapy e-learning program Pscribe has unique features that cannot be found in other programs. Implementing it in medical education might reduce the number of prescription errors made by future physicians.

PS 149 Accuracy of D-dimer/fibrinogen ratio to predict developing of pulmonary thromboembolismin patients admitted to intensive care units.

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Aim: The aim of this study is to evaluate Ddimer/fibrinogen ratio (DDFR) in ICU patients as a rule-in diagnostic test for patients presenting symptoms and signs of PTE. **Introduction**: Pulmonary thromboembolism (PTE) has been known as the third cause of cardiovascular related deaths. PTE can increase D-dimer and decrease fibrinogen levels .But in settings such as Intensive Care Units (ICU) and other long-term and critically ill hospitalized patients, several factors can influence D-Dimer and fibrinogen concentrations that make them unreliable to diagnose PTE. The aim of this study is to evaluate the accuracy of D-dimer/fibrinogen ratio (DDFR) in ICU patients presenting symptoms and signs of PTE. **Methods**: Critically ill patients admitted to ICU that were suspected to PTE were included and then the definite diagnosis was established either by Angiography or CT- Angiography. D-dimer and fibrinogen was measured before treatment and other parameters were extracted from medical records. Patients with drug history of anti-coagulants, oral contraceptive, and previous history of PTE were excluded. Suitable analytic tests were performed by SPSS software. **Results**: Thirty-seven PTE and 33 non-PTE subjects were included. Mean value of D-dimer and fibrinogen was 3.97±3.22 µg/ml and 560.6±197.3 mg/dl, respectively. Significant higher values was seen in D-dimer (4.65±3.46 vs. 2.25±2.55 ug/ml,p=0.006) and DDFR (0.913±0.716 vs. 483±0.440 x 10^3,p=0.003) in PTE patients. ROC analysis showed 70.3% sensitivity and 70.1% specificity with D-dimer value of 2.43 ug/ml, (AUC=0.714,p=0.002); in contrast, DDFR had 70.3% sensitivity and 61.6% specificity with value of 0.417x10^3, (AUC=0.710,p=0.004), considered as the best cut-points. In backward stepwise regression analysis, DDRF (OR= 0.72,p=0.025), gender(OR=0.76,p=0.049) and WBC (OR=1.11,p=0.373) were modeled. (p= 0.029,R^2=0.577). **Conclusions**: Cut-points for D-dimer<0.4 and DDFR>0.25 x 10^3 can be used as fairly reliable test to rule-out and rule-in PTE in these patients. Thus, with use of D-dimer and DDFR some patients may no longer need any other expensive diagnostic tests such as angiography to confirm or rule-out PTE; though is not accurate enough and more studies are recommended.

PS 150 The study of association between factor V leiden mutation and G6PD deficiency.

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Aim: Regarding to the moderate prevalence of Factor V Leiden mutation and relatively high prevalence of G6PD deficiency in Western Iran, the aim of the present study was to assess the prevalence of these thrombophilic mutations among G6PD deficient individuals from this area. **Introduction**: G6PD (Glucose 6-phosphat dehydrogenase) deficiency is the most common erythrocyte enzymopathy in humans, estimated to affect 400 million individuals worldwide. Factor V Leiden is a variant of human factor V that causes a hypercoagulability disorder. And it is clinically important genetic mutation associated with increased susceptibility to venous thrombosis.The factor V Leiden gene variant is caused by a single point mutation at nt1691, leading to the replacement of arginine at position 506 in the activated protein C (APC) cleavage site of factor Va by glutamine. **Methods**: In this cross-sectional study,G6PD deficient individuals consisted of 57 school boys randomly selected from 6 high schools located in 3 educational areas of Kermanshah ,a province in west of Iran, and 3 girls, mean age of 15±3.08, referred to the clinic of Kermanshah University of Medical Sciences with history of favism and hemolytic anemia. An age and sex matched group of healthy individuals, 95 males and 15 females with the mean age of 16.19±2.17, from the Kermanshah Province of Iran were selected and along with G6PD individuals were studied for factor V Leiden mutation. Healthy individuals consisted of school boys and blood donors. All of G6PD individuals and controls were not consanguineous. DNA was extracted by phenol chloroform method. The activity of glucose-6-phosphate dehydrogenase was determined using the fluorescent spot test. G6PD mutations were identified by a combination of PCR-RFLP technique, single strand conformation polymorphism (SSCP) analysis and DNA sequencing. The factor V Leiden was detected by PCR-RFLP method using MnlI restriction enzymes, as previously described . Chi-square test is used for matching the variants and we utilized SPSS.V.12 for statistical application. **Results**: Among G6PD deficient individuals there were 3 males carrier of factor V G1691A giving the prevalence of 5% and allele frequency of 2.5%. Factor V G1691A mutation was detected as heterozygous in 3 males out of 110 healthy individuals indicating the prevalence of 2.7% and allele frequency of 1.3%. No homozygous factor V G1691A and was found. Overall comparing to the healthy individual the differences were not statistically significant. **Conclusions**: Compared with those without the mutation of factor V Leiden, heterozygous carriers have a 7-fold increased risk of venous thrombosis; homozygous individuals have a risk that is increased up to 100-fold. It has been indicated that the G6PD deficiency could be connected to the venous thrombosis. In the present study, the prevalence of factor V Leiden in G6PD individuals tended to be higher compared to controls (5% vs. 2.7%) but, the difference was not statistically significant. Briefly, our finding indicates that the prevalence of factor V Leiden in G6PD deficient individuals is not statistically different compared to normal subjects and G6PD deficiency is not associated with this thrombophilic mutation in Western Iran.

PS 162 NOWADAYS ENTEROSCOPY IN DIAGNOSIS OF SMALL BOWEL CROHN'S DISEASE.

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Aim: The aim of the study is to evaluate the diagnostic capabilities of videocapsule endoscopy (VCE), single- and double-balloon enteroscopy in diagnosis of small bowel Crohn's disease. **Introduction**: Crohn's disease is a chronic disease which is predominantly observed in the developed countries and may affect any part of the gastrointestinal tract from mouth to anus. Up to 30% of patients diagnosed with Crohn's disease have only small-bowel involvement (Bourreille A. et al., 2009). The modern endoscopic techniques of the small-bowel imaging such as videocapsule and balloon-assisted enteroscopy (BAE) allow to visualize any part of small bowel mucosa and play a significant role in patients with suspicion on Crohn's disease. **Methods**: From V.2003 to V.2011 we observed 52 pts. with suspected small bowel Crohn's disease; 8 (15,4%) of them were operated urgently because of complications: bleeding (2), secondary appendicitis (4), perforation (1), bowel obstruction (1). Another 44 pts (m-23, f-21, ranged from 15-72 years, mean age 37,5±12,0 years) underwent complex examination including 19 VCE and 50 balloon-assisted enteroscopies (from which 8 interventions as a follow-up) for the last 3,5 years. VCE was performed in 32 pts. (incl. 7 after VCE); there were 22 peroral examinations in 21 pts, 28 transrectal in 21 pts and both of them in 10 pts. **Results**: Typical Crohn's disease appearances (ulcers, pseudopolyps, edema, mucosal hyperemia, scars, pseudostrictures or strictures) were found in 15 (34,1%) pts (incl. 4 pts after VCE) from 44 who were examined endoscopically. In 8 pts we`ve revealed stenosis, in 4 of them we`ve passed through the ileocecal valve stenosis by bouginage with the enteroscope and were able to examine superior area (up to 35 cm of ileum). Beyond the stenosis area we took target biopsy. In all Crohn's disease was confirmed histologically in 5 (33,3%) pts. We haven`t found any

endoscopic signs of Crohn's disease in 17 pts. (no abnormalities found-6, enteritis-9, celiac disease-1, NSAID-ulcers - 1). Conservative treatment has been applied in 10 (66,7%) pts., including 1 pt, who underwent polypectomy because of polyp enlargement. Surgical intervention - in 5 (33,3%) pts. because of persisting clinical picture of bowel obstruction. There was a capsule retention in 2 (10,5%) pts. In 1 case the capsule was extracted using a polypectomy snare through the enteroscope perorally by laparoscopically assisted BE; in the second case the patient was operated on because of severe extended (15cm) stricture up to 6 mm. No side effects during diagnostic BE were observed. It was one complication - bleeding in 1 patient on the 4-th day after polypectomy of Crohn's induced polyp in ileum that was successfully stopped using argon plasma coagulation. **Conclusions**: The use of the new enteroscopic techniques substantially improves the diagnosis of small bowel Crohn's disease and allows to determine a proper treatment of this disease in time. This technology might unfortunately also bring complications, such as videocapsule retention and bleeding after polypectomy. BAE can be a useful method in VC retention cases to avoid operation and to provide
minimally invasive treatment.

PS 169 Can Type D personality contribute to cardiovascular risk in Major Depressive patients?

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Aim: The aim of our study was to investigate possible role of Type D personality as a risk factor for cardiovascular morbidity in MD patients compared to healthy subjects. **Introduction**: Type D personality is defined as the joint tendency towards negative affection and social inhibition. Large body of evidence consistently shows strong correlation between Type D personality and morbidity of cardiovascular diseases. On the other hand, Major depression (MD) is associated to various comorbid cardiovascular disorders, as well as Type D personality. **Methods**: Our investigation included 60 MD outpatients in remission and 55 mentally healthy controls. The Type D personality was measured by self-rating 14-item scale. Demographic and clinical data were obtained by interviewing the subjects and reviewing their medical records. Statistical analyses were performed using SPSS for Windows v. 13.0. **Results**: Nearly 2/3 of subjects were females in both study groups. MD patients and controls were age-matched (48.17 ± 9.55 vs. 42.17 ± 7.25, respectively). The prevalence of Type D personality was high in both patients and controls (81.7% vs. 77.6%, respectively), without statistically significant difference among study groups ($X2=0.28$, $p > 0.05$). Cardiovascular morbidity was significantly more prevalent in MD patients compared to healthy controls ($X2= 30.73$, $p < 0.001$). 56.7% of MD group had cardiovascular morbidity in contrast to 6.1% of controls. The predictive value of male gender, MD and the Type D personality for a cardiovascular risk was analyzed. The overall predictive model was statistically significant ($X2 = 25.348$, $p < 0.001$), and it explained 26.6 to 35.7% chance for a person to develop a cardiovascular condition. However, the only significant predictor of cardiovascular risk was the presence of Major depression (Exp B = 28.93, $p<0.01$). **Conclusions**: This was the first study exploring Type D personality in MD patients. Our results showed surprisingly high prevalence of Type D personality in both groups. Previous studies in general population indicate approximately 20% of Type D's. In contrast to other findings, Type D was not related to risk for cardiovascular morbidity itself. Our study suggests that Type D personality contributes to cardiovascular morbidity in approximately 1/3 male MD patients.

PS 180 Actions of immunomodulat ors of two pharmacological groups on eradication and factors of specific and non-specific immune resista nce in Helicobacter Pylori induced peptic ulcer disease.

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Aim: 1. To investigate the influence of immunomodulators Licopid®(a synthetic bacterial preparation) and Derinat®(a preparation of nucleic acid) in combination with antihelicobacter "quadro-scheme" therapy(QST) on efficiency of eradication in patients with peptic ulcer disease(PUD)caused by HP. 2. To reveal interrelation of changes of intensity of specific immunity(levels of Immunoglobulins and population T-lymphocytes) and non-specific immunity(lysozyme activity in saliva and gastric juice) for the given group of patients. **Introduction**: Appearance of widespread antibiotic-resistant H. Pylori(HP) strains and suppression of immune system by exo- and endogenous factors causes more difficulty in HP eradication using standard schemes of treatment. This research is conducted to study efficiency of immunomodulators combined with eradication therapy on efficiency of eradication of HP and improvement of immune system. **Methods**: 1. This research is carried out on 70 patients within the age range of 20-55 years with PUD of stomach caused by HP. 2. Depending on types of received therapy, three groups of patients have been allocated:-(a) Group A(control group) consisting of 20 patients receiving "quadro-scheme" therapy(QST) consisting of bismuth colloidal subcitrate, omeprazole, amoxicillin and furazolidon. (b) Group B of 25 patients receiving QST with Licopid® (c) Group C of 25 patients receiving QST with Derinat® 3. Before and six weeks after application of preparations, the histomorphological tests of biopsy material taken endoscopically from stomach, blood immune test and nephelometric test of saliva and gastric juice from patients are done. **Results**: Six weeks after treatment, bacterial contamination test reveals that absence of HP is essentially higher in Group B(94.8%) and C(95.3%) compared to Group A(73.7%). Coccal/Resistive forms of HP are absent in Group B and C whereas present in Group A(10.5%). Blood immune test revealed that levels of CD-3, -4 and -8 T-lymphocytes and Immunoglobulin A, M and G are increased in Group B and C, more pronounced for Group C. In contrast, their levels are decreased in control Group A. The maximal authentic increase of lyzosymal activity in saliva is observed in Group C(on 12.3 % compared to initial level) whereas in Group B its activity reliably increases(8.1%). Activity of lysozyme in gastric juice increased 7.4 % for Derinat® and 3.6 % at application Licopid®. In Group A, the lowest activity of lysozyme in saliva and gastric juice is marked. **Conclusions**: Application of immunomodulators on background of QST leads to increased eradication of HP and improved factors of specific and non-specific immune resistance, more pronounced at application Derinat®.

PS 183 STUDY OF I/D POLYMORPHISM OF ANGIOTENSINE CONVERTING ENZYME (ACE) IN CHRONIC HEPATITIS C: INFECTION, PROGRESSION AND RESPONSE TO THERAPY.

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Aim: To evaluate the association between ACE polymorphism and chronic hepatitis C (CHC) severity and response to antiviral therapy. **Introduction**: Angiotensin-II (AT-II) seems to play an important role in liver fibrogenesis through hepatic stellate cell proliferation and TGF-β1 up-regulation via AT-II type 1 receptor in vitro. Inhibiting the action of AT-II,results in a decrease of blood markers of hepatic fibrosis, TGF-β1 levels and hepatic fibrosis in NASH. The regulation of ACE (Angiotensin Converting Enzyme) activity by ACE I/D polymorphism in intron 16 is well-established, being the D allele associated with a higher serum ACE activity. **Methods**: Population samples: 127 CHC patients (38 females and 89 males - 50.2±12.3 and 43.4±10.3 years) and 643 controls (171 females and 472 males - 45.6±12.1 and 51.3±15.4 years). Exclusion criteria: alcohol intake . 40 g / day and metabolic disease. Liver fibrosis has been evaluated by biopsy and/or FibroScan (Peter Scheuer score) and steatosis (Brunt score) before and after treatment. Patients were divided in different groups according to fibrosis (0-1 vs 2-4); steatosis (0 vs 1-2 vs 3-4) and treatment response (sustained responder vs non-responders). ACE I/D polymorphism was evaluated by PCR. All statistical analysis were done using SPSS 16.0 and the significant level established for $p<0.05$.

Results: Genotype DD was significantly more prevalent among CHC patients compared to controls (72.4% vs 44.3%; OR=3.299, 95% CI [2.171-5.015]; p=0.000). After treatment the stage of liver fibrosis was associated with ACE I/D polymorphism with a higher frequency of ID and II carriers among patients with fibrosis 2 to 4 (48.5% vs 20.7%; OR=3.608, 95% CI [1.167-11.151]; p=0,044). No differences were found between steatosis groups and responders vs non-responders. **Conclusions**: Genotype DD was significantly more prevalent among CHC patients but ACE I allele may be a risk factor for liver fibrosis progression.

PS 183 Antibiotic resistance of Escherichia coli strains isolated from healthy food animals and tab water samples in Greece.

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Aim: The aim of our study was to investigate the antibiotic resistance of E. coli strains isolated from healthy food animals and from tab water samples in Greece. **Introduction**: In many European countries antibiotics are continuously added to animal feeds to promote growth and increase feed efficacy. Many retrospective studies proved that the introduction of an antibiotic in a farm increases not only the level of resistance of pathogenic, but also of commensal bacterial species. Studies on the transfer of commensal resistant bacteria from animals to human mainly concern Escherichia coli (E. coli). The latter is a symbiotic inhabitant of the intestine in all food animal species and it is also considered as the main indicator of fecal pollution in the microbiological surveillance of drinking water. **Methods**: Sixty five E. coli strains yielded from stools of healthy food animals were included in the study. The strains were categorized as follows: 19 from calves, 11 from birds, 19 from swine, and 16 from sheep. The sampling was performed directly from the rectum of the animals. Additionally 33 E. coli isolates cultured from tab water samples were also included in the study. Disc diffusion method was used to determine susceptibility to the following antibacterials: Penicillin (6μg), amoxicillin (25μg), tetracycline (30μg), spreptomycin (10μg), gentamycin (10μg), trimethoprime/ sulfamethoxazole (1,29/23,75μg), cefixime (5μg), ceptiofur (30μg), enrofloxacin (5μg), and chloramphenicol (30μg). Individual colonies were suspended in normal saline to 0.5 McFarland and using sterile swabs the suspensions were inoculated on Muller Hinton agar for 24 hr. E. coli ATCC 25922 was used as control strain. Breakpoints were determined according to EUCAST guidelines. **Results**: The 65 isolates of animal origin exhibited high resistances to 8 antibacterials. In details all 65 isolates were resistant to penicillin 42 were resistant to tetracycline, 27 to chloramphenicol, 39 to trimethoprim/sulfamethoxazole, 48 to streptomycin, 42 to amoxicillin, 23 to enrofloxacin and 22 to gentamycin. Twelve tetracycline resistant strains carried the tetracycline resistance gene tet(B). In contrast low resistances were observed to cephalosporin (7 to cefixime and 5 to ceptiofur respectively). Isolates from swine stools exhibited the highest antibiotic resistances. Briefly all 19 isolates were resistant to tetracycline, trimethoprim/sulfamethoxazole, streptomycin, and amoxicillin while 9 of 19 to enrofloxacin 15 of 19 to chloramphenicol, and 9 of 19 to gentamycin. The antibiotic profiles of the 33 E coli strains isolated from tab water samples were completely different. Resistances were observed only in 6 isolates to tetracycline and in 4 isolates to streptomycin. **Conclusions**: Food animals and tab water could be the vectors for the transmission of different bacteria to humans. Therefore we strongly support that the exposed resistances should be considered for further investigation.

NEUROSCIENCES *Session*

PS 5 Gamma knife treatment of growing Vestibular Schwannoma: Tumor control - A prospective study.

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Aim: In this study we wish to detail the success rate of GKRS, by comparing growth rates before and after GKRS treatment, and finding the proportion of tumors that get a successful treatment (defined as a negative or zero post-treatment growth rate). I**ntroduction**: Vestibular schwannoma (VS) is a benign tumor that arises in the eighth cranial nerve, giving symptoms of hearing loss, tinnitus, and vertigo. In general, it is a slow-growing tumor that in some cases even shows spontaneous regression. When and how to treat is debated, but in some centers, newly diagnosed small tumors are subject to conservative treatment, i.e. serial MRI scans without active treatment. In cases where the tumor has shown rapid growth in serial scans, or has become relatively large, treatment is usually offered. Two primary treatment methods prevail: microsurgery, where the tumor is physically removed; and gamma-knife radiosurgery (GKRS), where a gamma ray radiation dose is delivered to the tumor. This radiation dose, in theory, causes tumor growth arrest. **Methods**: Between 2000 and 2006, 347 patients were diagnosed with VS. From these, 159 (46%) received treatment by the end of 2007, while the remaining 190 (54%) were treated conservatively. A total of 41 (22%) of these conservatively treated patients later received GKRS treatment due to growth of the tumor, as detected by the serial MRI scans. These 41 patients were included in the study, and were followed for a minimum of two years after treatment. The tumor volume on both pre-treatment and posttreatment images were measured, and mixed effects models were used to analyze the growth rates before and after treatment. We also conducted a logistic regression analysis to determine whether the age of the patient or the growth rate before treatment are related to achieving successful treatment. **Results**: A mean pre-treatment growth rate corresponding to a volume doubling time (VDT) of 1.38 years was found, and a post-treatment VDT of -19.6 years. While the post-treatment growth rate is not significant, the difference in growth rate compared to the pre-treatment period is. A tumor control rate of 65% was found. Neither age nor growth rate was related to tumor control, with p-values >0.079. However, we do find that a tumor of volume 2.0 cm^3 has 11.43 times the odds of tumor control as that of an otherwise identical tumor with a volume of 1.0 cm^3. **Conclusions**: We found a lower tumor control rate for GKRS treatment of VS than previously reported. This finding is likely because other studies have included patients that had no growth potential, while our study includes only patients who have had previously documented growth. Larger tumors have a significantly greater odds of tumor control than smaller tumors. Details will be presented at the conference.

PS 20 Brain Derived Neurotrophic Factor (BDNF) impairs recovery of synaptic transmission after hypoxia, by a mechanism dependent on glutamate NMDA receptors.

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Aim: Access the role of NMDA receptors upon the noxious effects of the neurotrophin BDNF on synaptic transmission after hypoxia. **Introduction**: The pathology of stroke is dependent on the molecular events that determine neuron death or neuron survival. Brain Derived Neurothrophic Factor (BDNF), a member of the neurotrophin family, has been pointed out as an essential factor for the regulation of neuronal survival, differentiation, and cell death events. In addition to these long-lasting actions, BDNF has been shown to have presynaptic regulatory effects as well as postsynaptic regulatory actions. Relevant to our work is the ability of BDNF to selectively and rapidly enhance phosphorylation of NMDA receptors thus raising their opening probability and opening frequency. An ongoing project at our unit has shown that in hypoxic conditions BDNF impairs recovery of synaptic transmission on reoxygenation. This observation led us to investigate if the BDNF-induced impairment on the recovery of synaptic transmission after hypoxia is related to the activation of the NMDA component of the glutamatergic response, which is known to be involved in neuronal damage as a consequence of ischemic or hypoxic episodes. **Methods**: Field-excitatory post-synaptic potentials (fEP SP) were recorded from the CA1 area of hippocampal slices, taken from male Wistar rats (4-6 weeks old), in a recording chamber for submerged slices and continuously superfused with gassed bathing solution at 32ć. Drugs were added to the superfusion solution and hypoxia was induced by changing to an equivalent solution equilibrated with 95% N2 plus 5% CO 2. An hypoxic period of 90 min was used, followed by a reoxygenation period of 30 min. The neuroprotective effect of adenosine was prevented in all experimental conditions by adding DPCP X (50nM), an adenosine A1 receptor antagonist. AP-V(50µM), a NM DA receptor antagonist was also present to prevent NM DA receptor activation. In test slices, BDNF (20ng/ml) was added to the bathing solution for at least 20 min before hypoxia, being present up to the end of the recording period. Recovery from hypoxia in slices in the absence (control) and presence (test) of BDNF was compared. The data are expressed as mean ± SEM and mean differences were evaluated by unpaired t-test. Values of $p < 0.05$ were considered to represent statistically significant differences. **Results**: In control conditions, i.e. when only DPCPX and APV were present, the mean fEPSP slope measured at the end of reoxygenation was 98.01 ± 3.692% of baseline values, (N=8). When BDNF was added, mean fEP SP slope measured at the end of reoxygenation was 102.6 ± 5.792 % of baseline values (N=10). Statistical Analysis of mean fEP SP slope at the end of reoxygenation in BDNF and control conditions showed no statistically significant differences (P = 0,5336), suggesting that the impairment of recovery from hypoxia caused by BDNF requires activation of NMDA receptors. **Conclusions**: NMDA receptor antagonism prevents the noxious effects of BDNF upon synaptic transmission after hypoxia, thus implying NMDA receptors in the underlying mechanism of BDNF action.

PS 29 Influence of sex steroids on the neurochemical organization of the lateral posterior parvicellular division of the hypothalamic paraventricular nucleus

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Aim: This study aims to examine if there are sex differencs in the neurochemical phenotype of one of the pre-autonomic divisions of the hypothalamic paraventricular nucleus (PVN) that might contribute for the sexually dimorphic activity of the adrenal cortex. I**ntroduction**: It is well established that sex streoids influence the production of glucocorticoids by modulating the activity of the key elements of the hypothalamic-pituitary-adrenal axis (HP A axis). Females are known to produce more corticosterone than males, both in basal and in stress conditions, and this discrepancy is usually associated to sex-related features of the HPA axis. However, there is clinical and preclinical evidence of dissociation of adrenocorticotrophic hormone (ACTH) and glucocorticoid production, particularly during disease, which suggests that the activity of the adrenal cortex is regulated by factors, other than ACTH produced by the pituitary gland. The lateral posterior parvicellular division of the PVN (PVNlp) is one of the autonomic-related descending projections and its neurons express receptors for sex steroids. We will focus on this PVN division in order to find out if its neurons display gender-related differences that might influence the apparent dimorphic physiology of the adrenal cortex. **Methods**: Six-month-old male and female Wistar rats were maintained throughout the experiment under standard laboratory conditions. Solid diet and water were available ad libitum until the day of sacrifice. Fifteen days before the end of the experiment, half of the males and of the females were submitted to gonadectomy under deep anesthesia. Thirteen days later, the four groups - intact

males (n=6), intact females (n=6), orchidectomized males (n=6) and ovariectomized females (n=6) - were stereotaxically injected in the lateral ventricle with colchicine. After perfusion, the hypothalami were processed for immunocytochemistry. The total number of neurons immunoreactive for corticotrophin-releasing hormone (CRH), vasopressin (VP) and oxytocin (OXT) in the PVNlp was estimated using the optical fractionators method. Data were analyzed by using a 2-way analysis of variance with sex and gonadectomy as the independent variables. Whenever significant effects were detected, the Tukey HSD post hoc test was performed. **Results**: There were no sex differences in the total number of VP and OXT neurons. However, and in contrast to OXT neurons, the number of VP neurons was significantly reduced in gonadectomized males and females. The total number of CRH neurons was sexually dimorphic, with males containing approximately twice the number in females. The number of CRH neurons was significantly reduced by gonadectomy in males, but not in females. **Conclusions**: These results clearly show that the neurochemistry of the PVNlp differs between male and female rats, and that these sex differences are dependent on the circulating levels of sex steroid hormones. This indicates that the PVNlp might play a significant role in the establishment of the sexually dimorphic pattern that might characterize corticosterone production.

PS 77 Influence of sex steroid hormones in the aging process of the female hippocampal CA1 region

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Aim: This study aims to examine if the loss of regular estrous cyclicity plays a role in the establishment of the structural changes in the hippocampal CA1 region during aging. I**ntroduction**: Cognitive functions progressively decline in aging and hormone replacement therapy seems to improve memory and to reduce the risk of dementia in postmenopausal women. In rodents, beginning in middle age (9-12 months), estrous cycles begin to become irregular, and this transitional process is comparable with the menopausal transition in women. However, an important distinction is that the the loss of reproductive cycles in middle-aged rats does not occur with concomitant decline in estradiol concentrations but, instead, they become acyclic and have chronically high estradiol concentrations. Therefore, we tested the hypothesis that long-term repeated administration of estradiol and progesterone might prevent the decline of cognitive functions and the associated changes in the morphology of hippocampal neurons. For this purpose, we focused on the CA1 hippocampal region because its neurons are recognized targets for estrogens. **Methods**: Wistar female rats were allocated to the following groups: i) Adult group: control rats killed at 6 months; ii) Intact old group ("old"): after sham-ovariectomy at 12 months of age, rats received no further treatment; iii) Ovariectomized group ("OVX"): after ovariectomy at 12 months of age, rats received no further treatment; iv) Hormone replacement group ("HR"): after ovariectomy at 12 months, rats received a weekly s.c. injection of 10 µg 17beta-estradiol in 100 µl sesame oil followed, 48 h later, by 500 µg progesterone in 100 µl sesame oil. At the end of the experiments (6 or 24 months), spatial learning and spatial memory were evaluated using the Morris water maze. Then, rats were anesthetized and perfused. After being isolated, the hipoccampal formations were Golgi impregnated. The dendritic trees of CA1 pyramidal cells were drawn and the dendritic branching density, number of dendrites per cell, total dendritic length and mean length of the terminal segments estimated. **Results**: All old female rats showed impaired spatial learning and memory. In old rats there was a trend towards an elongation of the dendritic trees compared to adult, OVX and HR rats. In addition, the number of dendritic branches in the proximal part of the apical trees and in the distal part of the basal arborizations was higher in old females than in the remaining groups. **Conclusions**: These results demonstrate that the presence of persistently high circulating levels of estrogens in old females is a determining factor for the development of age-related changes in hippocampal CA1 pyramidal cells.

PS 79 Investigation into the T-lymphocyte response following Abeta42 immunisation in human Alzheimer's disease

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Aim: To investigate whether the (i) meningoencephalitis and (ii) pathological modifications following immunisation are CD4+ T-lymphocyte mediated. I**ntroduction**: Alzheimer's disease (AD) is a neurodegenerative disease associated with cognitive impairment and is characterised pathologically by amyloid-beta (Abeta) and tau protein deposits within the brain. The amyloid hypothesis places abnormal aggregation of Abeta at an early point in the pathogenesis of the disease, upstream of tau aggregation. Given the escalating social and economic burden of AD, an immunisation strategy has been developed to stimulate the clearance of Abeta deposits, with the ultimate aim being to improve cognitive function. Despite promising animal studies demonstrating Abeta removal and cognitive improvement, a human clinical trial was terminated because of a meningoencephalitis side-effect that was suspected to be T-lymphocyte mediated. Preliminary data indicates that CD8+ T-lymphocytes do not mediate the meningoencephalitis or the pathological modification following Abeta1-42 immunisation; however the role of CD4+ T-lymphocytes is unknown. **Methods**: CD3 (marker of CD4+ and CD8+ T-lymphocytes) immunohistochemistry was performed on formalinfixed paraffin-embedded sections from 28 control unimmunised (cAD) and 16 immunised AD (iAD) cases (AN1792, Elan Pharmaceuticals) in the medial frontal gyrus, superior middle temporal gyrus and inferior parietal lobule. T-lymphocytes were manually quantified with the location of T-lymphocytes being noted in areas of white matter, grey matter (including perivascular or parenchymal) and the meninges. Statistical analysis was performed using SPSS, and data compared to CD8+ T-lymphocytes and other pathological features previously obtained. **Results**: Analysis reveals no significant difference in the number of CD3+ T-lymphocytes between cAD and iAD cases. The CD3+ T-lymphocyte count in our meningoencephalitis case exceeds the respective upper quartiles for all anatomical areas in both cAD and iAD cases. In cAD cases, grey matter CD3+ T-lymphocyte number is significantly correlated with phosphorylated tau load (P=0.01). This correlation disappears after immunisation. Instead, grey matter CD3+ T-lymphocyte number was significantly correlated with a microglial marker of inflammation (P=0.007). **Conclusions**: TIn relation to the previous CD8 data, we can conclude that (i) CD4+ T-lymphocytes are involved in the meningoencephalitis side-effect and, (ii) T-lymphocytes are not implicated in the pathological modification following immunisation. The precise role of T-lymphocytes will determine if future therapies circumvent or exploit this adaptive immune response.

PS 101 Impact of neonatal administration of a neurotrophic-factor enriched diet on rat development

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Aim: To evaluate the effect of several diets enriched with neurotrophic factors on prosaposin, BDNF and TrkB receptor levels in the hypothalamus of mice. I**ntroduction**: Neurotrophins (NTs) such as Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), NT-3 and NT-4 have an important role in pre- and post-natal brain development. Neurotrophin receptors include two different types - the p75 and the Tropomyosin-Kinase (TrkA, TrkB and TrkC)(Hefti, 1997-Annu Rev Pharmacol Toxicol 37:239). BDNF in particular, through activation of TrkB receptors has been shown to have a key role in the central nervous system (CN S) development, neuronal homeostasis and cerebral processes related to synaptic plasticity, such as long-term potentiation (Tyler et al., 2002-Learn Mem 9:224; Yamada et al., 2002-Life Sci 70:735). In fact, changes in BDNF expression during development are associated with impaired brain development (Nishigori et al., 2008-Reprod Sci 15:895). Prosaposin, is another molecule with neurotrophic properties, essential for human neuronal cell survival, differentiation and maintenance, particularly in early

phases of brain development (Sikora et al., 2006-Acta Neuropathol 113:163). It has been described that the level of maturation of CN S in newborn rats in the first weeks of life is similar to a premature newborn human between 20-40 weeks post conception (Clancy et al., 2007-Neurotoxicology 28:931). Given that, neurotrophic factors and its receptors expression are crucial to the development of CNS. Since most of premature newborn human are fed with supplemented milk, we proposed to investigate the consequences of oral supplementation with different milk fractions, chosen by their neurotrophic properties, in the neonatal period on the hypothalamic levels of prosaposin, BDNF and its receptors. **Methods:** Sprague Dawley male rats were fed with different diets (water, 1, 2, 3) through a pipette from postnatal day (PN D) 2 through PN D10, 13, 15, 18, 21, 28, or 36. The rats were killed and the hypothalamus isolated, disrupted with a Teflon pestle in 0.32M sucrose-Tris pH 7.5 and supplemented with protease inhibitors. SDS-PAGE (12%), was used to separate proteins, which were transferred to nitrocellulose membranes. After blocking with 5% milk solution, blots were incubated overnight at 4ćC with primary antibodies for the proteins of interest. The membranes were incubated with secondary antibodies conjugated with horseradish peroxidase for 1h at room temperature. The proteins were detected using Super Signal substrate. **Results:** In hypothalamus taken from animals fed with water, there was a 2.2 fold increase in TrkB-FL levels from PN D13 to PN D28 and from PN D15 to PN D28 (n=4, p<0.01). Hypothalamus from PN D13 rats fed with diet 1 show a significant increase in BDNF levels (1.6 fold, n=6, p<0.05) and a slight increase in TrkB-FL receptor and prosaposine levels. **Conclusions:** We show that there are age-related changes in TrkB-FL receptor levels in the studied PNDs. We also demonstrated that an oral diet is able to significantly increase BDNF levels and slightly increase TrkBFL receptor and prosaposine levels. Since it is classically established that neurotrophins do not cross the blood-brain barrier, the present work demonstrates that peripherally administered substances can modulate the levels of neurotrophins in CNS. This evidence opens new perspectives on possible strategies to increase neurotrophins in the CNS.

PS 116 Effects of caffeine on startle reflex and prepulse inhibition (PPI) in the rats grouped by baseline PPI levels

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Aim: The purpose of this study is to investigate the role of caffeine, a widely used dopamine agonist and adenosine antagonist, on sensorimotor gating mechanism through a cross-species measurement termed prepulse inhibiton (PPI) in Sprague-Dawley rats. I**ntroduction:** The answer of an organism to a reflex-eliciting startle stimulus (pulse) is inhibited when it is preceded by a weaker stimulus (prepulse). This is termed prepulse inhibiton (PPI) and is a measurement of sensorimotor gating in central nervous system. This measurement has been found to be impaired in several neurological and psychiatric disorders, including schizophrenia, bipolar disorder, obsessive compulsive disorder and nocturnal enuresis. Direct or indirect agonists of dopaminergic system are widely used to generate decreases in PPI which can be reversed with antipsychotics. Since PPI level is also related to cognitive functionality of brain, not only antipsychotics but also widely used chemicals are analyzed as we intake them excessively on regular basis of our life. Caffeine is, surely, one of the most taken chemical which exists in coffee, tea, soda, chocolate etc. Even though we consume caffeine with lots of beverage and food today, prior studies which examine the relation of caffeine intake and PPI level were not so common and systematic in literature. **Methods:** All procedures used in this study were approved by the Local Ethical Committee. Twenty-four male adult (3-4 months) Sprague-Dawley rats were used in this study. Animals were handled and habituated to laboratory before starting the experiments. After that their baseline levels were measured. Rats have been grouped into as low, moderate and high groups according to their baseline PPI values at 78 dB prepulse stimuli. Following that caffeine (10 mg/kg) was administered subcutaneously to high and low groups. Right after the injection animals were placed to the Acoustic Startle Reflex System (SR-Lab, CA, USA). Startle magnitudes, PPI values and habituation responses were recorded during the study. **Results:** The caffeine administration did not make any significant effect on startle magnitude in both low and high group (p› 0.05). Caffeine had no effect on PPI in low group, however in the high group caffeine decreased PPI levels at 74 and 78 dB's of the two prepulse stimuli values (p<0.05). When the inter stimulus interval (ISI) between pre-pulse and pulse stimuli was decreased to 50 or increased to 500 ms, caffeine made no change in the low inhibition group, but it increased the PPI of the high inhibition group. When habituation responses were compared, caffeine did not make any significant effect on both low and high inhibitory groups. **Conclusions:** This study shows that the rats grouped by baseline PPI values give different responses to caffeine.

PS 117 Investigating the Role of Microglia in Gliomas Using an in vitro Model

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Aim: The aim of this study is to characterise an organotypic model of glioma using rat hippocampal slices, and to investigate the immune profile of this model. I**ntroduction:** Malignant gliomas are a heterogenous group of primary brain tumours, associated with a particularly poor prognosis. The role played by microglia in this condition is not fully understood, and has been the focus of recent research. Organotypic modelling has proven to be a useful in vitro tool to represent complex in vivo environments. **Methods:** Rat hippocampal slices were prepared and after 7 days, rat C6 tumour cells added to one group of theslice cultures. The trauma of the addition of cells was replicated in the sham group by the addition of cell free media, and a third group consisted of the control slices, to which nothing was added. At different time-points (D1, D3, D5 and D7), the cultures were stopped and immunohistochemistry undertaken on fixed slices using Iba1 (marker of microglia), CD68 (marker of microglial phagocytosis), and Ki67 (marker of proliferation). At the same time-points, RNA extraction, reverse transcription and gene amplification was performed to detect cytokines (IL1β, IL6, IL10, TNFα and TGFβ). **Results:** We reproduced an in vitro model of brain tumour, and have shown an increase in Iba1+ microglia associated with glioma cells (p=0.001). In addition the phagocytic activity of microglia, as determined by the ratio CD68:Iba1 (%), was significantly increased in the glioma model over the time of the experiment and compared with control groups (p<0.001). An increase in cell proliferation in the glioma group was observed at all time-points using the marker Ki67 (p<0.001). We also observed that the levels of mRNA of pro-inflammatory cytokines IL1β, IL6 and TNFalfa were reduced in the tumour group relative to control groups (p=‹0 .001). The levels of mRNA of TGFβ were found to be significantly higher than control on days 3 and 7 (p=0.001 and p=0.038 respectively). **Conclusions:** In our organotypic model of brain tumour, we have observed that glioma cell proliferation is associated with proliferation of microglia, an increase in microglia phagocytic activity, and a cytokine profile which overall is anti-inflammatory. Thus our model supports the role of microglia promoting tumour growth, and is suitable for further investigation and manipulation of the role of microglia in glioma.

PS 137 The effects of Abeta42 immunisation on microglia and neuroinflammation in human Alzheimer's disease

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Aim: To investigate the effects of Abeta42 immunisation on microglial activation and the consequences this has for neuroinflammation in the immunised AD brain. This will be done using Iba-1; a protein that has proven itself in animal and human studies to be a reliable and specific marker of microglia. I**ntroduction:** Alzheimer's disease (AD) is the leading cause of dementia worldwide and affects 35 million people. For over a century its pathogenesis and the factors involved in its inexorable progression have remained enigmatic. Although senile plaques and tau pathology are regarded as disease hallmarks,

clearance of some of these features following immunisation does not alter cognitive decline or mortality. Therefore other pathological features have come to the forefront. Microglial activation evoked by amyloid-beta (Abeta) has been implicated as the trigger for neuroinflammation. Evidence suggests that this inflammation is detrimental and contributes to the neurodegenerative process. Interestingly, studies following immunisation with Abeta42 have proposed a beneficial role for microglia in the clearance of the very plaques that activated them. **Methods**: Immunostaining against Iba-1 was performed in 11 immunised AD cases (iAD - AN1792, Elan Pharmaceuticals) and 28 unimmunised AD (cAD) cases. This was quantified by two methods (manual and computer analysis) and correlated with previous pathological data from the laboratory. **Results**: 1. Iba -1 protein load (%) is significantl y lower in iAD vs . cAD cas es (p=0.002). 2. There is no significant difference in the number of Iba-1 positive cells between iAD and cAD groups (p=0.435).

PS 163 Effect of hemopressin peptide fragments on G-protein activation in rat membrane homogenate

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Aim: Here we describe the detailed in vitro biochemical characterization of hemopressins towards opioid and cannabinoid receptors using receptor binding and G-protein activation assays performed in rat brain membrane. I**ntroduction**: The endogenous ligands of cannabinoid (CB) receptors are of lipid origin. A possible nonapeptide ligand for CB1 cannabinoid receptors was suggested from rodent brain or adipose tissue named as hemopressin with PVNFKFLSH sequence (Dale et al., 2005.). Hemopressin was shown to be selective ligand to CB1 cannabinoid receptors. This peptide derived from the alfa-chain of hemoglobin, also has a hipotensive effect, and inhibits peripheral hyperalgesic responses. **Methods**: Hemopressin nonapeptide and its C-terminally truncated heptapeptide fragment (PVNFKFL) were synthesized by solid phase peptide synthesis. **Results**: Using of opioid radioprobes revealed no binding affinity towards this system, while GTPïS assay, which mesures the agonist mediated G-protein activation, has demonstrated that hemopressins could activate the G-proteins. The stimulatory effect was inhibited by the specific cannabinoid receptor antagonist AM251, but not by the opioid receptor antagonist Naloxone. **Conclusions**: Our results indicate that hemopressins selectively interact with G-proteins coupled to the CB1 cannabinoid receptors.

PS 171 Synergistic roles of the proteasome and mitochondria in alpha-synuclein oligomerization: implications in Parkinson's disease

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Aim: The main goal of this work is to study the involvement of mitochondrial deregulation in the Ubiquitin-Proteasome System (UP S) impairment in different Parkinson´s Disease (PD) models. We will also evaluate the interplay between UPS and mitochondria in ubiquitin dependent alpha-synuclein clearance. I**ntroduction**: PD is the most common progressive neurodegenerative movement disorder, characterized by the selective loss of nigrostriatal dopaminergic neurons and the presence of intracellular insoluble proteinaceous inclusions, known as Lewy Bodies (LBs). Although PD etiopathogenesis remains elusive, the leading hypothesis for the death of specific groups of neurons establishes that mitochondrial dysfunction, UPS impairment, and protein ologomerization are major events that act synergistically causing this devastating disease. **Methods**: To characterize this process we used three different PD cell models: SH-SY5Y ndufa2 knockdown (KD) cells, PD cybrids and peripheral blood cells (PBC) of patients with diagnostic of PD. Each one was compared with the correspondent cellular control: SH-SY5Y cells, control cybrids and PBC of individuals who does not suffer of PD. For each model we proceed to study the mitochondrial electron transport chain (ETC) complex I activity, determined with NADH-ubiquinone oxidoreductase activity assay; measure 20S and 26S proteasome chymotrysin-like activity, using fluorimetric proteasomal activity analysis; and quantify ubiquitination and alpha-synuclein aggregation by Western Blot (WB). The first two cell models were incubated with lactacystin to work as a negative control of proteasomal function. Incubation with NDUFA antibody was used to quantify, by WB, NDUFA protein KD in SH-SY5Y ndufa2 KD cells. MTT reduction test was used to measure the cell viability. **Results**: Our data show that: mitochondrial ETC complex I activity was reduced in the three PD models used, as in SH-SY5Y cells treated with lactacystin; there was no significant reduction of 26S proteasome chymotrysin-like activity in the three PD models, but there was a surprising increase of 20S activity, transversal to the three models; SH-SY5Y ndufa2 KD cells and PD patient's lymphocytes shown a leaning to increased ubiquitination and alpha-synuclein oligomerization. Lactacystin concentration used does not affect cell viability. **Conclusions**: Our results suggest that mitochondrial dysfunction, transversal to these three chronic PD models, upregulate UPS function in an AT P independent manner. Mitochondrial dysfunction, through reduction of AT P synthesis and increase of ROS production, may increase protein missfolding and affect the ATP dependent degradation process of UPS, that is also the most susceptible to oxidative stress, resulting in ubiquitinated species accumulation. As a possible cell response, 20S proteasome chymotrysin-like activity seems to be up-regulated which could mean a mechanism of protein aggregation rescue, even if it does not stop alpha-synuclein oligomerization as shown in this work results. In the other way, inhibition of UPS in SH-SY5Y cells proved to impair mitochondrial function. These results suggest that the cross-talk between mitochondria and proteasome is likely to be a two ways dead-road inside the cell. This line of investigation together with the study of other protein quality control systems, as autophagy, may open a new window to PD therapeutics.

PS 185 Effect of the amyloid-beta peptide in GABA release from hippocampal synaptosomes: modulation by BDNF

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Aim: To determine the effect of amyloid-beta (Abeta) peptides in gamma-aminobutyric acid (GABA) release from rat hippocampal synaptosomes and how it is modulated by brain-derived neurotrophic factor (BDNF). I**ntroduction**: Alzheimer's disease (AD) is the main cause of dementia in the elderly, and the hippocampus is one of the most vulnerable brain regions (1). While the cause of AD is uncertain, several lines of evidence suggest Abeta peptides as having a causal role in its pathogenesis (2). Recent findings support a key role for GABAergic dysfunction in AD (1); however, the effect of Abeta upon GABA release in the hippocampus still remains unclear. BDNF is an endogenous glycoprotein from the neurotrophin family which affects neuronal growth, survival and differentiation (3). It also plays a role in synaptic plasticity and neuronal protection (4). BDNF inhibits GABA release from isolated hippocampal nerve terminals (synaptosomes) (3), but its influence on Abeta-induced dysfunction has not yet been elucidated. **Methods**: Rat hippocampus slices were incubated for 60min with or without Abeta (25µM) and 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 (20ng/ml), it was applied before the 2nd stimulation period. The eluent was collected in 2min fractions for liquid scintillation counting, as were the filters at the end of each experiment. BDNF effects were calculated as changes in S2/S1 ratios as compared with controls (no BDNF added) in the same synaptosomal batch. Abeta effect was calculated as changes in S1 area as compared with controls (no Abeta

incubation). Experiments were only considered for further analysis if the S2/S1 ratio in the absence of BDNF was between 0.8 and 1.2. Data is presented as mean±SEM for n experiments. The significance of the differences was calculated with the Students' T-test.. **Results**: The amount of GABA released during S1 from synaptosomes prepared from slices incubated with Abeta (1.69±0.19, n=18) was not significantly different ($p>0.05$) from that obtained in control conditions (1.54±0.13, n=14). BDNF decreased GABA release by 26.8±8.1% (n=5) in control conditions and by 21.1±2.6% in synaptosomes prepared from slices incubated with Abeta. Both BDNF-induced decreases were significantly different ($p<0.05$), but there was no significant difference between the decrease in control conditions and the decrease in synaptosomes prepared from slices incubated with Abeta ($p>0.05$). **Conclusions**: As reported in the past (Canas et al, 2004 [3]) and now seen by us, BDNF significantly decreases GABA release from synaptosomes prepared from hippocampal slices. Abeta treatment did not affect GABA release as well as it did not influence the inhibitory effect of BDNF.

PS 192 Effects of the antioxidant treatment with EGCG in the noradrenergic neurons of the A5 and A7 during Diabetes

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Aim: Evaluate the effects of antioxidant treatment with Epigallocathechin Gallate (EGCG), a potent antioxidant present in green tea, in the noradrenergic neurons of the A5 and A7 pontine cell groups in STZ-diabetic rats and in behavioral pain responses. I**ntroduction**: Diabetic neuropathy is a common complication of diabetes, which is accompanied by changes at the central and peripheral nervous system and altered pain sensations, namely chronic pain. Streptozotocin (STZ)-diabetic rats were shown to present neuronal hyperactivity at the spinal dorsal horn along with decrease in the numbers of noradrenergic neurons of the A5 and A7 pontine cell groups. Noradrenergic neurons from A5 and A7 project to the spinal dorsal horn and modulate nociceptive transmission by releasing noradrenaline and, accordingly, diabetic rats present lower levels of noradrenaline and higher behavioral responses to pain. The mechanisms underlying the decrease in the numbers of noradrenergic neurons in the brainstem during diabetes remain unclear. Since preliminary data shows that diabetes induces oxidative stress damage in neurons of those areas, we designed a study to evaluate the effects of antioxidant treatment with Epigallocathechin Gallate (EGCG), a potent antioxidant present in green tea, in the noradrenergic neurons of the A5 and A7 pontine cell groups in STZ-diabetic rats and in behavioral pain responses. **Methods**: Diabetes was induced by intraperitoneal injection of Streptozotocin (STZ) in male Wistar rats. A group of STZ rats was submitted to a ten week treatment with EGCG, administered in aqueous solution (2g/l; STZ+EGCG) since the third day after the induction of Diabetes. The other experimental groups were kept with normal ingestion of water during the same period (STZ+water and CTR). The behavioral responses to pain were evaluated before and in the end of the treatment, using the paw pressure test and the Von Frey test to determine mechanical hiperalgesia and tactile allodynia, respectively. In the end of this treatment, the animals were sacrificed by vascular perfusion. Noradrenergic neurons were identified by immunoreactions against Tyrosine Hydroxylase(TH), involved in the synthesis of noradrenaline, in transversal sections of the brainstem. The immunoreactive neurons were quantified separately in A5 and A7 of each experimental group. Data was compared with ANOVA followed by Tukey's post hoc test for multiple comparisons. **Results**: Rats treated with EGCG had hiperglicemia during all the experimental period and the treatment with EGCG didn't affect the values of glicemia. Mechanical hiperalgesia and tactile allodynia was observed in the non treated STZ rats. STZ rats treated with EGCG shown less severe mechanical hiperalgesia and normal tactile sensitivity. Occurred severe loss of noradrenergic neurons in the A5 and A7 of the non-treated STZ rats, in contrast with the STZ rats treated with EGCG where this lost was prevented. **Conclusions**: This study demonstrates that the antioxidant treatment with EGCG prevents loss of noradrenergic neurons in the A5 and A7 during diabetes, therefore contributing to an improvement of the behavioural responses to pain. The increase of antioxidant ingestion or its supplementation can be presented as preventive measures to neurodegenerative processes that affect noradrenergic neurons of the brainstem involved in pain modulation and, consequently, to changes in the painful sensitivity during diabetes.

PS 196 Antioxidant treatment of diabetic animals with EGCG prevents painful diabetic neuropathy and loss of serotoninergic brainstem neurons involved in descending modulation

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Aim: This study aimed to evaluate the effects of the antioxidant treatment with Epigallocatechin gallate (EGCG) on behavioral pain responses and on serotoninergic neuronal population of the RVM in streptozotocin (STZ)-diabetic rats. I**ntroduction**: Diabetes is accompanied by several complications which may include painful diabetic neuropathy. It is responsible for spontaneous pain, mechanical hyperalgesia and tactile allodynia and affects more than 25% of diabetic patients with neuropathy. Previous findings showed that during diabetes the numbers of serotoninergic neurons in the rostroventromedial medulla (RVM) decrease probably due to oxidative stress and neuronal death. Since serotoninergic RVM neurons are involved in descending nociceptive modulation, it is likely that this accounts for painful diabetic neuropathy. **Methods**: Diabetes was induced in male Wistar rats (250-300g) by an intraperitoneal injection of STZ. Control rats (CTR) received only the vehicle solution. Three days later, a group of STZ rats started a treatment with an aqueous solution of EGCG (2g/l; STZ+EGCG) during 10 weeks while the remaining experimental groups were given water (STZ+water and CTR). Before onset of treatment and at its completion, behavioral evaluation was performed using the paw pressure test and the dynamic plantar aesthesiometer for determination of mechanical hyperalgesia and tactile allodynia, respectively. RVM serotoninergic neurons were identified by immunohistochemistry against tryptophan hydroxylase (TpH), the rate-limiting enzyme in serotonin synthesis. The number of immunorreactive neurons for TpH (TpH-IR) was counted in RVM, which encompasses the raphe magnus nucleus and the gigantocellular pars alpha area. Means were compared by One-Way Analysis of Variance (ANOVA) followed by Tukey post hoc test for multiple comparisons. **Results**: STZ rats showed hyperglycaemia three days after injection and EGCG treatment had no effects on the hyperglycaemic condition. Treatment with EGCG prevented tactile allodynia and mechanical hyperalgesia detected in untreated STZ rats, along with the decrease in the number of TpH-IR neurons at the RVM. **Conclusions**: The results of the present study show that EGCG treatment prevents serotoninergic neuronal loss in the RVM in diabetes, which may explain the benefits of this treatment in behavioural response to pain. This study suggests that antioxidants may prevent the pathophysiological changes of serotoninergic descending pain modulation pathways during diabetes, giving rise to new perspectives on the development of therapeutic strategies for the treatment of painful diabetic neuropathy.

PS 203 Apoptosis upregulation in multiorgan lesions in tuberous sclerosis

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Aim: The aim of this study was to confirm whether apoptosis upregulation is characteristic to all types of TS-associated lesions. I**ntroduction**: Tuberous sclerosis (TS) is a disorder manifesting with formation

of benign tumors or hamartomas in numerous organ systems: e.g. heart, brain, skin, lungs etc. The disease is known to be caused by the mutation in one of two tumor-suppressor genes: TSC1 or TSC2, encoding hamartin or tuberin, respectively. In our recent studies we found that apoptosis may be upregulated in tumors forming in the progression of TS. **Methods**: We used samples characteristic for tumors or lesions occurring in TS: cardiac rhabdomyomas, subependymal giant cell astrocytomas (SEGA), subependymal nodules, renal angyomyolipoma and ungula fibromas. As positive control served samples of healthy tissue from respective organs. We evaluated the protein lysates by Western Blotting, using antibodies raised against apoptosis-related proteins, i.e. Bax and Bcl-2. **Results**: We demonstrated strong Bax expression in all evaluated samples, but not in healthy tissue controls. At the same time, Bcl-2 levels were not significantly altered. Equal protein loading was confirmed with tubulin staining. **Conclusions**: Proapoptotic events are sometimes found in specific types of tumors. Interestingly, however, all five types of lesions characteristic for TS show intensified apoptosis. This is unusual, since some of these lesions, e.g. ungual fibromas, are rather treated as benign tissue architecture disorders and do not form tumors. Also, some of these lesions, i.e. cardiac rhabdomyomas, subside spontaneously, while others, like SEGA, tend to grow with age and can finally transform into tumors requiring surgical intervention. Why apoptosis seems to outweigh proliferative processes in some TS-associated lesions, while it succumbs in others is a matter of debate.

PS 205 Tumorogenesis pathways in focal cortical dysplasia II B.

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Aim: The aim of this study was to evaluate activation of protein kinase B/Akt and extracellular signalregulated kinase (Erk) pathways in FCD II B and compare these results with activation of tumorogenic pathways in TS. **Introduction**: Focal cortical dysplasia (FCD) is a benign form of brain cortex deformation, belonging to the group of neuronal migration disorders occurring in the developing encephalon. Histopathologic image is characterized by aberrated brain cortex architecture. Clinically, the most frequent symptoms include drug-refractory epilepsy. From among four types of FCD, type IIB is the most advanced one, characterized by the presence of dysmorphic or immature neurons and balloon cells, called Taylor cells. The presence of the latter type is also typical for tumors occurring in tuberous sclerosis (TS), thus it is sometimes assumed that FCD IIB may be a local form of TS. **Methods**: We used samples of FCD II B, subependymal giant-cell astrocytomas (SEGA) and subependymal nodule (SEN), which is another brain lesion characteristic for TS. The research was performed by Western Blot method, using antibodies specific for phosphorylated kinases of Akt and Erk pathways. Brain samples excised during lobotomy were used as negative control. **Results**: Activation of the following proteins has been demonstrated: Erk, eIF4E; S6K1; Akt; GSK3ï¢; PDK1; MEK; RSK1. Also, we showed increased expression of cyclinD1 in tumor samples. **Conclusions**: 0Proteins phosphorylated in FCD IIB samples point to activation of Akt and Erk pathways. Also, activation of the above pathways in lesions occurring in TS, i.e. SEGA and SEN, has been demonstrated. Our results confirm similarity between FCD II B and TS.

PS 217 L-DOPA UPTAKE IN A CELLULAR MODEL OF DOPAMINERGIC NEURONS

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Aim: To characterize the transporter involved in L-DOPA uptake in these cells. **Introduction**: L-3,4-dihydroxyphenylalanine (L-DOP A) is the immediate product of the rate-limiting step in catecholamine biosynthesis and the precursor of all the endogenous catecholamines. Treatment with L-DOPA remains to date the most effective treatment for the slowness of movement, increased muscle tone and tremor, that are typical of Parkinson's disease (PD). The human neuroblastoma cell line SH-SY5Y has been widely used as a cellular model of dopaminergic neurons for PD research. In this study we investigated the transporter involved in L-DOPA uptake in these cells. **Methods**: L-DOPA levels in SH-SY5Y cells were evaluated by high performance liquid chromatography with electrochemical detection. Results are presented as arithmetic mean ± standard error mean. **Results**: SH-SY5Y cells take up L-DOPA in a time dependent (linear until 6 min) and concentration dependent (2.5-2500 µM) manner. Non-linear analysis of the saturation curves for L-DOPA revealed a KM (µM) of 570 ± 97 and a Vmax (nmol/mg protein/6 min) of 611 ± 34. The uptake of L-DOPA (2.5 µM) was reduced by the inhibitor of the L-type amino acid transporters 2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH , 0.1-1000 µM) (IC 50 = 47 ± 2 nM; Emax = 24 ± 10 % control uptake) and by neutralamino acids (1 mM), but not by the inhibitor of the A-type amino acid transporters N-(methylamino)-isobutyric acid (MeAIB, 0.1-1000 µM), nor by the acidic and basic amino acids (1 mM). L-DOPA uptake (2.5 µM) was unaltered by lowering the pH from 7.4 to 6.2. In the absence of Na+ there was a 20% reduction in the Vmax values for L-DOPA uptake. Accumulation of L-DOPA in SH-SY5Y cells was largely inhibited by the L-isomers of the small and large neutral amino acids (alanine, serine, threonine, cysteine, leucine, isoleucine, phenylalanine, methionine, and tyrosine), histidine, tryptophan, valine, asparagine and glutamine. Whereas the amino acids glycine, proline and the basic amino acid arginine also produced an inhibition of L-DOPA uptake, albeit minor, the basic amino acids lysine and cystine, and acidic amino acids aspartate and glutamate, did not inhibit the uptake of L-DOPA. **Conclusions**: L-DOPA uptake in SH-SY5Y cells was sensitive to inhibition by BCH, but not to MeAIB, and was moresensitive to inhibition by neutral than to basic or acidic amino acids. Although most of L-DOPA was entering the cells in a Na+-independent manner, a minor component of L-DOP A uptake (25%) was found to require extracellular Na+. In general, these findings support the view that L-DOP A may be transported by systems Bo (Na+-dependent) and L (Na+-independent). The fraction of L-DOP A that is handled through system L is through a high-affinity (KM values in the µM range) and pH-insensitive transport, which are characteristic of the LAT1.

PS 224 Addressing cell proliferation in the subependymal zone in the experimental autoimmune encephalomyelitis murine model of multiple sclerosis

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Aim: To address whether current treatment for multiple sclerosis, namely with natalizumab, influences cell proliferation in the subependymal zone (SEZ), in the experimental autoimmune encephalomyelitis murine model of multiple sclerosis (EAE). I**ntroduction**: The subependymal zone (SEZ) is one of the major sites of neurogenesis in the adult brain. While thesecells usually migrate through the rostral migratory stream towards the olfactory bulb, they can also bemobilized to other brain regions and differentiate into various cell types. Such seems to be the case in EAE where cells originating from the SEZ have been shown to originate oligodendrocytes that may regenerate damaged neurons. **Methods**: EAE was induced in female SJL mice. All mice were weighed and scored daily for the appearance and severity of the symptoms. Treatment with natalizumab (5mg/Kg), a humanized monoclonal antibody used as therapeutic approach to multiple sclerosis (MS) that blocks the passage of T and B cells from the immune system to the brain parenchyma start when the first sympthoms appeared. The control group received control rat IgG (control for the treatment). A control group for the EAE induction was also performed. Animals were sacrificed when symptoms were reverted in the treatment group, brains frozen and cut in 20 µm

slices for immunohistochemistry against Ki-67, a cellular proliferation marker. Sterological analysis was performed in the SEZ to determine proliferation and ectopic migration. **Results**: Present data show that natalizumab successfully reverted the EAE phenotype. Preliminary data suggest that the group induced with EAE and injected with IgG (that mimics a normal onset of the disease) showed a decreased number of Ki-67+ cells in the SEZ but showed an increase of its number in SEZ vicinity. Treatment with natalizumab leads to an increase in the proliferation rate of the SEZ and a decrease in the non-tangential migration of the cells from the SEZ comparatively with the IgG injected group. **Conclusions**: CNS lesions, as MS, modulate cell proliferation in the SEZ. We used a EAE relapse and remission model and the modulation of SEZ proliferation seems to be different from the already described EAE chronic models. It was also observed an increase in cell proliferation in the vicinity of SEZ, which may occur in order to repair the damaged foci. Finally, Natalizumab seems to increase cell proliferation in SEZ but slightly decreases in the vicinity.

PS 230 The antioxidant treatment with EGCG prevents pain and oxidative stress damage at the spinal cord of STZ-diabetic rats

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Aim: This study aimed to evaluate the effects of the treatment with Epigallocatechin Gallate (EGCG), a potent antioxidant present in green tea, at the behavioral pain responses and oxidative stress damage at the spinal cord of STZ-diabetic rats. I**ntroduction**: Diabetic neuropathy is a common complication underlying type I diabetes mellitus. Painful diabetic neuropathy - which is characterized by spontaneous pain, mechanical hyperalgesia and tactile allodynia - is a major disabling problem. The spinal dorsal horn is an important pain transmission relay and was shown to be affected during diabetes. Spinal nociceptive neurons were shown to be hyperactivated in STZ-diabetic rats, which seems to contribute to altered pain sensations. The mechanisms underlying spinal impairments remain unclear. Nevertheless it is likely that oxidative stress contributes to that impairment, based on previous findings showing increased oxidative stress damage at the spinal dorsal horn in STZ-diabetic rats and its reversal by an antioxidant treatment with alpha-lipoic acid, along with amelioration of pain responses. **Methods**: Diabetes was induced by intraperitoneal injection of STZ (60 mg/dl) in male Wistar rats. Control animals (CTR) received the vehicle solution. Three days post-injection one STZ-diabetic group started to receive EC GC (2g/L; STZ+EGCG) in the drinking water and the other maintained the normal water consumption (STZ+H20) during 10 weeks. Mechanical hyperalgesia and tactile allodynia were behaviourally evaluated by Randall-Sellito and dynamic plantar aesthesiometer, respectively, before diabetes induction, at 4 weeks post-injection and in the end of the treatment. Then, the animals were sacrificed and spinal cords were removed. Spinal sections were immunoreacted against 8-hydroxy-2'-deoxyguanosine (8-OH -dG), the marker of oxidative stress damage. The expression of 8-OH -dG was quantified by densitometry in 10 randomly taken spinal sections from the L4-L5 segment. Means were compared by ANOVA followed by the Tukey post hoc test for multiple comparisons. **Results**: STZ rats developed hyperglycemia, which was maintained until the end of the experiments and was not affected by treatment with EGCG. The paw withdrawal thresholds evaluated by Randall-Sellito test were significantly lower in STZ+H20 than in CTR and STZ+EGCG rats. Moreover, STZ+H20 rats developed tactile allodynia, which was not detected in CTR and STZ+EGCG rats. Expression of 8-OH –dG was significantly higher in STZ+H20 than in CTR and STZ+EGCG rats. **Conclusions**: These findings show that the treatment with EGCG prevents the oxidative stress damage at the spinal dorsal horn of STZ-diabetic rats. Moreover, it ameliorated the mechanical hyperalgesia and prevented the development of tactile allodynia, which is likely to be in part mediated by its spinal effects. The pain-related molecular and functional effects of EGCG at the spinal dorsal horn during diabetes will be studied in the future. Antioxidant treatment with EGCG should be, then, considered for the treatment of diabetic neuropathy.

PS 260 The involvement of nitric oxide in the antinociception induced by sucrose in mice

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Aim: In the present study we examined the modulation of tail-flick response by long term sucrose ingestion in mice and evaluated the possible involvement of L-arginine-NO pathway in this effect. The involvement of opioid receptors in sucrose analgesia was also examined. I**ntroduction**: Sweet-substance-induced analgesia has widely been studied, and the investigation of the neurotransmitters involved in this antinociceptive process is an important way for understanding the involvement of the neural system in controlling this kind of antinociception. Sucrose is a naturally occurring sweetener with analgesic effects in newborns. In systematic reviews, single doses of sucrose have been reported to reduce pain in newborns undergoing commonly performed medical procedures. National and international pain management guidelines promote the widespread use of sucrose. Sucrose has also shown to provoke analgesic effects when ingested for a relatively long period of time (14 days). This analgesic effect is dependent on the number of days of sucrose intake. Although not fully understood, the mechanism of action is thought to involve activation of the endogenous opioid system through taste. It has been suggested that there is a role for nitric oxide (NO) pathway on modulation of pain and inflammation in anim als and humans. However, both inhibitory and promotive actions of NO in nociception and pain have been reported. **Methods**: Male albino mice were randomly distributed into six groups of ten. The groups received tap water, tap water along with daily intraperitoneal injections of naltrexone (20mg/kg), tap water along with daily injections of N-nitro-L-arginine methyl ester (L-NAME) (10mg/kg), sucrose, sucrose along with daily injections of naltrexone, and sucrose along with daily injections of L-NAME respectively for 12 days. Baseline tail flick latencies (TFLs) were taken prior to starting treatment. After 12 days of treatment, TFL measurement was repeated and the mean values were calculated and compared. **Results**: The group receiving sucrose showed significant ($p<0.001$) antinociception compared to those receiving tap water alone or along with L-NAME or naltrexone. Administration of L-NAME with sucrose had no significant effect on sucrose antinociception. The group receiving sucrose along with naltrexone had %13 lower TFLs in comparison with those receiving sucrose alone ($p<0.05$). However, the former group also showed %15 higher values compared to the control group ($p<0.05$). There was no significant difference between groups receiving tap water alone or along with L-NAME or naltrexone. **Conclusions**: Sucrose ingestion showed marked analgesia in mice which was partially inhibited by opioid receptor antagonist but unmodified by the nitric oxide synthase inhibitor. Our findings support the hypothesis that sucrose may facilitate the release of endogenous opioid peptides that may ultimately be responsible for the observed antinociceptive effect. Contrary to previously studied pain models, the NO-cGMP had no role in the thermal hyperalgesia induced by the tail flick test. Based on our findings we recommend further studies on the involvement of NO in nociception in other animal and pain models.

PS 262 Could we use cortical connectivity analysis to monitor the anesthetic depth? A preliminary study.

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Aim: This preliminary study aims to explore the possibility of monitoring the anesthetic depth by analyzing the dynamics of cortical connectivity by means of electroencephalography. I**ntroduction**: Cortical connectivity is considered to be one of the most sensitive parameters that assess neural function. In this study we evaluated the changes in cortical connectivity induced by nociceptive stimulation during anesthesia in rats. **Methods**: In this experiment we used 5 Wistar rats of approximately 250-300g each. We induced and maintained anesthesia with chloral hydrate. The depth of anesthesia was estimated with the frontal median frequency (MEF). We main-

tained a basal ME F of 2 Hz. During the experiment we acquired the cortical electric activity through chronically implanted electrodes at the dura mater level. We used Biopac MP 150 systems for data acquisition from 2 montages on each side (frontal and parietal on both hemispheres). After a baseline recording of 5 minutes, we applied a nociceptive stimulus by mechanically clamping the left hind leg for a 1 minute timeframe. Afterwards, the electroencephalographic signal corresponding to the right hemisphere was analyzed. The cortical connectivity was assessed using the fronto-parietal index of ME F (ratio of the median frequencies) and the Pearson coefficient between the frontal and parietal electrocorticographic montages.The experiment was approved by the Ethic Committee of "Carol Davila" University of Medicine and Pharmacy. **Results**: For the baseline recordings, the fronto-parietal correlation calculated with the Pearson coefficient was 0.3 +/- 0.03 and the fronto-parietal index was 0.76 +/- 0.06. During the timeframe corresponding to the nociceptive stimulus the Pearson coefficient was 0.29 +/- 0.02 and the fronto-parietal index was 0.8 +/- 0.03. Comparison between these results show no statistical significance using the Student T-test ($p>0.05$). **Conclusions**: Our results show no significant change in cortical connectivity during nociceptive stimulation. Additional experiments should be carried through for further conclusive results.

PS 269 Prrxl1 Transcriptional Activity is Modulated by Phosphorylation.

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Aim: To further characterize the nature and sites of Prrxl1 post-translational modifications and their impact on its function. **Introduction**: The establishment of synaptic connections between the PNS and CNS is a well-researched theme, and many molecules have been identified as guidance factors for the correct synapse. Prrxl1 is a homeodomain transcription factor essential for the connectivity and survival of nociceptive neurons in the mouse embryo dorsal root ganglion (DRG) and spinal cord (SC). Prrxl1-/- mice display abnormalities in the spatio-temporal patterning of nociceptive afferent projections to the SC dorsal horn (dSC), defects in the development of dSC neurons and post-natal death of DRG small neurons (1,2). Prrxl1 displays a multiple band pattern on western-blots (WB) (3), which is abrogated by incubation with a phosphatase. **Methods**: Mice were dissected and nuclear extracts of DRG and dSC of mouse embryos (from embryonic day E12.5 to post-natal day P14) were prepared using a Triton-sucrose buffer and analysed by WB. This time-course analysis was further completed by 2D-electrophoresis of embryonic and post-natal dSC extracts. Furthermore, nuclei of ND7/23 cells (a DRG-derived cell-line with nociceptive properties) and embryonic dSC were incubated with a Ga3+ Immobilized metal ion affinity chromatography resin, with affinity for phosphopeptides. Constructs corresponding to truncated versions of Prrxl1 were generated by molecular cloning, and their WB band pattern, transcriptional activity and DNA-binding activity were assessed by luciferase-reporter assays and by a modified ELISA. CN Br Chemical cleavage of recombinant protein was performed to enhance this analysis. Moreover, site-directed mutagenesis was performed for an evolutionarily conserved putative phosphorylation site which was subjected to the same analysis. **Results**: Prrxl1 is highly phosphorylated and its phosphorylation state varies in a time and tissue specific manner. Phosphorylation seems to occur throughout the protein, clustering in the N-terminus (encompassing the DNA-binding domain) and in the C-terminus (containing a conserved putative regulatory domain). Analysis of evolutionary conserved sites has revealed a phosphorylation site in the homeodomain, whose mutation impairs transcriptional activation and DNA-binding. Luciferase reporter assays of truncated versions of Prrxl1 have identified the N-terminus as sufficient for the DNA-binding and transcriptional activity of Prrxl1. **Conclusions**: Altogether, our results show that Prrxl1 is phosphorylated at multiple sites. Moreover, these phosphorylations probably play a fundamental role in the regulation of Prrxl1 transcriptional activity in nociceptive neurons.

PS 278 Modulation of BDNF effect on glutamate release in Synaptosomes by amyloid-beta peptide.

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Aim: To study of the effect of the amyloid-beta (Abeta) peptide in glutamate release from rat hippocampal presynaptic terminals (synaptosomes) and how amyloid-beta peptide modulate brain-derived neurothrophic factor (BDNF) effect on glutamate release in synaptosomes. **Introduction**: Accumulation of amyloid-beta peptides is a constant feature associated with the Alzheimer's disease (AD). These peptides form aggregates and lead to degeneration of neurons in the neocortex, enthorhinal cortex and hippocampus, which use glutamate as a neurotransmitter. It has been proposed that a sustained increase in extracellular glutamate levels may be associated with the cognitive deficits and loss of neurons observed. Also, studies with hippocampus slices showed that Abeta peptide inhibits the uptake of glutamate and increases its extracellular levels (Kabogo et al., 2010). BDNF is an important neurotrophin for regulation of neuronal survival and differentiation. BDNF is also implicated in the modulation of synaptic transmission and plasticity. This neurotrophin is found decreased in AD (Peng et al., 2005), which leads to wondering if its action is modulated by the presence of Abeta. **Methods**: Slices were obtained from Wistar rat hippocampus: half were incubated for 1 hour with ï¢-amyloid 25-35 (25µM) and the remaining were the control experiment. They were then homogenized and centrifuged. The precipitate of the last centrifugation was resuspended in a Percoll solution and centrifuged. Then the supernatant was centrifuged two times with KHR solution. The synaptosomes were incubated with 0.2 mM [3H]-glutamate for 5 min and then distributed in perfusion chambers with glass microfiber filters. After 20 minutes washout samples were collected during 40 minutes with 2 minutes intervals. The synaptosomes were stimulated for 2 minutes with a concentrated solution of K+ added in min 5 (stimulation period, S1) and 29 (stimulation period, S2), to induce an increased release of glutamate. 20 ng/mL of BDNF (brain derived neurotrofic factor) were added in the 9th minute and its effect was quantified by the percentage change in the ratio S2/S1. **Results**: In synaptosomes with Abeta, the ratio S2/S1 (93.5%) decreased when compared to the control situation. Analyzing only S1 (without-the effect of BDNF), in the presence of Abeta there is a decrease of glutamate release. BDNF increased glutamate release by 21.2±4.5% (n=4) in control conditions and by 13.9 ± 20.9%(n=4) in synaptosomes prepared from slices incubated with Abeta ($p<0.05$ as compared with control). **Conclusions**: Regarding the effect of amyloid-beta protein in the release of glutamate, incubation of slices with amyloid-beta protein did not significantly alter the release of glutamate by synaptosomes. Given that the uptake inhibitors do not increase the release of glutamate in the superfusion technique this result was to be expected. Clarifying this relationship might be useful for a better understanding of the pathophysiology of AD and thus contribute to new therapeutic approaches. BDNF causes a smaller increase in the glutamate release in synaptosomes of slices with Abeta, when compared to slices with no Abeta. Therefore, this may indicate that Abeta might have a modulator effect of the BDNF effect.

PS 292 Mapping glycine receptor (GlyR) and glycine transporters (GlyT1 and GlyT2) in primary cortical astrocytes.

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Aim: Investigate the expression and subcellular localization of glycine receptor (GlyR) and transporters (GlyT1 and GlyT2) in astrocytes, derived from the cerebral cortex. Evaluate developmental changes, at mRNA and protein levels, between astrocytes at 10, 18 and 24 days in vitro (DIV). **Introduction**: GABA and Glycine are the main inhibitory

neurotransmitters in the central nervous system (CN S). Glycine-mediated neurotransmission, although well established at the caudal regions of the CNS, has been disregarded in the brain. However, in recent years neuronal glycine receptor (GlyR) expression was found in several brain regions, including hippocampus and cortex. GlyR form pentameric channels permeable to chloride ions. Neuronal GlyR is composed by subunits α (1-3) and β, but little information about glial GlyR is available. Other important elements of glycine-mediated synaptic activity are the glycine transporters (GlyT), which ensure glycine removal from the synaptic cleft. GlyT1 is located at the cellular membrane of glial cells, while GlyT2 is mostly present in glycinergic terminals. Given that glycine-mediated transmission is considered a potential alternative therapeutic target for the treatment of epilepsy, it is crucial to better understand the components of glycinergic transmission in the brain, in both neuronal and non neural cells. **Methods**: Primary astrocytic cultures were prepared from P0-P2 Sprague-Dawley pups. To assess mRNA expression of GlyR subunits, total RNA was extracted, reverse transcribed and amplified with specific primers for α1, α2, α3 and beta transcripts. Quantification, relative to transcript expression at 10 DIV, was performed with Pfafll's equation by normalization with an internal control gene, β-actin. Immunofluorescence studies were performed by plating the astrocytes in PDL-coated coverslips. At 10, 18 and 24 DIV cells were fixed (4% paraformaldehyde), permeabilized (0.1% Triton X-100, 0.1% gelatin in PBS), blocked (0.25% gelatin in PBS) and incubated with the primary and with the fluorescent-labelled secondary antibodies. Astrocytes were immunolabelled with Glial Fibrillary Acidic Protein (GFAP) and GlyR or GlyT. Nuclei were stained with DAPI (1:15000) and autofluorescence was removed by a brief incubation in 100 mM CuSO4. Images were acquired on an inverted confocal laser scanning microscope. **Results**: The qPC R results indicate that only GlyRα2 and GlyRβ subunit transcripts are present in astrocytes. At the protein level, immunofluorescence studies at the different developmental stages show that astrocytes express GlyRα2 mainly in the cell body. Double immunolabelling of astrocytes with GFAP and GlyT confirmed GlyT1 and GlyT2 expression and revealed developmental changes in their subcellular localization. At 10 DIV both transporters are mostly confined to the cell body, while at 18 and 24 DIV they can be found in the cell membrane and in many astrocytic processes. **Conclusions**: This study confirms the presence of GlyR in cortical astrocytes, both at mRNA and protein levels. It also shows a developmental regulated subcellular localization of GlyT. This knowledge is important to promote further investigation on the functional meaning of astrocytic GlyR and GlyT during both physiological and pathological processes.

ONCOLOGY & MOLECULAR BIOLOGY *Session*

PS 45 Heterologous Expression of Plasmodium falciparum IMPDH in *E. coli*.

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Aim: The study aims to investigate the heterologous expression of Plasmodium falciparum IMPDH in E.coli in the hope of obtaining a sufficient protein expression level that can be used for inhibitors screening. The hypothesis of the investigation is that the uptake of Plasmodium falciparum Inosine Monophosphate Dehydrogenase (IMPDH) gene into E.coli will lead to significant production levels of the parasite's IMPDH enzyme. I**ntroduction**: Malaria is a widespread vector-borne infectious disease that is caused by the parasite Plasmodium spp. Currently, there are a variety of anti-malarial drugs in clinical-use. Despite the availability of a wide range of anti-malaria drugs, the rapid development of drug resistance among parasites means that there is always a need of better anti-malaria drugs. Therefore, continuous research has been done by scientists to identify new malaria drug targets that allow the development of better anti-malaria therapies. One of the potential malaria drug targets is the parasite's IMPDH enzyme. IMPDH is essential for Guanosine Monophosphate (GMP) synthesis and subsequently the synthesis of Guanosine Triphosphate (GTP). Inhibition of IMPDH via an inhibitor can cause disruption of intracellular biochemical reactions and this will eventually lead to death of the parasite. **Methods**: Electrotransformation of pET-46 plasmid containing His-tagged Plasmodium falciparum IMPDH gene was done on E.coli strain BL21 DE3 Codon Plus which contains DE3 prophage that carries a T7 polymerase expression cassette, as well as LacI operon. The bacteria was then grow in culture and was induced with IPTG to allow the production of Plasmodium falciparum Inosine Monophosphate Dehydrogenase. Gravity-flow chromatography was then used to purified the proteins presence in the culture. The purified protein fractions were analysed using SDS-PAGE and the activity of the enzyme was analysed using fluorescence spectrometer. The experiment was repeated in E.coli strain Rosetta P-Lysate DE3 and E.coli strain JMP 109 DE3. The influence of different post-induction incubation temperature on the production of Plasmodium falciparum IMPDH was also investigated. Dot-Blot analysis, PCR and GFP purification were set up as control experiment. **Results**: SDS-PAGE analysis showed no significant indications that were able to prove the presence of Plasmodium falciparum IMPDH enzyme. In addition, Fluorescence spectrometer assays also demonstrated inconsistent and negligible enzyme activity. **Conclusions**: From the investigation, it can be concluded that the Plasmodium falciparum IMPDH gene does not express well in E.coli. The results were not sufficient to indicate the presence of Plasmodium falciparum IMPDH. In the control experiments, small amount of expressions were present albeit inconsistent. The study also proved E.coli to be an incompatible model organism for heterologous expression of Plasmodium falciparum IMPDH. Without a well-match expression system, Plasmodium falciparum IMPDH could not be evaluated as a suitable research target for potential drugs. Future investigations of alternative expression systems such as yeast may enable the identification of a suitable heterologous expression system for the Plasmodium falciparum IMPDH enzyme, facilitating the development of IMPDH as a tractable drug target.

PS 49 Studies on the putative involvement of an effect in metabolic substrate cellular uptake in the anticarcinogenic effect of clotrimazole at the intestinal epithelial level.

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Aim: The aim of this study was to investigate: (a) the anticarcinogenic effect of clotrimazole (CTZ) on tumoral and non-tumoral intestinal epithelial cell lines (Caco-2 and IEC-6 cells, respectively), by investigating its effect upon cell proliferation, viability and differentiation, alone or in conjunction with an inhibitor of mitochondrial oxidative phosphorylation (rhodamine123), and (b) the possibility of inhibition of the apical uptake of glucose or butyrate as a mechanism contributing to the anticarcinogenic effect of CTZ in Caco-2 cells. **Introduction**: Colorectal cancer is one of the most common solid tumours worldwide. Butyrate (BT) is one of the main end products of anaerobic bacterial fermentation of dietary fibre in the human colon and is an important metabolic substrate in normal colonic epithelial cells. BT becomes less essential for growth of neoplastic cells, which show an increase in the rate of glucose uptake and glycolysis, producing excessive lactic acid. CTZ is an antifungal drug that has demonstrated anticancer activity by inducing detachment of some glycolytic enzymes from cytoskeleton, thus inhibiting glycolysis. **Methods**: The effect of CTZ on cell proliferation, viability and differentiation, 3H-deoxyglucose (3H-DG), 3H-0methyl-glucose (3H-0MG) and 14C-butyrate (14C-BT) uptake and mRNA expression levels of glucose transporters was assessed. **Results**: In Caco-2 cells, CTZ showed anticarcinogenic activity, decreasing cellular viability and proliferation, and increasing cell differentiation. The effect of CTZ upon cell proliferation and viability was greatly potentiated in the presence of rhodamine123. In IEC-6 cells, CTZ also decreased cellular viability and proliferation, but increased cellular DNA synthesis rate and had no effect on cell differentiation. Exposure of Caco-2 cells to CTZ (10 μM) for 1 and 7 days increased (by 20-30%) the uptake of the glucose analogs 3H-deoxyglucose (3H-DG) and 3H-0-methylglucose (3H-0MG), respectively, but had no effect on the uptake of 14C-BT. The effect of CTZ upon 3H-DG and 3H-0MG uptake showed concentration-dependency and was maximal at 10 μM. CTZ did not alter the pharmacological characteristics of 3H-DG and 3H-0MG transport, but produced significant changes at the level of mRNA expression of facilitative glucose transporter 2 (GLUT2) and Na+-dependent glucose co-transporter (SGLT 1). **Conclusions**: CTZ exhibits anticarcinogenic effect both in tumoral (Caco-2) and non-tumoral (IEC-6) intestinal epithelial cell lines. In Caco-2 cells, the anticarcinogenic effect of CTZ was strongly potentiated by inhibition of oxidative phosphorylation. Moreover, stimulation of glucose membrane uptake might be a compensation mechanism in response to the inhibition of glycolysis induced by CTZ.

PS 94 PgP modulators may mediate Imatinib Resistance in Chronic myeloid Leukemia.

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Aim: The aim of this study is evaluated the potential therapeutic of Reversin 205 in overcoming the resistance to Imatinib. **Introduction**: Chronic myeloid leukaemia (CML) is a mieloproliferative disorder characterized by the presence of the BCR-ABL gene fusion, which encodes an oncoprotein with a deregulated tyrosine kinase activity. This molecular event contributes to the enhancement in cellular proliferation and resistance to apoptosis, becoming the main therapeutic target of the disease. In fact, the first-line treatment is Imatinib, a specific tyrosine kinase inhibitor that blocks the BCR-ABL tyrosine kinase activity. Although the good results with Imatinib, the development of drug resistance is a reality and can be mediated by different pathways, besides the BCR-ABL mutations, like the increase in expression of drug efflux ABC (ATP biding Cassette family) transporters. Since Imatinib is a substrate for P-glycoprotein (PgP) and ABCG2 (BCRP) transporters, decreasing their actions may induce drug accumulation in cancer cells. Reversin 205 is a PgP modulator and can be a new approach to overcome resistance to Imatinib. **Methods**: For this purpose, we used a CML cell line, the K562 cells, and generated two sub-cell lines resistant to Imatinib the K562-RC and K562-RD cells. To obtain these resistant cells, K562 cells were

exposed to the drug following two strategies: by continuous exposure of increasing concentrations of Imatinib (K562-RC) and by discontinuous exposure, with interchanged mediums with and without Imatinib (K562-RD). The half maximal inhibitory concentration (IC50) of K562 sensitive cells for Imatinib is 75nM, whereas for K562-RC cells this value is increased to the double (150nM) and in the case of K562-RD cells this value is 250nM. To evaluate the effect of Reversin 205 on cell viability, all the different cell lines treated in absence and presence different concentrations of the drug, were analysed by the Resazurin Assay. Cell death was determined by optical microscopy (May-Grunwald staining), by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining and by the expression levels of the activated caspase 3. The functionality of PgP was assessed using radiosensitizer (99mTc Sestamibi) kinetic studies. Drug efflux transporters expression levels, namely PgP and BCRP, was evaluated by FC using monoclonal antibodies. **Results**: We observe in K562-RD and K562-RC resistant cells an increase in PgP expression compared with the sensitive cell line, which may contribute to the resistance to Imatinib. However, K562-RC has a higher expression than K562-RD. The alterations in expression were followed with a higher radiosensitizer profile. On the other hand, and concordantly with these results, we found that the IC50 at 24 hours of exposure to Reversin 205 was 25µM to K562, 20 µM to K562-RD and 15 µM to K562-RC. This compound induced cell dead by apoptosis in a time- and dose- dependent manner, confirmed by morphological analysis and by the increase in Caspase 3 expression. **Conclusions**: In conclusion, our results show that PgP expression may be involved in imatinib resistance which may be circumventing by the PgP modulator Reversin 205, suggesting that this compound could be used as a new potential approach in the treatment of resistant and sensitive CML.

PS 97 Immunophenotype features of blasts in patients with acute myeloblastic leukemia.

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Aim: The aim of this study was to evaluate the correlation between immunophenotipic findings and morphological subtype of leukemia according to FAB classification. **Introduction**: Acute myeloblastic leukemia (AML) represents a clonal expansion of immature myeloid cells in a bone marrow. Clinical feature of AML is the consequence of impaired hematopoesis, and it is expressed like anemic, infectious or hemorrhagic syndrome. With the usage of monoclonal antibodies, specific for particular antigens on surface of the blasts, it is possible to identify the cell type that is prevalent in the malignant population. **Methods**: In the aim of this study, we have examined 30 patients of both sexes (7 females and 23 males) mean age 55.33 ï,± 9.49 years, treated in a period between February and November of year 2010, at the Department of Hematology, Clinical Center of Nis. The diagnosis was made after bone marrow puncture and findings of more than 30% of the immature elements in myelogram. At the same time, the samples were also taken for immunophenotyping. Results are shown as a percentage of positive cells on a specific CD antigen, and a comparative analysis in relation to FAB subtype is performed. **Results**: The highest value of CD34 was detected in AML Mo, significantly higher than in M1 ($p < 0.05$), M2 ($p < 0.01$), also as in M3, M4 and M5 ($p < 0.001$). The highest value of CD33 was detected in AML M1, significantly higher than in Mo ($p < 0.05$). The highest value of CD14 was observed in AML M5, significantly higher than Mo and M3 ($p < 0.05$). **Conclusions**: Immunophenotyping is a valuable method for differentiation of certain cell types in AML, especially in cases of poorly differentiated forms, or with nonspecific cytochemical features. The greatest expression of CD34 was found in Mo, with the primitive progenitors. High expression of CD33 was found in subtype M1 and M2-M5 subtypes. CD13, as a "clean" myeloid marker was highly exposed in all myeloid subtypes, with maximum expression in M3, on the promyelocytes. CD14 was highly positive in the M5 and M4 subtypes, with the dominant population of monoblast.

PS 135 Characterizing a TET-repressible ErbB2-expressing fibroblast line as a possible model for testing ErbB2 targeted therapies.

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Aim: Our objective was to characterize an ErbB2 expressing cell line that can serve as a model for antitumor therapies to better understand the keys of successful therapy in animal models. We've investigated the SADR cell line which is a derivative of NIH3T3 fibroblasts stably transfected with the ErbB2 gene under the negative control of tetracycline. **Introduction**: ErbB2 is a receptor tyrosine kinase underlying bad prognosis in several human malignancies. Trastuzumab, a humanized anti-ErbB2 antibody, is a specific targeted therapy against these tumors, with a history of both success and a high rate of therapy resistance. **Methods**: We studied the sensitivity of SADR cells to short (5 minutes) and long (3 days) trastuzumab (0.1 mg/ml) treatment in terms of proliferation, cell cycle, ErbB2 expression and phosphorylation, apoptosis, and cell morphology in native and in ErbB2-deprived (tetracycline treated) SADR cells. As in initial experiments we found a drift in the rate of proliferation inhibition, we also examined how the parameters in question changed during the aging of the cell line: experiments were made with cells from 3rd, 10th and 20th passages. Proliferation was assessed both from mitochondrial activity (MTT assay) and from cell culture impedance (xCELLigence system). DNA, ErbB1, pErbB2 and Annexin V were fluorescently labeled and measured in flow cytometry. For further characterization, we also assessed mRNA expression using a whole mouse genome chip. **Results**: The proliferation rate upon ErbB2 deprivation by tetracycline decreased at all passages, coherent with the proliferation advantage from excess ErbB2. In these cells, trastuzumab did not decrease the proliferation further, nor did it affect the cell cycle, thus confirming specific trastuzumab action targeting ErbB2. In native SADR cells not treated with tetracycline, trastuzumab caused a G1-block that was correlated with the passage number, also enhanced short term ErbB2 phosphorylation, and decreased ErbB2 expression in the long run. While in the earliest passage minimal effects were observed upon trastuzumab treatment, by the 20th passage all ErbB2-related effects have manifested. Apoptotic rate was low (under 1%), and treatment did not alter that. Long term trastuzumab treatment decreased the focus formation and increased spreading. The mRNA expression showed that both trastuzumab treatment and ErbB2 deprivation increase the expression of genes with antiangiogenic effect (thrombospondin, adamts5, connective tissue growth factor) and downregulate the level of those enhancing cell adhesion and focus formation. These confirm the observed morphological changes and the in vivo inhibition of tumor progression as well. **Conclusions**: The SADR cells appear to be not only a therapy target model, but also a model of addiction to the ErbB2 oncogene at the cellular level. The experiments also draw attention to the stability problems of transfected cellular systems.

PS 138 RNAi INDUCED DOWN-REGULATION OF FLT 3 EXPRESSION IN ACUTE MYELOID LEUKEMIA AND INCREASE SENSITIVITY TO the PROTEASOME INHIBITOR MG262.

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Aim: The aim of this study was to analyze the efficacy of a siRNA anti-FLT3 in monotherapy and in combination with the proteasome inhibitor (PI), MG262, in hematologic malignancies,particularly in AML. **Introduction**: Acute Myelogenous leukemia (AML) is one of the most common type of hematological malignancies, which is

characterized by the block in cell differentiation, increase in cell proliferation and accumulation of hematopoietic immature cells of the myeloid lineage in bone marrow and peripheral blood. FMS-like tyrosine kinase 3 (FLT 3) is a tyrosine kinase (RTK) receptor involved in the survival, proliferation and differentiation of hematopoietic stem cells. Constitutively activation of FLT 3 has been reported in approximately 30% of AML. This aberrant activation is responsible for predicting poor clinical outcome and may constitue a new therapeutic approach in this leukemia. **Methods**: For this purpose, we used an acute promyelocytic leukemia cell line, the HL-60 cells. The cells were culture in the absence and presence of 100 nM of the siRNA anti-FLT 3, delivered to cells by cationic liposomes, and/or with MG262 ranging from 1 nM to 100 nM. To evaluate the effect of siRNA-FLT 3 and MG262 on cell viability and proliferation, Resazurin and the Trypan Blue exclusion were used. Cell death was determined by optical microscopy (May-Grunwald staining) and by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining and the activated caspase 3 expression levels. We also analyzed FLT 3 expression using an antibody anti-FLT 3 by FC. **Results**: Ours results show in cells treated with 100nM of siRNA-FLT 3, after 24 hours of transfection, about 10% of cell death and 60% of grow inhibition. On the other hand we found that the half maximal inhibitory concentration (IC50) at 48 hours of exposure to MG262 was approximately 25 nM. However, when we combine the RNAsi-FLT 3 with 5 nM of MG262, we observe an increase in the antiproliferative effect and in the percentage of cell death (80% and 45%, respectively), which suggest that RNAsi may increase the sensitivity to the proteasome inhibitor MG262. Besides that, the cytotoxic effect is mediated mainly by apoptosis in a time-and dose-dependent manner. **Conclusions**: In conclusion, our results suggest that there is a synergistic effect between siRNA-FLT 3 and MG262, demonstrating that the inhibition of multiple pathways becomes more efficient, illustrating the potential benefit of new combined targeted therapeutic approaches for AML treatment.

PS 142 QUANTIFICATIO OF SURVIVIN-IMMUNOPOSITIVE CELL OF LYMPH FOLLICLES IN DIFFERENT TYPE OC CHRONIC TONSILLITIS.

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Aim: The aim of this paper was to show possible differences in the intensity of expression of this marker in the both CHT and RT by measuring numeral areal density of survivin-immunopositive cells in the germinative centers and the mantle zones of the lymph follicles in the chronic tonsillitis. **Introduction**: Chronic inflammations are frequent pathological states of the palatine tonsils. Specific entities are chronic hypertrophic tonsillitis (CHT) and recurrent tonsillitis (RT). During the inflammation in the lymph tissue are expressed many both pro- and anti-apoptotic factors, amongst which is survivin. Molecular mechanisms by which surviving acts are not so well known, but his role in the inhibition of apoptosis is known. **Methods**: Material consisted of tonsils taken after tonsillectomy from patients of both sexes, age between 10 and 29 years: 5 tonsils with RT and 5 tonsils with CHT. 5µm thick serial paraffin tissue slices were stained on hematoxylin-eosin and immunohystochemicall metod LSAB+/HRP with aplication of the antibody for survivin. Determination of numerical areal density (NA) of survivn-immunopositive cells, that is, average nummber of cells on 1mm2 tissue, in the lymph follicles of tonsils: germinative centres and mantle zones. The digital pictures were taken with Olympus BX-50. For the quantification of the cells program Image J was used. **Results**: By comprising the NA of survivin-immunopositive cells in the germinative centers and mantle zones of the lymph follicles of the tonsils with CHT and RT we found out that there is a statistically important difference in their number in the mantle zones, while there is no such difference in cell count found in the germinative centers. **Conclusions**: Survivin is expressed in the mantle zones and the germinative centers of the lymph follicles in the RT and CHT. Difference in the number of these cells in the mantle zones in RT and CHT might indicate that there is a different type of mechanism of expression of the secondary immune response in these two conditions.

PS 143 Significance of histopathological examination in diagnostics of chronical periapical lesions.

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Aim: Determine histopathological diagnosis of chronical periapical lesions, compare them with clinical diagnosis and classify them by size. **Introduction**: Preliminary diagnosis of chronical periapical lesions is determined by clinical symptoms and radiographs, but for definitive diagnosis histopathological examination is necessary. **Methods**: The examination included 34 patients with chronical periapical lesions diagnosed by clinical examination and radiographs. Removed chronical periapical lesions are processed by classical histological technique and histologicaly analyzed using hematoxylin & eosin stain. **Results**: By histological analysis is found that 53% of chronical periapical lesions are periapical granulomas and 47% radicular cysts. 70% of lesions were size < 9 mm, 18% 9 to 20 mm and 12% > 20 mm. After histopathological examination is determined that in 26% of all cases the clinical diagnosis were wrong. **Conclusions**: Statistically significant difference between clinical and histopathological diagnosis was confirmed.

PS 147 Effects of Chelidonium majus ethanolic extracts on viability and proliferation of different cell types in vitro.

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Aim: The aim of this study was to examine the effects of Chelidonium majus extracts on cell viability and proliferation of two different cell lines Hela and MDCK, respectively. **Introduction**: Chelidonium majus is the plant from the family Papaveraceae and its use in traditional medicine has been known since an ancient times. This plant is very rich in alkaloids and the most present are isoquinoline alkaloids such as sanguinarine, chelidonine, chelerythrine, berberine and coptisine. Other components that are isolated from this plant are flavonoids and phenolic acids. Extract of this plant and its components have several important pharmacological activities such as antiviral, antimicrobial, anti-inflammatory and antitumor activity. **Methods**: Two different ethanolic extracts of aerial parts of the plant C. majus were examined, one prepared by Soxhlet apparatus and the other one by ultrasonic procedure. They were diluted in the culture media IMDM (Iscove's Modified Eagle Medium) and examined in the following effective concentrations: 10 mcg/ml, 20 mcg/ml, 50 mcg/ml, 100 mcg/ml and 250 mcg/ml, for each type of extraction. The cells were seeded into the 96 well plates and cultivated for 24h in Dulbecco's Modified Eagle Medium (DMEM) at 37 °C in humidified 5% C02 atmosphere. Cells were incubated with extracts for 24h in the viability assay and 72h in the proliferation assay. Cells only with medium were used as a control. After an incubation period MTT test was performed. **Results**: In the presence of most of the examined concentrations, cells of both cell lines had a viability close to the negative control or slightly to moderately reduced. In this assay HeLa cells were more sensitive than MDCK cells which is especially pronounced at the highest concentration of both extracts. In the proliferation assay there are distinct differences in the effects of examined concentrations on this two cell types. All concentrations, except the lowest one, had antiproliferative activity on HeLa cells in a dose dependent manner. Only two of the highest examined concentrations of both extract types had an antiproliferative effect on MDCK cells in a dose dependent manner. Ultrasonic extract had a weaker antiproliferative effect on both cell lines than the extract prepared by Soxhlet apparatus. **Conclusions**: TBoth types of extract in examined concentrations markedly stronger act by reducing cell viability and proliferation of HeLa cancer cells compared to noncancerous MDCK cells.

PS 153 Vitamin C: a new use for a well-known compound?

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Aim: The main purpose of this study is to assess the potential of vitamin C as a new approach on cancer treatment. **Introduction**: The role of antioxidant vitamins, diet and lifestyle modifications in modulating human cancer incidence has drawn significant attention from basic and clinical scientists. Vitamin C has been long known for its anti-oxidant properties that may be related with is significant benefit on cancer, as a chemoprotective agent and/or as a therapeutic approach. Although the results are scattered, and no unifying hypotheses to explain the mechanisms of action of these vitamin in vitro or in vivo have been proposed. Vitamin C exists in two forms: ascorbate (AA) and dehydroascorbate (DHA). This water-soluble vitamin is a potent antioxidant, but, depending on concentration, may also have pro-oxidant and even mutagenic effects in the presence of transition metals. Myelodysplastic syndromes (MDS) are a heterogeneous stem cell bone marrow disorders characterized by dysplasia, peripheral cytopenias, ineffective haematopoiesis due to excessive apoptosis and abnormal proliferation of blasts in bone marrow. These diseases affects older adults and are associated with high risk of progression to acute leukaemia with a short overall survival and resistance to conventional therapies.. For most MDS patients the only potentially curative treatment is bone marrow transplant. On the other hand, Diffuse Large B Cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) accounting for up to 40% of all NHL cases. It is a clinically aggressive disease with an incidence peak at around 60 years old with mean 5-year-survival of only up to 50%. Its current therapeutic approach is based in chemotherapy regimens that despite major advances are yet highly toxic and with a fairly reduced efficacy. **Methods**: To achieve our goal we used well-established MDS and DLBCL cell lines, the cells F36P and FARAGE , respectively. We incubated these cell lines in the absence and presence of AA and DHA, in single dose, in a daily dose administration and in association with conventional anticarcinogenic agents (cytarabine, ARA-C; doxorubicin, DOX0). Cell viability and death was determined by the trypan blue assay, optical microscopy and by flow cytometry (FC) using annexin-V/propidium iodide stain and caspase 3 expression levels. We also evaluated mitochondrial membrane potential, reactive oxygen species levels (hydrogen peroxide, H_2O_2; superoxide anion, $O_2^{\bullet-}$) and the antioxidant defense, Reduced Glutathione (GSH), by FC using JC1, 2,7-diclorofluorescein, dihidroetidium and mercury orange, respectively. **Results**: Our results show that both AA and DHA induce a decrease in cell viability and proliferation in a time, dose and cell type dependent manner with IC50 values ranging from 1,5 mM with AA and 1 mM for the FARAGE cells and values of 2,5 mM (AA) and 1 mM (DHA) for the F36P cells. In the presence of AA and DHA, cells exhibited typical apoptotic morphology that may be related with the observed increase in caspase 3 expression levels. We also observed that Vitamin C induced apoptosis by increased ROS production and mitochondrial membrane potential depolarization. **Conclusions**: In conclusion, our results suggest that Vitamin C may be used as a new therapeutic approach on cancer treatment, namely in MDS and DLBCL in monotherapy or as adjuvant to conventional chemotherapy.

PS 156 Abnormalities lymphocyte populations in haematological malignancies.

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Aim: The aim of this study was evaluate the role of human lymphocytes subpopulations in evolution and prognosis of several haematological malignancies, namely chronic lymphocytic leukaemia (CLL), multiple myeloma (MM), myelodisplastic syndromes (MDS) and chronic myelogenous leukaemia (CML). **Introduction**: For a long time, scientists believed that the immune system acted to fight cancer development. However, recent findings demonstrate that the immune system also acts to promote cancer progression. In fact, it has been proposed that when some types of immune cells encounter tumour cells, they can also cause inflammation. However, when compared to normal injury, these immune cells often do not withdraw, but rather generate an enduring chronic inflammation, stimulating cell proliferation and activating angiogenesis, invasion and metastization. Human lymphocytes are classified in three main populations according to their biological function and cell surface antigen expression: T lymphocytes, B lymphocytes and natural killer (NK) cells. T lymphocytes can be subdivided as well in functionally different populations. The most clearly defined of these are helper/inducer T cells and suppressor/cytotoxic T cells. B lymphocytes are the producers of antibodies; they mediate humoral immunity particularly effective against toxins, whole bacteria, and free viruses. NK cells mediate cytotoxicity against certain tumors and virus-infected cells. Abnormalities in lymphocyte populations have been documented in patients with haematological malignancies. However, the role of these abnormalities is not clarified. **Methods**: To attaint this objectives we studied a total of 47 newly diagnosed patients (CLL=5 pts; MM=18 pts; MDS=10 pts; CML= 11 pts) and 3 healthy donors. Using flow cytometry, we determined simultaneously in peripheral blood and/or bone marrow the major lymphocyte subpopulations, including the total number of T lymphocytes (CD3+), B lymphocytes (CD19+) and natural killer cells (CD3-/CD56+) as well as helper/inducer (CD3+/CD4+) and suppressor/cytotoxic (CD3+/CD8+) T lymphocyte subsets. **Results**: Our results show that CLL patients have a significant increase of CD8+ T cells in peripheral blood compared to healthy controls. On the other hand, CD4+ T cells were decreased in CLL, MM and CML. In this context, CD4+/CD8+ ratio were decrease in CLL, MM and CML patients, while an increase were observed in MDS, suggesting that the abnormal lymphocytes distribution may contribute to the neoplastic process. **Conclusions**: Since haematological malignancies evolution may be associated with a number of phenotypic and functional alterations in lymphocytes populations, cellular immunity might influence patient outcome, response to antineoplastic treatment and, consequently, the prognosis.

PS 161 The effect of chenodeoxycholic acid on butyrate uptake in non-tumoral and tumoral intestinal epithelial cell lines.

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Aim: The aim of this study was to characterize the effect of the bile acid CDCA upon BT uptake and upon cellular viability, proliferation and differentiation, in non-tumoral (IEC-6) and tumoral (Caco-2) intestinal epithelial cell lines. **Introduction**: Colorectal cancer (CRC) is one of the most common solid tumours worldwide. Although the causes of CRC are multifactorial, a diet high in dietary fibre and low in saturated fats is associated with a reduced risk of this pathology. Butyrate (BT), one of the main end products of dietary fibre bacterial fermentation in the human colon, is known to play an important role in colonic epithelium homeostasis, being able to prevent/inhibit colon carcinogenesis. BT is transported into colonic epithelial cells mainly by two carriermediated transport systems, the monocarboxylate transporter 1 (MCT1) and the sodium-coupled monocarboxylate transporter 1 (SMCT1), both being proposed to function as tumour suppressors. Epidemiological and experimental studies (both in vitro and in vivo) suggest that bile acids may play

a role in the aetiology of CRC. A significantly higher concentration of the primary bile acid chenodeoxycholic acid (CDCA) was observed in patients with CRC/adenoma, and CDCA was also shown to be tumour promoting in animal studies. **Methods:** 14C-BT uptake by Caco-2 and IEC-6 cell lines was quantified by liquid scintillometry, mRNA expression levels of MCT1 and of SMCT1 transporters were quantified by qRT-PCR and the effects of CDCA and BT on cell viability, proliferation and differentiation were quantified by the lactate dehydrogenase assay, sulforhodamine B assay and alkaline phosphatase activity assay, respectively. **Results:** Incubation with CDCA for 2 days did not affect 14C-BT uptake in Caco-2 cells while in IEC-6 cells resulted in a marked inhibition of 14C-BT uptake in a concentration-dependent manner (IC50=120 μM) and this effect was quantitatively similar from 1 to 7 days of exposure. CDCA (100 μM) acted as a competitive inhibitor of 14C-BT uptake for low concentrations of 14C-BT and showed no effect on cell viability (LDH and MTT assays) or proliferation (SRB assay). CDCA decreased both MCT1- and SMCT1-mediated 14C-BT transport and caused significantly increased SMCT1 mRNA expression levels. 14C-BT uptake was found to be stimulated by CaM, CaMKII and PKC and inhibitied by PKA. Inhibition of 14C-BT uptake by CDCA was dependent on CaM, MAP kinase (Erk 1/2 and p38 pathways) and PKC activation. Furthermore, CDCA was able to partially reduce the BT-mediated decrease in cell proliferation and the increase of differentiation of IEC-6 cells. **Conclusions:** In conclusion, our results suggest that inhibition of BT uptake in the intestinal epithelium may contribute to the procarcinogenic effect of the primary bile acid CDCA at this level.

PS 182 Clinical Relations of Methotrexate Pharmacokinetics in Pediatric Osteosarcoma.

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Aim: Our objectives were to analyze the relationship between exposure to HD-MTX and toxicity, and to assess correlations between pharmacokinetic parameters and the survival of osteosarcoma. **Introduction:** High-dose methotrexate (HD-MTX) with leucovorin rescue is widely used to treat osteosarcoma, which predominantly occurs in children. Methotrexate efficiency and toxicity is unpredictable and shows large variability.Peak serum concentrations of methotrexate and the area under the concentration-time curve (AUC) have been reported to correlate with outcome, however, no such relations have been set up in the regard of toxicity. **Methods:** Pharmacokinetic data of 101 patients treated with 865 HD-MTX courses were evaluated. Pharmacokinetic parameters (MTX clearance and AUC) were calculated based on methotrexate serum levels measured at 6, 24, 36, 48 hours after the initiation of the infusion by high pressure liquid chromatography (HPLC) technique. Clinical data were collected by retrospective chart review. Toxicity parameters were categorized according to Common Toxicity Criteria and MTX dose intensity was calculated. Data were analysed by Student's t-test, Mann-Whitney U test and chi square test (StatSoft's STATISTICA v8.0). Event-free survival (EFS) and overall survival (OS) were estimated according to the Kaplan-Meier method. All results with a p-value of less than 0.05 were considered statistically significant. **Results:** Patients with serious hepatotoxicity had significantly higher mean peak MTX concentrations, 24 h and 48 h MTX serum levels and AUC(0-48h), and significantly lower MTX clearance. No significant association was found between toxicity and age, gender, presence of metastases or histologic tumor response. Patients with higher 24h and 48h methotrexate serum levels had significantly better overall and eventfree survival. Higher dose intensity was associated with a greater probability of event-free survival. There was no association between presence of toxicity and survival. **Conclusions:** Higher MTX exposure leads to higher incidence of hepatotoxicity. Higher serum concentrations at 24 and 48 hours result in a better 5-year OS and EFS. These results suggest that higher MTX exposure may lead to serious side effects, but it also improves treatment outcome.

PS 187 Establishment of direct bladder cancer xenografts in nude mice.

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Aim: The aim of this project was to develop a direct xenografic model of invasive urothelial bladder carcinoma to study the tumoral response to cisplatin and gemcitabine in vivo. To guarantee the utility and validity of this model, the reproducibility and maintenance of the phenotype of the original tumor were assessed through the comparison of the histologic type and grade and expression of several tumor markers (immunohistochemistry). With this model we will be able to select the best cancer treatment based on responsiveness of individual tumors and have the possibility of tailor treatment to individual patients—using effective agents while sparing unnecessary ones. **Introduction:** Transitional cell carcinomas (TCC) are the most common urothelial tumors in western countries and approximately 25% of patients will, at the time of diagnosis or later, present with muscle invasive or locally advanced bladder cancer. The standard therapy for these patients is radical cistectomy and chemortherapy with therapeutic regimens containing cisplatin, MVAC (methotrexate, vinblastine, dosurubicin and cisplatin) or GC (gencitabine and cisplatine). However both regimens carry risk of significant toxicity and toxic deaths and a substantial number of patients will suffer from adverse reactions without achieving any benefits. Unpredictable outcomes from empiric therapy in patients with the same tumor type and stage is a widely recognized and frustrating problem for patients, their caregivers, and medical professionals There has been a long-standing hope that new markers and approaches could be used to optimize therapy for each patient. Established human cancer cell lines, primary cultures of patient tumor specimens and animal models have been used to evaluate the activity of chemotherapeutic agents. However, these models showed some limitations in terms of correlation with clinical results and it's predictive value The use of direct xenografting of human cancers allows the transplantation of all cellular fractions of the tumor, maintaining the original cell heterogeneity, tumor phenotype and malignat potential. **Methods:** Tumor fragments of invasive bladder cancer obtained after patient cystectomy were implanted subcutaneously into nude mice, and xenograft tumors were passed through 2 more mice generations before drug testing. At each passage, histopathology, CD147, TP5, P63, CD20, and Ki-67 molecules were examined by immunohistochemical analysis to evaluate the preservation of original features and tumor. **Results:** Tumor growth was observed in 1 mice (11%, 1/9) after 13 weeks of lag period, and reached a volume of 1,5 cm3 at 18 weeks, when it was excised and implanted in new mice. Histological and immunohistochemically, the tumor developed was similar to the original tumor. At the moment, this xenograft was passed from 2 generations. In the second generation, tumor growth was observed in 2 mice (66%, 2/3) at 5 and 9 weeks following transplantation. **Conclusions:** With these preliminary results we proved that it is possible growth urothelial bladder tumors in nude mice maintaining the original phenotype and immunohistochemical staining pattern. However more studies will be needed to understand the factors behind the low tumor take and eventually improve the success of xenotransplant. At the end of the project we hope to find some association between the molecular markers analyzed and the aggressiveness and chemo-resistance of the tumor.

PS 190 Expression of Metalloproteinases-2, -8 and -9 in Monoclonal Gammopathies.

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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. **Introduction:** 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. Multiple myeloma is a B-cell cancer 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 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 α 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. Moreover, malignant cells can be detected in peripheral blood of many patients with MM, suggesting migration of myeloma cells outside the bone marrow. However, the role of MMPs in the development of MM is poorly understood. **Methods:** A total of newly diagnosed 5 MGUS patients and 7 symptomatic MM patients 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. **Results:** Our preliminary study, shows that MM patients have higher percentage of PC expressing MMPs when compared to MGUS patients. On the other hand, 60%, 80% and 60% of MGUS patients and 50%, 100% and 88% of MM patients have positive staining for MMP-2, MMP-8 and MMP-9, respectively. In this subset of patients, 60% of MGUS and 100% of MM patients are at least positive for two MMPs. However, intracellular MMPs expression levels were higher in MGUS. **Conclusions:** 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 191 The RAS/RAF and AKT/mTOR pathways as therapeutic targets in diffuse large b-cells lymphoma.

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Aim: The aim of this study is to evaluate the potential therapeutic of an mTOR inhibitor (Everolimus) and a RAS/RAF pathway inhibitor (L744, 832) in DLBCL. **Introduction:** Diffuse large B-cell lymphoma (DLBCL) is a common type of non-Hodgkin lymphoma, accounting for about 25-30% of all cases in the western countries. It is a clinically aggressive lymphoma in which the patients have a five-year survival rate of 50%. Several intracellular pathways are related to lymphomagenesis and two of the most frequently involved are the BCR/PI3K/AKT/mTOR pathway (B-Cell-Receptor/ Phosphatidylinositol-3-kinase/AKT protein kinase B/Mammalian Target of Rapamicin) and RAS/MAPK pathway (MAPK, Mitogen Activated Protein Kinase, associated with the RAS-RAF proteins). On the other hand, RAS proteins are activated by farnesylation mediated by the farnesyltranferase enzyme. **Methods:** For this purpose we used the a DLCL cell line, the Farage cells, cultured in the absence and presence of several concentrations of Everolimus and of L744,832, in monotherapy and in association with each other and with conventional chemotherapy drugs (Vincristine). Cell growth and viability were evaluated by the rezasurin assay. The effectiveness of the drugs was determined by dose-response curve and IC50. Cell death was investigated by optical microscopy using the May-Grunwald staining, and by flow cytometry, through the annexin-V and propidium iodide double staining. The mechanisms involved in the antiproliferative effect and in cell dead were analyzed by flow cytometry by the expression of Cyclin D1 and proteins involved in apoptotic pathways, namely, Caspase 3, Cytochrome c, Lamin A/C, Bcl-2 and Bax. **Results:** Our preliminary results show that Everolimus and L744,832, induced cell death in a time- and dosedependent manner, with IC50 values of 500nM after 48h for Everolimus and ranging from 50μM to 75μM after 24h for L744,832. These compounds induced cell dead mainly by apoptosis that may be mediated by caspase 3. **Conclusions:** In summary, our results suggest that Everolimus and L744 might be used as a new approach on DLCL treatment.

PS 204 Implication of mammalian target of rapamycin in children with Choroid Plexus Papilloma.

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Aim: The main target of the Project was to confirm activation of kinases belonging to extracellular signal -regulated kinase (Erk) pathway, e.g. pMEK, TRKA/B and mammalian target of rapamycin (mTOR) effectors: 4EBP1 and pS6K wchich are often found in brain tumors. **Introduction:** Choroid plexus papilloma (CPP) is a rare intracranial tumor located in the ventricular system of the choroid plexus, mostly in lateral ventricles. It may disrupt flow of cerebrospinal fluid and be the reason of increased intracranial pressure. CPP commonly affects young children under 5 years of age and accounts for 0.9-2 % of all brain neoplasms. At least 50% of cases are found in children under 2 years. The tumor may be formed by epithelial cells of choroid plexus. So far, there were no reports on the activation of Erk (extraxellular signal -regulated kinase) in CPP, while this kinase triggers mTor signaling cascade and takes part in carcinogenesis in many types of brain tumors. **Methods:** We evaluated activation of kinases belonging to Erk pathway as well as mTor signaling effectors: 4EBP1 and ribosomal protein S6 by Western Blot method, using phosphospecific antibodies, binding only phosphorylated, active protein forms. The research was performed on human choroid plexus papilloma tumors resected during elective surgery. **Results:** We demonstrated activation of mTOR kinase pathway and related Erk signaling pathway. Also, we confirmed increase of phosphorylation of 4EBP1 and pS6K responsible for protein translation. Excessive activation of mTOR downstream effectors may result in uncontrolled proliferation. **Conclusions:** mTOR kinase is a central regulator of cellular state and processes like: autophagy, angiogenesis and proliferation. Erk pathway lays upstream of mTOR kinase. Excessive activation of this kinase may lead to uncontrolled proliferation and result in tumorgenesis. So far, there have been no data on mTor activation in CPC. In the current study we focused on confirmation of Erk pathway wchich may be a possible mechanism of tumorgenesis in CPP.

PS 206 Epigenetic modifications in Hepatocellular carcinoma: The potential role of epigenetic modulators on HCC therapy.

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Aim: The aims of this work are to study the epigenetic modifications in cell cycle regulators and apoptosis related genes in Hepatocellular carcinoma and the potential therapeutic role of epigenetic modulators on this kind of solid tumor. **Introduction**: Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer related deaths, with the heaviest burden on Southeast Asian and African countries, due to high rates of chronic Hepatitis B Virus (HBV) infection. The tumor usually presents in an advanced stage, when surgical resection is non-curative. Thus, research on new, more effective chemotherapeutic approaches is necessary. Besides the already known genetic mutations found on HCC cells, it has recently been accepted that epigenetic gene expression modifications may play a pivotal role on hepatocarcinogenesis. These mechanisms involve methylation of CpG islands and histone deacetylation, altering the expression of several genes, namely tumor suppressor genes and oncogenes. These mechanisms, opposite to genetic mutations, are reversible, so that they may be explored as new therapeutic approaches on hepatocellular carcinoma. In fact, drugs targeting these mechanisms are already available for the treatment of Myelodysplastic Syndromes. **Methods**: By using a methylation-specific PCR protocol, we were able to find epigenetic alterations on some cell cycle regulator genes and apoptosis related genes on three different HCC cell lines, HUH-7, HEPG2 and HEP3B. Additionally, we proved the efficacy of trichostatin (a histone deacethylase inhibitor drug) and decitabine (a hypomethylating drug) on reducing cell viability on HCC cell lines by using the Alamar Blue reduction assay. **Results**: Our preliminary results show that the gene coding to the p16, DAPK and DKK family proteins are methylated in the different HCC cell lines and the epigenetic modulators, trichostatin and decitabine, induced a decrease in cell proliferation and viability in a dose and time dependent manner. **Conclusions**: This study reinforces the theory that epigenetic modifications are involved in hepatocarcinogenesis and shows that epigenetic modulators (epidrugs) may be useful as new therapeutic approach in hepatocellular carcinoma.

PS 207 Nordihydroguaiaretic acid as a new therapeutic approach in cancer.

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Aim: The aim of this study was to test the cytotoxic and antiproliferative effects of NGDA in different tumor cell lines namely in Hepatocellular carcinoma (HCC) Acute Myeloid Leukemia (AML). **Introduction**: Nordihydroguaiaretic acid (NDGA) is a phenolic compound produced by Larrea tridentata, the creosote bush of Mexico and the American southwest. This compound is known for its anti-oxidant properties. NGDA eliminates reactive Oxygen Species (ROS) in vitro; inhibits 5-lipoxygenase reducing the synthesis of leukotriens, leading to the reduction of the inflammatory pathways. It has also shown that this compound inhibits cell growth and induces apoptosis in different types of cancer, in both cell culture and animal models. However, the mechanism involved in its anti-tumorigenic and anti-proliferative effects are not clear. **Methods**: For this purpose, we used 3 HCC cell lines, Huh-7, Hepg-2 and Hep3b, and a AML cell line, HL-60. The antiproliferative effect was assessed by the Alamar Blue assay and cell death by optic microscopy and flow cytometry using Annexin V and Propidium iodide. We also assessed intracellular ROS levels by flow cytometry using specific fluorescent probes, DHE and DCF-DHA, respectively for superoxide anion and peroxides. **Results**: Our results showed that NGDA have antiproliferative and cytotoxic effects in a dose, cell and time dependent manner. The cell death occurred in all the cell lines, preferentially by apoptosis. An increasing of intracellular levels of ROS was observed that may mediate cell death. **Conclusions**: Our results suggest that NGDA may constitute a new potential therapeutic approach in both HCC and ML treatment owing to its pro-oxidant properties when used in high concentrations.

PS 208 Suicide gene therapy in hepatocellular carcinoma - In vitro studies.

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Aim: The aim of the present study is to test the therapeutic potential of gene therapy using suicide genes (HSV-TK/ GCV or CD/ 5-FC) in monotherapy and in combination with conventional anticarcinogenic agents (Doxorrubicin) and new targeted drugs (proteasome inhibitor MG-262) in the hepatocellular carcinoma cell lines, HUH-7 and HepG-2. **Introduction**: Hepatocellular carcinoma (HCC) is the most frequent liver tumor in the world. It is often diagnosed in advanced stages of the disease and current treatments such as surgery, radiation and chemotherapy, have significant limitations in terms of efficacy and patients survival making HCC a disease without effective therapeutic options and poor prognosis. Although there are undergoing several preclinical studies and phase I/II/III clinical assays with drugs that target specific signaling pathways, growth factor receptors and/or tumoral microenvironment the results are not totally conclusive and the combination of gene therapy and conventional chemotherapy as been poorly studied in HCC. Suicide gene therapy involves the delivery of a suicide gene into target cells, making them sensitive to an appropriate prodrug. This delivery can be made by viral or non-viral vectors, cationic liposome/DNA complexes (lipoplexes) emerged as promising systems due to their low toxicity and immunogenicity, lack of pathogenicity and versatility. Herpes Simplex Virus thymidine kinase (HSV-TK)/ ganciclovir (GCV) and the bacterial cytosine deaminase (CD)/ 5-fluorocytosine (5-FC) systems are the most commonly used. **Methods**: To attain this purpose, the HCC cell lines, HUH-7 and Hepg-2, were submitted to suicide gene therapy (HSV-TK/GCV or CD/5-FC) mediated by the Tf-lipoplexes, using increasing concentrations of GCV and 5-FC both as single agents and in association with MG-262 and Doxorrubicin, during different periods of time. Cell viability was evaluated by the Alamar Blue assay. **Results**: Results show that the delivery of HSV-TK gene to the cells, mediated by the Tf-lipoplexes and followed by ganciclovir treatment, resulted in decreased viability in monotherapy, depending on GCV dose and incubation time. A synergetic antiproliferative and cytotoxic effect was observed when used the combination of HSV-TK/GCV with MG-262 or Doxorubicin Similar results were obtained when the suicide gene therapy approach CD/5-FC were used in both cell lines. **Conclusions**: These results suggest that suicide gene therapy HSV-TK/GCV or CD/5-FC may constitute a new potential therapeutic approach in HCC not only in monotherapy, but also in association with conventional therapies or new targeted drugs.

PS 209 The importance of signaling pathways in Chronic Myeloid Leukaemia: Ras/MAPK, PI3K/mTOR and Proteasome Pathways as therapeutic targets.

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Aim: The aim of this study was evaluated the potential therapeutic of the farnesiltransferase (L744832), mTOR (Everolimus) and proteasome inhibitors (MG262) respectively, alone and in combination with Imatinib in Chronic Myeloid Leukemia. **Introduction**: Chronic myeloid leukaemia (CML) is a mieloproliferative disorder characterized by the presence of the BCR-ABL gene fusion that encodes an oncoprotein, with a deregulated tyrosine kinase activity. The first-line treatment is Imatinib, a specific tyrosine inhibitor that blocks BCR-ABL tyrosine kinase activity. This oncoprotein activates multiples pathways in cell, responsible for particularly tumor cells characteristic, namely the high cellular proliferation and resistance to apoptosis. One of these pathways is the RAS/MAPK pathway which is responsible for cell proliferation and survival. The activation of RAS proteins requires the attachment of a farnesyl group that is mediated by the farnesyltranferase enzyme. Other pathways include de activation of the PI3K/AKT/mTOR pathway (Phosphatidylinositol-3-kinase/AKT protein kinase B/Mammalian Target of Rapamicin). On the other hand, the escape to apoptosis is also a problem. The proteasome inhibitor MG262, a bortezomib analogue, acts directly by blocking the proteasome 20s subunit triggering apoptotic mechanism. So, the knowledge of the cell signaling pathways involved in CML may contribute to the development of new therapeutic approachs in this kind of leukaemia. **Methods**: For this purpose, we used a CML cell line in blast crisis, the K562 cells. Cells were cultured in absence and presence of different concentrations of L744832, Everolimus and MG262 alone, and in association with Imatinib. To evaluate the effect of these inhibitors on cell viability we used the Resazurin Assay. Cell death was determined by optical microscopy (May-Grunwald staining), by flow cytometry (FC) using the Annexin V and Propidium Iodide double staining, and by the expression levels of the activated caspase 3. The effect of the drugs in cell cycle was determined by flow cytometry using Propidium Iodide incorporation. **Results**: We found that the half maximal inhibitory concentration (IC50) at 24 hours of exposure to L744832 was 25µM. To Everolimus and MG262 after 48 hours of treatment, the IC50 range between 15 to 25 µM and 15 to 25 nM, respectively. The association of lower doses of these inhibitors with 1 nM of Imatinib, which IC50 value was 75 nM, shows an additive/synergist cytotoxic effect. Combined therapy reveals to be a more effective strategy since the effects are greater with the use of smaller doses. These compounds induced cell dead by apoptosis in a time- and dose- dependent manner. **Conclusions**: In conclusion, our results suggest that inhibition of others pathways beyond BCR-ABL oncoprotein could be used as a new potential approach in the treatment of CML.

PS 94 AZFb microdeletions and oligozoospermia - How does it work?.

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Aim: On this work, our aim was to identify the deletion patterns and its breakpoints in patients with "atypical" microdeletions in AZFb and AZFc and, when possible, to characterize the recombination mechanisms underlying these microdeletions. **Introduction**: Microdeletions in AZFa, AZFb and AZFc regions lead to different patterns of male infertility, from oligozoospermia to azoospermia. Intrachromosomal recombination mechanisms were identified in patients with microdeletions in AZFb and AZFc. However, some AZFb deletions could not be explained through these mechanisms. Furthermore, severe oligozoospermia cases are normally associated to AZFc and not with AZFb deletions in the Y chromosome, as presented in this study. **Methods**: Two patients presenting severe oligozoospermia and two with azoospermia were identified as having atypical AZFb+c deletion patterns, after Y chromosome microdeletions analysis. The definition of those microdeletions and the fine characterization of the respective breakpoints were performed using STSs-PCR and DNA sequencing. **Results**: From the four patients studied, and according to Y chromosome structure and nomenclature, we identified three different deletion patterns: P5/P1 distal (50%; 2/4); P5/P1 proximal (25%; 1/4) and IR4/distal-P2 (25%; 1/4). **Conclusions**: Analysis of these patients allows us to present a putative region that should be involved in spermatogenesis conservation.

PS 216 NEW TARGETED THERAPIES IN DIFFUSE LARGE B-CELL LYMPHOMA - THE ROLE OF NF-KB PATHWAY.

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Aim: The aim of this study was to evaluate the therapeutic potential of an IkB kinase inhibitor (Partenolide) and a proteasome inhibitor (MG262) in DLBCL. **Introduction**: Diffuse Large B-cell Lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL), accounting for about 30-40% of all cases. It is a clinically aggressive lymphoma with a five-year survival rate of 50%. The nuclear factor-kB (NF-kB) pathway is essential in lymphomagenesis, being active in several NHLs. The implication of this pathway confers cancer cells a survival advantage by inhibiting apoptosis. After activation, NF-kB translocates from the cell cytoplasm to the nucleus, where it participates in gene transcription. On account of that, the deregulation of NF-kB pathway plays a role in the development of several NHLs, being critical for DLBCL, in particular to the activated B-cell-like (ABC) DLBCL subtype. The inhibition of the NF-kB through the introduction of an IkB-super-repressor or by inhibiting IkB proteasome degradation may be new therapeutic strategies in this kind of lymphomas. **Methods**: We used the DLBCL cell line Farage, tested in absence and presence of several concentrations of Partenolide and MG262, used in monotherapy and in association with each other and with conventional chemotherapy drugs (Vincristine). Cell viability was determined by the Alamar Blue® test and the efficacy of the drugs evaluated by dose-response curves and IC50. The type of cell death was investigated by flow cytometry (with Annexin-V and Propidium Iodide double staining) and optical microscopy (using May-Grünwald Giemsa staining). The mechanisms involved in drugs cytotoxicity were analyzed by flow cytometry through the expression of proteins involved in apoptosis, namely Caspase 3 and Cytochrome c. **Results**: Our preliminary results showed that Partenolide and MG262 induced cell death mainly by apoptosis in a time- and dose-dependent manner, attaining the IC50 value of 10,0 µM after less than 24 hours for Partenolide and of 25 nM after 24 hours for MG262. **Conclusions**: Our investigation suggests that Partenolide and MG262 might be used as new therapeutic approaches in Diffuse Large B-Cell Lymphoma.

PS 226 Biophysical aspects of nucleolar-targeting peptides.

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Aim: The main aim of this work is to unravel some of the important features of nucleolar-targeting peptides (NrTPs) responsible for their efficient translocation. **Introduction**: Cell-penetrating peptides (CPPs) were first identified two decades ago and since then new classes and applications are constantly being discovered. CPPs are short cationic peptides that can serve as vehicles for the delivery of different molecules and particles into cells [1]. The efficiency and non-invasive nature of peptide-mediated cellular transport provides a promising tool for biomedical research. NrTPs are peptides derived from crotamine, the main compound of a South-American rattlesnake (Crotalus durissus terrificus) venom. These peptides have the ability to translocate into cells and exhibit preferential nucleolar localiza-

tion [2]. Although NrTPs have already been classified as CPP, their mode of action at the molecular level is not, clear, yet. **Methods**: The work included the characterization in aqueous medium, quenching studies and quantification of partition into bilayers membranes. **Results**: This work allowed us to study the photophysical properties of peptides NrTP1, NrTP2, NrTP4 and crotamine. NrTP1, NrTP2 and NrTP4 have essential the same spectral profile and molar absortivity as free tyrosine. Crotamine, on the other hand, reveals close proximity with tryptophan spectrum (twice the molar absorptivity due to the presence of two Trp residues). The quenching studies revealed a linear relation between acrylamide concentration and fluorescence, therefore indicating that there were no major peptide aggregates in solution. In the presence of lipid vesicles there was a decrease in the KSV. **Conclusions**: Results obtained suggest high affinity towards lipid bilayers, especially zwitterionic (POPC) and negative (POPG) charged ones, which is in agreement with the behaviour of most CPP. Moreover, quenching studies already predicted some degree of interaction, as KSV drops upon addition of lipid vesicles. Although these results only concern the basic and essential characterization of NrTP they are definitely the starting point for further studies that can ultimately lead to the production of a versatile therapeutic tool.

PS 240 The additional value of a family history of cancer in the development of pelvic high grade serous carcinoma in BRCA1 and BRCA2 mutation carriers.

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Aim: The current study aims at evaluating the additional value of a family history on the development of pelvic serous carcinoma in BRCA1 and BRCA2 mutation carriers, regarding risk of developing the disease, as well as age of onset. **Introduction**: Approximately 5% to 10% of all ovarian cancer cases have a genetic predisposition. The BRCA1 and BRCA2 genes are tumor suppressor genes in which mutations can cause the hereditary breast-ovarian cancer syndrome, conferring a high risk of developing breast, ovarian, tubal and primary peritoneal cancer, especially at a young age. Family history is also known as a strong risk factor for developing ovarian cancer in the general population. However, the impact of a family history on the risk of ovarian cancer among carriers of a BRCA mutation is still unclear. **Methods**: Mutation carriers from families followed-up at the University Medical Center Groningen between 1996 and 2007 were selected. The occurrence and age of onset of breast, contra lateral breast and ovarian cancer was recorded. The impact of family histories on the incidence and age of onset of breast and ovarian cancer was analyzed through Cox-regression. **Results**: 1018 patients from 201 different families were included. A total of 51 pelvic carcinoma cases were diagnosed: 40 in BRCA1 and 11 in BRCA2 mutation carriers. The risk of developing the disease was significantly higher among patients who had first-degree relatives with ovarian cancer before the age of 50 (HR = 1.73, 95%CI = 1.17 - 2.56) and those who had first-degree relatives with ovarian cancer at any age (HR = 2.04, 95%CI = 1.46 - 2.86). First or second-degree relatives with breast cancer did not significantly increase the risk of developing pelvic carcinoma. **Conclusions**: This is the first retrospective multicenter cohort study modeling the influence of a family history of cancer on the risk of developing pelvic carcinoma for women with a proven BRCA1 or BRCA2 mutation in a European population. Our findings demonstrate that ovarian cancer cases among first-degree relatives, at any age, do increase the risk of developing pelvic carcinoma in BRCA1 and BRCA2 mutation carriers substantially. This knowledge is important when counseling these patients.

PS 252 A drug Delivery system derived from Dengue Virus Structural proteins. A focus on siRNA Therapy.

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Aim: Development of drug delivery system with clinical relevance for the therapy of pathologies at a molecular level using Dengue Virus (DENV) structural protein fragments. Specific objectives: 1. To evaluate the Lipid membrane partition and the translocation capability of Dengue Virus C protein derived peptides, pepM and pepR as well as ssDNA complexes, using Lipid vesicles as biomembrane models. 2. To study the transfection capability of DENV derived peptides of a ssDNA or a plasmid in cell lines. 3. To study of the siRNA therapy approach using pepM and pepR as transfection agents using cell lines models for specific pathologies. **Introduction**: Due to the demand on more specific and effective therapies for molecular based therapies, a plethora of drug delivery systems have been described and developed during the last years to improve the pharmacological availability of the drug inside the cell. Cell Penetrating Peptides (CPP's) are one of delivery systems for plenty of molecules, currently being used as novel therapeutic approaches for the treatment of numerous therapies. One of the first peptide described was the HIV Trans-Activator of Transcription protein (TAT). Our group evaluated the function of two conserved domains, one hydrophobic related to lipid membrane interactions (MBS) and other positively charged assigned to viral ssRNA binding of Dengue Virus Capsid Protein (DENV C protein)using synthetic peptides, pepM (MBS) and pepR (RBS), whose molecular features suggested new CPP system. **Methods**: In this work, fluorescence spectroscopy techniques together with the quantitative analysis using biophysical models were used, as well as confocal microscopy on cell lines (Baby Hamster Kidney, BHK, cells) and blood cells. The study was carried out using DENV C protein synthetic peptides, a ssDNA primer and Large Unilamelar Vesicles (LUV) as viral genome and cellular membrane models. For confocal microscopy, full lenght purified DENV C protein was also used. **Results**: Both pepM and pepR, and corresponding ssDNA complexes, revealed affinity for membranes. Partition constant showed that membrane affinity increases when the content in negatively charged lipids on membranes increases, a known feature during endosome maturation. PepM crosses lipid membranes and transporting ssDNA mainly due its high membrane affinity and hydrophobic profile. For pepR studies, no translocation profile using membrane models was observed, indicating that it is not by lipid membrane diffusion. Confocal microscopy correlates the affinity of DENV C protein peptides to cell membranes and ability to translocate along the lipid membrane delivering a cargo to the cytoplasm. PepR showed translocation capability alone and with ssDNA cargo. Together with the biophysical studies it may be dependent on an endocytosis mechanism. **Conclusions**: Both peptides have shown CPP capability by delivering a ssDNA molecule to the cell cytoplasm. Studies with different cargoes such as plasmids, antibodies, proteins or siRNA would evaluate the plethora of potentialities for these CPPs. In the future it may be used as a research tool as a transfection agent and/or in vivo imunohistochesmistry techniques. Additionally, it may be a potential approach for breakthrough therapies for several molecular diseases by the delivery of pharmacological molecules such as siRNA.

PS 254 Characterization of Ceramide Kinase-Like (CERKL) in the Mammalian Retina.

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Aim: The CERKL gene encodes for ceramide kinase-like, a novel protein of unknown function. CERKL mutations are associated with a severe retinal phenotype. The purpose of this work is to characterize CERKL's retinal expression pattern and function. **Introduction**: Hereditary retinal degeneration (HRD) is a clinically and genetically heterogeneous group of diseases that cause visual loss due to progressive loss of rod and/ or cone photoreceptor cells in the retina.

The RP26 locus was originally mapped to human chromosome 2q31-q33 in a large Spanish family. The underlying gene was identified and named ceramide kinase-like (CERKL). To date, nine CERKL mutations have been reported, all of which are responsible for a distinct form of HRD, characterized by early macular involvement with roughly parallel cone and rod loss, resulting in a deficit in both peripheral and central vision. CERKL is a homolog of the ceramide kinase (CERK) protein, and both proteins harbor a kinase domain related to the diacylglycerol kinases (DAGK) and a pleckstrin homology (PH) domain. In addition, a CERK-specific region that is downstream from the catalytic core and bears a putative Ca2+/calmodulin binding motif is also present in CERKL. Several studies have been conducted to prove biochemical similarity between CERK and CERKL enzymatic activities. However, so far there has been no evidence that CERKL phosphorylates ceramide or any other lipid substrate in vitro or in vivo. **Methods**: RT -PCR analysis was conducted in order to asses the expression levels of Cerkl in the developing mouse eye as well as to reveal the unique splice variants of Cerkl in the mouse retina. A specific anti-CERKL antibody was used to study the localization of the endogenous CERKL protein in the mouse retina. A calcium-overlay assay was used to determine whether CERKL actually binds calcium. GST pull-down assay will be used to investigate a possible interaction between CERKL and calmodulin. To identify CERKL-binding proteins we used the Ras-Recruitment system. **Results**: RT -PCR analysis revealed that in the mouse retina, Cerkl is highly expressed, while expression of its homolog, Cerk, is relatively low. The exon structure of human and mouse CERKL genes is highly conserved. Four different Cerkl splice-isoforms were identified in the adult retina (variants a'-d'). In the embryo, five different isoforms were detected (isoforms a', d'-g'). One of the embryonic isoforms (isoform g') included a novel exon of 156bp (exon 12). Therefore, the total number of exons in the mouse Cerkl gene is 14, as in the human ortholog. In the mouse retina CERKL is located in the ganglion cell layer, in amacrine cells of the inner nuclear layer, and in cone photoreceptors. Based on a calcium-overlay assay, CERKL does not appear to bind calcium directly. One of the identified CERKL-binding proteins is a ubiquitously expressed calcium-binding protein. Interaction between CERKL and this protein was confirmed by a co-immunoprecipitation assay. **Conclusions**: The severe retinal phenotype associated with human CERKL mutations indicates that this gene plays a crucial role in retinal activity. The high expression level of CERKL in cones correlates with the CERKL associated phenotype in humans, which involves severe cone degeneration. CERKL's localization in the retina as well as the identified calcium-binding partner indicate that CERKL may require an interaction with one or several calcium-binding proteins, in order to sense changes in calcium concentration as part of the phototransduction cascade.

PS 258 POLYMOR PHISM OF GLUTAT HIONE S -TRANSFERASE M1 AND INDICATORS OF OXIDATIVE DNA DAMAGE IN PATIENTS WITH URINARY BLADDER CARCINOMA.

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Aim: The aim of this study was to examine the role of GSTM1 polymorphism in onset of urinary bladder carcinoma, through the association of polymorphism of GSTM1 and indicators of oxidative damage to DNA in patients with urinary bladder carcinoma. **Introduction**: Exposure to potential carcinogens is an etiological factor in 60-70% of patients with carcinoma of urinary bladder. Carcinogens in the organism are subject to biotransformation and detoxification reactions, in which the important role belongs to glutathione S-transferase M1 (GSTM1). In addition, GSTM1 protects oxidatively damaged nitrogenous bases of nucleic acids by its selenium-independent glutathione peroxidase activity. Homozygous GSTM1*0 (frequently referred to as GSTM1-null genotype) individuals show loss of GSTM1 enzymatic activity, which may result in increased damage to DNA molecule. **Methods**: The case-control study had included 80 patients with urinary bladder carcinoma, with additional 60 persons in the control group. GSTM1 polymorphism was determined using polymerase chain reaction (PCR). The degree of DNA damage was measured by the level of 8-hydroxydeoxyguanosine (8-OHdG), which was screened in urine samples using ELISA Kit (Competitive Enzyme-Linked Immunosorbent Assay). The effect of smoking and occupational exposure on the level of DNA damage had also been evaluated. **Results**: In the control group, 53% of subjects had shown GSTM1-positive genotype (homozygous and heterozygous for the GSTM1*1 allele), and 47% of them had GSTM1-null genotype (homozygous for GSTM1*0 allele). In 39% of patients with urinary bladder carcinoma, there is a GSTM1-positive genotype (homozygous and heterozygous for the GSTM1*1 allele), and in 61% GSTM1-null genotype (homozygous for GSTM1*0 allele) was confirmed. Statistical analysis has not shown the differences in concentrations of DNA damage marker (8-OHdG) between smokers and nonsmokers, neither in patients occupationally exposed to chemical carcinogens in relation to persons who were not exposed ($p1 = 0.097$, $p2 = 0.446$). In patients with GSTM1-null genotype, obtained values of 8-OHdG were twice as high as in patients with GSTM1 positive genotype (7.52 ± 1.52 vs. 3.49 ± 0.93. Ng / mg creatinine) and this difference is highly statistically significant ($p = 0.002$). **Conclusions**: The distribution of GSTM1-null genotype in the control group corresponds to the expected frequency of this gene in the Caucasian population. Although the distribution of GSTM1-null genotype among patients with urinary bladder carcinoma was higher than in control group, the difference was not statistically significant. There is a significant correlation between GSTM1-null genotype and the degree of DNA damage.

PS 268 Prolonged ingestion of epigallocatechin gallate prevents diabetes induced vascular modifications in the corpus cavernosum of the rat. Immunofluorescence characterization of VEGF, VEGFR1 and VEGFR2 expression.

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Aim: To study whether the consumption of epigallocatechin gallate (EGCG) solution during 10 weeks prevents diabetes induced structural and molecular changes in blood vessels of CC of rats. **Introduction**: Diabetes type 1 is a very common chronic disease that frequently initiates in youth. Patients suffering from this disease, have an increased risk of developing complications such as erectile dysfunction, which can have a great impact in sexual intercourse. This is due, in part, to marked oxidative stress in affected cells, induced by the increasing production of ROS (reactive oxygen species) that causes damage in blood vessels of corpus cavernosum (CC), decreasing erectile capability of penis. Thus, we hypothesise that treatment with antioxidants in the initial phase of diabetes type 1 may protect CC from structural and molecular changes induced by oxidative damage. In support of this hypothesis our previous study demonstrated that green tea long-term ingestion led to a decrease of vascular endothelial growing factor (VEGF) and its receptor VEGFR2 expression in CC of aged rats. **Methods**: Wistar rats were divided into three groups (n=8): one control group of non-diabetic rats (C), one injected intraperitoneally with a streptozotocin solution (STZ) and another injected with STZ and treated with an antioxidant present in green tea (EGCG- epigalocatequin galact) solution (2g/L) (STZ/EGCG). The rats were sacrificed 10 weeks after the STZ injection. The CC was removed and immediately fixed and processed for dual-labelling immunofluorescence detection of the endothelial protein PECAM (platelet/endothelial cell adhesion molecule) and α-actin, and VEGF and their respective receptors VEGFR1 and VEGFR2. All the sections were mounted and observed in an Apotome fluorescence microscope (Carl Zeiss MicroImaging GmbH). Besides that, we performed a morphometrical analysis of smooth muscle content in CC after imunohistochemical detection of α-actin (present in smooth muscle) employing the program ImageJ® (National Institutes of Health, Maryland, USA). **Results**: The expression of PECAM and VEGFR2 was restricted to the endothelium and of the α-actin to smooth muscle layer in corpus cavernosum's blood vessels in all experimental groups. One the other hand, the expression of VEGF was observed both in the endothelium and on smooth muscle, often

colocalizing with VEGFR1. Morphometrical study demonstrated that STZ rats presented a reduced layer of perivascular smooth muscle (12,8 +3,5) when compared with that observed in group C (26,2+3,5) or STZ/EGCG (23,8+5,5) animals. **Conclusions:** The data here reported suggest that EGCG consumption prevent structural diabetes-induced modifications of the blood vessels in the CC. The role of VEGF needs to be further evaluated namely in the perspective of a target to prevent erectile disfunction during diabetes.

PS 273 Effects of chronic ingestion of high-fat diet and energy restriction on the expression of VEGF, VEGFR1 and VEGFR2 in aged rat myocardium.

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Aim: To analyse the effects of high-fat diet regular consumption and energetic restriction on the expression of VEGF and VEGFR1 and VEGFR2 in the myocardium of aged rat. **Introduction:** Cardiovascular diseases (CVD) are a leading cause of death in the aged population. Regular intake of hyperlipidic diet and obesity can induce endothelial dysfunction that always precedes the onset of CVD and atherosclerosis. The heart is an organ where the damages caused by high fat diet lead to failure, that are due, in part, to structural modification, such as fibrosis, and to changes in the expression of angiogenic factors and their specific receptors. Herewith we characterize the expression of vascular endothelial growth factor (VEGF) and its membrane receptors VEGFR1 and 2 in the heart of young and aged rats treated with high-fat diet and energy restriction. **Methods:** Adult male Sprague-Dawley rats (8-wks old), individually housed in appropriated cages with free access to tap water and maintained under a 12h light-dark cycle at standard temperature (20-22ćC) and humidity (40-60%), were divided into three groups (n=12): a control group of rats fed ad-libitum with a standard rodent chow with 7% of energy supplied from fat (C); a group of rats with free access to a rodent purified diet with 45% of energy supplied from fat (HF) and another subjected to energy restriction (75% of daily individual intake of C rats) (ER). In each group, 6 rats were sacrificed by decapitation when they reached the ages of 6 and 18 months. The heart was removed and divided in two fragments that were immediately fixed in 10% buffered formalin, or frozen at -80ćC until analysis. Dual-labelling immunofluorescence detection of the endothelial protein PECAM (platelet/endothelial cell adhesion molecule) and α-actin, VEGF and VEGFR1 or VEGFR2 was observed in an Apotome fluorescence microscope (Carl Zeiss MicroImaging GmbH). Besides that, we performed to morphometrical analysis of smooth muscle content in the myocardium after imunohistochemical detection of α-actin) employing the program ImageJ® (National Institutes of Health, Maryland, USA). Analysis of VEGF and VEGFR2 was also performed by Western blotting followed by pixel computerized quantification (Scion Image®). **Results:** The expression of PECAM was restricted to the endothelium and of the α-actin to smooth muscle layer in myocardium's blood vessels in all experimental groups. VEGF and its receptors were detected in the cardiomyocytes, however no quantitative differences were detected neither with aging nor with consumption of high-fat diet or energetic restriction. Morphometrical study concerning the perivascular smooth muscle layer demonstrated a significant decrease in the treated groups when compared with age-matched controls. **Conclusions:** The data suggest that aging and diet composition could modulate vascular mechanisms of the heart, nevertheless further molecular studies will be necessary to elucidate this hypothesis.

PS 277 The role of Pim1 in melanoma progression.

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Aim: In order to identify the role that cytoplasmic serine/threonine kinase Pim1 has in promoting melanoma progression, regulation of melanoma cell proliferation by Pim1 was investigated using Pim1 inhibitors. In addition, the regulation of Pim1 expression by PI3K signalling was investigated. To translate the findings into therapeutic potential, this study goes on to investigate whether Pim1 and SMI-4a can be combined with PI3K or MEK inhibitors to yield a synergistic increase in the inhibition of melanoma cell proliferation. **Introduction:** Melanoma is the deadliest type of skin cancer with malignant (stage 4) melanoma having a 5 year survival rate of less than 20%. Pim1 has recently been identified as a PI3K signalling dependent gene that is up regulated in melanoma cells but its role in melanoma progression has to date been only superficially characterized. Pim1 promotes cell proliferation and inhibits apoptosis by activating and inhibiting Cdc25a and Bad respectively. Pim1 has also been associated with regulating Akt (PKB) and PRAS40 activity and inhibiting the expression of the cell cycle inhibitor p27Kip1 through directly promoting the stability of the substrate targeting subunit (p45Skp2) of the E3 ubiquitin ligase SCFSkp2 responsible for targeting p27Kip1 for degradation. The databases COSMICTM and CONANTM record that Pim1 over expression is in fact implicated in a variety of cancers. **Methods:** Human melanoma cell lines MV3, SKMEL28 and WM2664 were cultured in supplemented DMEM. Estimates of melanoma cell proliferation were obtained through absorbance measurements of melanoma cells treated with Pim1 inhibitors 4-[3-(4-Chlorophenyl)-2,1-benzisoxazol-5-yl]-2-pyrimidinamine (Pim1) or [(5E/Z)-[[3-(Trifluoromethyl)phenyl] methylene]-2,4-thiazolidinedione] (SMI-4a) and stained using crystal violet solution. Protein lysates, extracted using a NP40 lysis or RIPA buffer, were resolved in Bis-Tris Novex or SDS Acrylamide gels and estimates of protein expression were obtained using ECL or li-cor detection systems. RNA interference of Pim1 was carried out by transfection of ONTARGET plus Smart Pool Pim1. **Results:** The PI3K inhibitor reduced melanoma cell proliferation by ~75% but had no effect on Pim1, p45Skp2 or p27Kip1 expression. SMI-4a inhibits p45Skp2 expression and Akt and PRAS40 phosphorylation in a dose dependent manner. SMI-4a promotes p27Kip1 expression but has no effect on melanoma cell proliferation. Pim1 inhibits melanoma cell proliferation by ~30% and p27Kip1 expression but has no effect on p45Skp2 expression or Akt and PRAS40 phosphorylation. Attempted knock-down of Pim1 inhibited Pim1 expression by ~10% and had no effect on melanoma cell proliferation, p45Skp2 expression or PRAS40 phosphorylation. The combinations of Pim1 inhibitors with PI3K or MEK inhibitors reduced melanoma cell proliferation by 55-70%. **Conclusions:** Pim1 and SMI-4a are less effective than the PI3K inhibitor at reducing melanoma cell proliferation. Pim1 is confirmed to regulate the expression of p45Skp2 and suppress p27Kip1 expression, suggesting Pim1 is able to promote melanoma progression. Pim1 is also confirmed to regulate Akt and PRAS40 phosphorylation but was not found to be regulated by PI3K signalling or to regulate its own expression in melanoma cells. Combinations of Pim1(2) or SMI-4a with PI3K or MEK inhibitors demonstrated no enhanced inhibition of melanoma cell proliferation compared to the effect of the PI3K or MEK inhibitor alone.

PS 280 Xanthohumol-supplemented beer affect skin wound-healing in diabetic rats.

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Aim: This study aims to evaluate modulation of angiogenesis, inflammation and oxidative stress, after chronic consumption of a XN-supplemented beer, in diabetic rats. **Introduction:** In recent years there has been a growing interest in phenolic compounds as they seem to protect of the establishment of several highly prevalent pathologies, such as cancer, diabetes and cardiovascular diseases, having in common dysfunctional angiogenesis and inflammation. Special attention has been given to xanthohumol (XN), a polyphenol well-known for its anticancer and antioxidant potential, that has also demonstrated other interesting biological properties. Diabetes is responsible for metabolic dysfunction leading to inflammation and oxidative stress, resulting in deleterious actions on endothelium. Therefore, consumption of polyphenol-enriched food and beverage

could improve vascular dysfunction that occurs in diabetic tissues. **Methods:** This study aims to evaluate modulation of angiogenesis, inflammation and oxidative stress, after chronic consumption of a XN-supplemented beer, in diabetic rats. Healthy Wistar rats and streptozotocin (STZ)-induced diabetes mellitus type 1 Wistar rats consumed water (Control and STZ-Control, respectively), 5% ethanol (STZ-E), stout beer (STZ-Stout) or stout beer supplemented with 10 mg/L XN (STZ-Stout XN), during 4 weeks. Then, skin wound-healing assay was performed, and animals continued the treatment for 7 days. Wound tissue was collected to evaluate vessel number (vWF staining). Liver, kidney and muscle were removed to assess local oxidative stress by hydrogen peroxide (H_2O_2) production. VEGF, NO release and N-acetylglucosaminidase (NAG) content were measured systemically. Student's t-test or ANOVA followed by the Bonferroni test were used for statistical analysis. **Results:** Results obtained demonstrated that in STZ-Stout XN the number of vessels decreased in the wound area. VEGF levels, NAG content and NO released were also reduced systemically when compared to STZ-Stout group. The same profile was noticed for local production of H_2O_2 that was reduced in STZStout XN group. **Conclusions:** These findings suggest that consumption of a XN-supplemented beer decreased angiogenesis, inflammation and oxidative stress in diabetic animals. Based on the health-promoting properties of XN, the production of a beer enriched in this polyphenol would be of huge interest to the brewing industry, for the benefits this could bring to consumer's health.

PHYSIOLOGY & INMUNOLOGY *Session*

PS 18 POSTJUNCTIONAL α AND β-ADRENOCEPTOR-MEDIATED RESPONSES IN THE AORTA OF NEWBORN AND YOUNG RABBITS.

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Aim: To evaluate α and b-adrenoceptor-mediated effects at the postjunctional level in the aorta of newborn and young rabbits. **Introduction**: Postjunctional α-adrenoceptors are innervated receptors responsive to the neurotransmitter noradrenaline, whereas b2-adrenoceptors function as hormone receptors responsive to adrenaline from the adrenals. Canine and ovine blood vessels express fully developed α-adrenoceptor-mediated responses at birth. In 1-week old rabbits, b-adrenoceptor-mediated responses are fully functioning and b-adrenoceptor-coupled intracellular responses are present in newborn rats. In canine saphenous vein, at birth, there is a lack of b-adrenoceptor mediated responses. α and β -adrenoceptormediated effects were never evaluated in newborn rabbits. **Methods**: Aortas of anesthetized newborn (postnatal day 1) and young (3 to 4 months old) rabbits were dissected, removed, transversely sectioned, mounted in isolated-organ baths and connected to isometric force transducers. Dose-response curves to isoprenaline and phenylephrine were determined. Data was fitted to sigmoid curves by nonlinear regression (GraphPad Software, La Jolla, CA, USA). Statistical analysis was performed by Student's t-test. The F-test was used to analyze the statistical significance of differences in the values of Hill slope. P<0.05 was the chosen level of significance. **Results**: The maximal contraction to phenylephrine was lower in neonates than in young rabbits (0.54±0.12 mN/mg, n=12 vs 1.00±0.16 mN/mg, n=18. P<0.05) but the potency of phenylephrine was higher in neonates (EC50=0.38±0.05 μM, n=12) than in young rabbits (1.11±0.17 μM, n=18). Complete relaxation to isoprenaline of phenylephrine-contracted aortic rings occurred only in newborn rabbits. The maximal relaxation in young rabbits was 21±4 %. Isoprenaline potency was higher in neonates (EC50=15.0±2.8 nM, n=10) than in young rabbits (EC50=190±42 nM, n=8). In young rabbits, the Hill slope was not different from unit (0.98±0.02, n=8). In newborn rabbits, the Hill slope was significantly higher than 1 (1.64±0.23, n=10). **Conclusions**: Postjunctional α-adrenoceptor-mediated responses in rabbit aorta are already developed at birth, similarly to other species. In rabbits, β-adrenoceptor-mediated responses are present at birth and develop earlier than in dogs. α-adrenoceptor-mediated responses seem to correlate with the release of noradrenaline, present at birth in all species. The maturation of β2-adrenoceptor-mediated responses seem to correlate with the adrenaline secretion which is developed in all species except for the dog Since in newborn rabbits the Hill slope for the β-adrenoceptor-mediated dose-response curve is different from unit we hypothesize that a different population of β-adrenoceptors is present at birth to disappear later during postnatal maturation.

PS 53 Neuregulin attenuat es right ventricular hypertrophy and dysfunction in an experimental model of pulmonary hypertension.

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Aim: This study analysed the effects of NRG-1 chronic treatment in an animal model of PAH and HF. **Introduction**: Neuregulin (NRG)-1 is implicated in the preservation of left ventricular (LV) performance in pathophysiological conditions [1]. Nevertheless, the role of NRG-1 in right ventricular (RV) failure secondary to pulmonary arterial hypertension (PAH) is still unknown. This study analysed the effects of NRG-1 chronic treatment in an animal model of PAH and HF. **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 (4ug/Kg/day, ip) or vehicle. The study resulted in 4 groups: control (n=10); control+NRG (n=10); MCT (n=10); MCT+NRG (n=10). Echocardiography, RV invasive hemodynamic evaluation and sample collection for vascular, morphometric, histologic and molecular studies were performed 25 to 28 days after MCT administration. **Results**: MCT group developed PAH, as shown by the increase in RV maximum pressure (MCT vs control: 63±3 vs 34±3mmHg) and by the decrease in cardiac output (MCT vs control: 34.4±4.4 vs 64.6±3.4mL/min) which were both attenuated in the MCT+NRG group (53±3mmHg and 52.2±1.6mL/min). Ecocardiographic data confirmed these results and showed a marked dilatation of the RV , and a decrease in the pulmonary artery acceleration time in the MCT group, changes that were also reduced by NRG-1 treatment. Animals from the MCT group developed RV hypertrophy (RV weight/tibia length ratio MCT vs control: 0.08±0.002 vs 0.05±0.003 g/cm) and pulmonary congestion (lung weight/tibia length ratio MCT vs control: 0.7±0.03 vs 0.4±0.03g/cm), both changes were minimized by the NRG-1 treatment (0.06±0.002 g/cm and 0.6±0.03 g/cm, respectively). Histological analysis also revealed a decrease of RV cardiomyocyte hypertrophy and fibrosis in the MCT+NRG group in comparison with MCT group. The RV of MCT group animals presented an increased expression of brain natriuretic peptide and endothelin(ET)-1 (17.5 and 5.0 times vs control, respectively). These changes were attenuated or reversed in the MCT-NRG group (brain natriuretic peptide expression increased only 5.6 times vs control, and ET-1expression did not change). The MCT group presented endothelial pulmonary vascular dysfunction (38.2±5.4% vs control 86.3±1.8%) of relaxation of a phenylephrine contracted arterial ring in response to acetylcholine), effect that was also attenuated in the MCT+NRG group 51.0±5.7%). **Conclusions**: NRG-1 chronic treatment significantly reduced the severity of PAH and RV hypertrophy, as well as the expression of genes associated with overload and ventricular hypertrophy. These findings suggest that the NRG-1 pathway has a relevant role on the pathophysiology of PAH and right ventricular HF, representing a potential therapeutically target.

PS 100 The effect of oxidati ve stress upon the absorption of folic acid in Caco-2 cells.

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Aim: The aim of this study was to investigate the effect of oxidative stress upon the uptake of FA by human intestinal epithelial cells (Caco-2 cells). **Introduction**: Excessive reactive oxygen species (RO S) in the cells, either because they are formed in excess or because they are not adequately removed by the antioxidant defense machinery, cause oxidative stress, leading to cellular damage. In agreement with this, RO S have been implicated in the initiation and progression of a number of pathologies, including some conditions that affect the intestine. Folic acid (FA) plays a fundamental physiological role in one-carbon metabolism. An adequate supply of FA is therefore necessary for normal human health. Humans cannot synthesize FA and thus depend on dietary sources of this vitamin, and so any impairment in the process of intestinal absorption of FA may induce a whole-body deficiency state. **Methods**: For this purpose, we investigated the effect of a 1h incubation with increasing concentrations of tert-butylhydroperoxide (TBH) upon oxidative stress markers and upon viability and proliferation. Induction of oxidative stress was evaluated by measuring lipid peroxidation (TBARS assay) and total (GSx), oxidized (GSSG) and reduced (GSH) glutathione levels. Cellular viability and proliferation was measured by quantification of extracellular lactate dehydrogenase activity and sulforhodamine B assay, respectively. **Results**: TBH (3000 μM) induced a decrease in total and reduced gluthatione levels and an increase in lipid peroxidation products, while maintaining cell viability and proliferation. Based on these results, TBH 3000 μM was chosen for the following experiments. Analysis of time-course of 3H-FA (10 nM) uptake by Caco-2 cells showed a reduced intracellular maximal accumulation

(Amax) and a reduced rate constant for outward transport (kout) of 3H-FA over time in TBH-treated cells. Analysis of saturation curves at 4°C shows that TBH did not affect non-carrier mediated 3H-FA uptake. Analysis of saturation curves at 37ćC shows that uptake of 100 µM 3H-FA was significantly increased in TBH-treated cells. For concentrations of 3H-FA up to 10 µM, we observed no differences between the two groups in carrier-mediated 3H-FA uptake. 3H-FA uptake was strongly pH-dependent, being acidic pH-stimulated, both in control and TBH-treated cells. Finally, 3H-FA uptake was similarly reduced by DIDS (100 µM), 5-metyltetrahydrofolate (1 µM), pemetrexed (0.2 µM) and methotrexate (1 µM) in control and TBH-treated cells. **Conclusions**: Based on our results, we conclude that Caco-2 cells submitted to treatment with 3000 µM TBH for 1 h constitutes a good cellular model to study the effects of oxidative stress. We also conclude that TBH (3000 µM) distinctly modulates the membrane transport of low (10 nM) and high concentrations (100 µM) of 3H-FA. Namely, oxidative stress (a) increases the efflux of low concentrations of 3H-FA, probably by affecting the activity of the efflux transporters multidrug resistance protein (MRP) and/or breast cancer resistance protein (BCRP), and (b) increases uptake of high concentrations of 3H-FA, by increasing the activity of a transport mechanism with an unknown identity at present.

PS 159 The role of polymorphisms in cytokine encoding genes as risk factors for autoimmune thyroiditis.

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Aim: The general aim is to go further in the understanding of AT at the genetic level and use this knowledge to achieve a better management of the patients. We will evaluate the role of genetic polymorphisms in IL6, TNFA, IL1B and IFNGR1 as risk factors for the development of Graves disease and Hashimoto's thyroiditis. **Introduction**: Autoimmune thyroiditis (AT) comprises a set of diseases including Hashimoto's disease and Graves disease. Hashimoto's thyroiditis and Graves' disease are characterised by the reactivity of autoantigens, causing, respectively, inflammatory destruction or autoimmune stimulation of the TSH (thyroidstimulating hormone) receptor. AT (and particularly Hashimoto's thyroiditis) is the most common thyroid disease and is the leading form of autoimmune disease in women. Cytokines are crucial in the regulation of immune and inflammatory responses and therefore are potential candidate genes as risk factors in the development of autoimmune thyroiditis. **Methods**: Our project is a case-control study comprising 1075 subjects. The control group included 667 DNA samples obtained from Portuguese healthy blood donors. The case group included 408 subjects, of which 79 were diagnosed with Graves disease and 329 with Hashimoto's thyroiditis. Cytokine gene polymorphisms were analysed by real time polymerase chain reaction using TaqMan genotyping assays. **Results**: A significant association was found between the A allele in TNFA-308G>A (OR =1.69, CI 1.27-2.27, p=0.0004), the allele C in IL6-174G>C (OR =1.33, CI 1.01-1.75, p=0.04) and risk for Hashimoto's thyroiditis. The homozygous genotype CC of IFNGR1-56T>C was also significantly associated with Hashimoto's thyroiditis (OR =0.67, CI 0.47-0.95, p=0.02), but in this case exerting a protective effect. Significant associations were also observed for the risk to develop Graves disease: TNFA-308G>A (OR =1.70, CI 1.03-2.81, p=0.04), the allele C in IL6-174G>C (OR =1.84, CI 1.11-3.05, p=0.02). The individuals carrying the homozygous genotype CC of IFNGR1-56T>C (OR =0.48, CI 0.24-0.99, p=0.03) have a lower risk to develop Graves disease . We did not observe any significant associations between the IL1B-511C>T and any type of autoimmune thyroiditis. **Conclusions**: This study reports significant associations of genetic variants in TNFA, IL6 and IFNGR1 with the risk to develop Hashimoto's thyroiditis and Graves disease, highlighting the relevance of polymorphisms in inflammation-related genes as molecular markers for autoimmune thyroiditis.

PS 177 The role of motivational component in energy provision of mental activity.

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Aim: Evaluation of changes of brain haemodynamics and metabolism of an organism at rest and at mental activity, and revealing of interrelation between various productivity of carrying-out of the task and haemodynamic and metabolic characteristics both at students with various structure of motivation and a functional condition. **Introduction**: The main indicator of any activity is its success. There are many features and reasons which provide on success of activity. One of the main factors is motivation to carrying-out of activity and to success achievement. It`s known that any activity is impossible without energy expenditure. We hypothesize that motivational component as the mechanism which provides on high results of any activity participates in forming of definitive level of energy expenditure. In additional we hypothesize that successful and unsuccessful persons have different final energy expenditure according to their structure of motivation. **Methods**: We used following methods in our research: the metabolography, based on incomplete gas analysis, the rheoencephalography for evaluation of a brain blood supply level as obligatory parameter of mental work carrying-out, the method of the heart rhythm mathematical analysis, the Gerbachevsky`s multifactorial psychodiagnostic motivational test, test "Quantitative relations" (D.Y. Raygorodsky). Our research was involved 23 students, including 12 women and 11 men from 19 to 20 years old. Data are expressed as median and range. Data were analysed by Mann-Whitney U test, Student t-criteria, Wilcoxon T-criteria. A p-value ‹0.05 was considered statistically significant. **Results**: We have allocated 11 successful and 12 unsuccessful students according to the data of the test "Quantitative relations". We observed increase of resting metabolic rate at 6 persons from group of successful students, at 10 studets from group of unsuccessful students. We also registered decrease of resting metabolic rate at 5 persons in successful group, at 2 persons in unsuccessful group (p‹0,05) The rheographic index, the characteristic of blood supply, received by means of a rheoencephalography method, has increased in both cases. Parameter ascending statistically significantly for pools of the left internal somnolent and vertebral arteries in case of successful students and for pools of both hemispheres in case of unsuccessful students (p‹0,01). Motives of achievement prevail at successful persons, motives of an avoidance prevail at unsuccessful persons (p ‹0,05 concerning motive of competition, motive of an avoidance). **Conclusions**: We assume that decrease of resting metabolic rate at successful students is possible due to redistribution of energy supply towards working structures of a brain. This kind of expenditure is essentially less in comparison with expenditure at physical activity. The rheographic index is an astable indicator at unsuccessful students. Parameters of functioning of an organism are more optimum for achievement of concrete result at successful persons.

PS 18 Functional ontogenesis of postjunctional angiotensin II receptors in the rabbit aorta.

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Aim: The aim of the present study was to evaluate aorta angiotensin II-mediated responses at postjunctional level of newborn and young rabbits. **Introduction**: In the aorta of newborn sheep, angiotensin II-mediated contraction (postjunctional response) is absent or diminished compared to adult animals(1).In contrast, the aorta of near-term mice already contracts to angiotensin II in a concentration-dependent way(2). However the maturation of postjunctional angiotensin II-mediated vasoconstriction at birth is unknown in rabbits. **Methods**: Aortas of anesthetized (xylazine 3 mg/kg and ketamine 22 mg/kg) male New Zealand newborn (postnatal day 1), young (3 to 5 months

old) and adult (14-16 months old) rabbits were dissected, removed and cut in rings which were mounted in isolated-organ baths and connected to isometric transducers. Non-cumulative dose-response curves for angiotensin II were determined. Concentration-response results were plotted and fitted to a sigmoid curve by non-linear regression (GraphPad Software, La Jolla, CA USA). Statistical analysis was performed by Student's t-test and by F-test (for Hill slope). $P<0.05$ was assumed to denote a significant difference. **Results**: Angiotensin II (1-300 nM) caused a dose-dependent contraction of the aorta in all groups. Angiotensin II potency was lower in adults ($EC50 = 9.66\times10\text{-}8 \pm 1.32\times10\text{-}8$ M, n=4) than in young rabbits ($EC50 = 1.48\times10\text{-}8 \pm 3.67\times10\text{-}9$ M, n=6). This loss of potency in adult rabbits suggests that young rabbits are the best control for this study. Maximal contraction to angiotensin II was lower in neonates than in young rabbits (neonates: Emax = 0.23 ± 0.07 N/g, n=5; young: Emax = 0.67 ± 0.12 N/g, n = 6). The potency of angiotensin II (neonate: $EC50 = 1.01x10\text{-}8 \pm 2.77\times10\text{-}9$ M, n=5; young: $EC50 = 1.48\times10\text{-}8 \pm 3.67\times10\text{-}9$ M, n=6) and the Hill slope of the concentration-response curve (neonate: Hill slope 1.07± 0.19; young: Hill slope 0.94±0.08) were not significantly different between neonatal and young rabbits. **Conclusions**: Postjunctional angiotensin II-mediated responses in aorta are already expressed in neonates when compared to young rabbits. As the potency of angiotensin II and the slope of the concentrationresponse curve were similar in neonates and young rabbits, it is suggested that the response is mediated by the same receptor, namely AT1, in both groups. The lower efficacy of angiotensin II at birth suggests that either the maturation of the excitation-contraction coupling or the receptor density is still incomplete. These results differ from those observed in sheep(1), but are in agreement with the results obtained in near-term mice(2), showing that the maturation of aorta postjunctional angiotensin II-mediated responses at birth are species dependent.

PS 198 Research of splenic lymphocyt e homing using in vivo cell competition method.

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Aim: We aimed to study the effect of cell immobilization and surface carbohydrate chain modulation on the splenic homing of lymphocytes in mice. **Introduction**: The spleen is the largest peripheral solitary lymphoid organ with a major lymphocyte homing activity. In contrast to lymph nodes and Peyer's patches, the spleen lacks high endothelial venules (HEV), and the exact details of lymphocyte recirculation both in humans and mice are not yet known. According to data from literature carbohydrate components are presumably playing a key role in the early adhesion of lymphocytes to the vascular endothelium. Our goal was to develop a method in which we could compare the migratory behaviour of two sets of lymphocytes within one organism. **Methods**: In our research we obtained lymphocytes from donor mice, and made them distinguishable with two different methods. One part of the cells were reacted with intracellular dye carboxy-fluorescein diacetate succinimidyl ester (CFSE), the other part was marked by non-specific biotinylation of their cell surface proteins. With flow cytometry and multicolour labelling microscopy we have verified that the labelling procedures have not influenced the adhesion and tissue specific localization of the cells. To justify the method's concept, we have studied the effect of different cell modifying substances: ConA lectin, binding to cell surface carbohydrate chains and colcemid (similar to colchicin) inhibiting microtubule polymerization, thus immobilizing cells. **Results**: We found that ConA lectin strongly inhibited CFSE-labeled lymphocyte entry into the spleen and almost completely prevented their migration to the white pulp compared to competitor cells. Colcemid treatment did not alter the number of CFSE-labeled lymphocytes entering the spleen significantly; however their migration in splenic tissues was greatly inhibited. **Conclusions**: According to our results, this method could be suitable for further research of splenic lymphocyte homing details.

PS 200 Ghrelin and obestatin decrease the IOP in a acute glaucoma model.

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Aim: The aim of this study is to evaluate the effect of the pro-ghrelin derived peptides in the intra-ocular pressure in animal models of acute glaucoma. **Introduction**: Ghrelin, des-acyl-ghrelin and obestatin are peptides derived from the pro-ghrelin. Ghrelin, an acylated 28 aminoacid peptide, is the endogenous ligand of the growth hormone secretagogues receptor-type 1a (GHSR-1a). Des-acyl-ghrelin lacks the acyl group and does not bind GHSR-1a. Obestatin is a 23 aminoacid peptide generated by an alternative splicing mechanism. These peptides act on many organ systems. In the eye, ghrelin promotes the relaxation of the iris' sphincter muscle independently from GHSR-1a and from nitric oxide (NO) and dependently on prostaglandins, while obestatin potentiates its cholinergic contraction. On the iris' dilator muscle ghrelin promotes relaxation, mediated by the GHSR-1a, and obestatin leads to a decrease in the basal tension. Ghrelin's aqueous humour levels were studied in patients with glaucoma. Glaucoma consists on an optic nerve damage and is the second cause of blindness all over the world, appearing subsequently to an increased intra-ocular pressure (IOP) in the majority of cases. Ghrelin's aqueous humour levels were significantly decreased in glaucoma. **Methods**: The first part of the experimental protocol was the calibration of the Tonovet® tonometer, used to measure the IOP, to the rabbit. The external calibration involved the cannulation of the eye's anterior segment with a 25G needle, connected to a pressure transducer and to a 0,9% NaCl reservoir, which enabled us to alter the IOP. Real IOP values varied from 5 to 60 mmHg and in each level IOP was measured with the tonometer. Afterwards, the real IOP was compared to the the tonometer measurements. In the second part of the protocol we generated acute glaucoma models in rabbits and studied the effects of ghrelin (10-4M, n=6), des-acyl-ghrelin (10-4M, n=7) and obestatin (10-4M, n=7) in the IOP, as well as the subcellular pathways involved. An intra-vitreal injection of 20% NaCl (50µL) was made in both eyes, in order to raise the IOP, being this an adapted glaucoma model. Then, one of the three peptides was sub-conjunctivally injected. Concerning the subcellular pathways, keterolac (a COX inhibitor; 30 mg/mL; 500µL; n=7) or L-NAME (a NO synthase inhibitor; 150mg/Kg; 500 µL; n=11) were sub-conjunctivally injected previously to both NaCl and ghrelin injection. All the results were compared to a control group which did not receive ghrelin, des-acyl-ghrelin or obestatin. **Results**: There is a linear correlation between the IOP measured by the Tonovet (Y) and its real value (X), being that the tonometer underestimated the real value ($Y= -0{,}331 + 0{,}750X$). Our results show that the NaCl injection increases the IOP from 9.9 ± 1.9 to 44.9 ± 4.1 mmHg. After that ghrelin promotes a decrease of 20,8 ± 5,0 mmHg (47,9 ± 11,6%); des-acyl-ghrelin does not significantly change IOP; and obestatin promotes a decrease of 15,8 ± 3,9 mmHg (37,5 ± 9,4%). When keterolac or L-NAME are added, ghrelin's effect is completely blunted. **Conclusions**: It is of major importance to calibrate the tonometer, in order to obtain real measurements. Concerning the experimental protocol, ghrelin leads to a decrease in the intra-ocular pressure, dependently on GHSR-1a and on NO and prostaglandins synthesis. Obestatin also promotes a decrease in the IOP.

PS 231 A Combined Cell Therapy and In-Situ Tissue-Engineering.

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Aim: To test the hypothesis that combined delivery of a novel in-situ polymerizable-hydrogel and cardiomyocytes into the rat myocardial infarction model will result in superior functional outcome than each intervention alone. **Introduction**: Myocardial cell-replacement strategies are emerging as novel therapies but are hahmpered by limited sources for human cardiomyocytes and by significant cell loss following transplantation. **Methods**: A photopolymerizable, biodegra-

dable, PEGylated-fibrinogen hydrogel was shown to be an effective carrier for cardiomyocytes [neonatal rat ventricular cardiomyocytes (NRV CMs) or human embryonic stem cell-derived cardiomyocytes (hESC-CMs)] both in-vitro and in-vivo. To determine the functional impact of this combined in-situ cell-delivery/tissue-engineering strategy, infarcted rat hearts were randomized to injection of saline, NRV CMs, biopolymer, or combined delivery of the biopolymer and NRV CMs. **Results**: Echocardiography revealed typical post-infarction remodeling in the saline-injected control group [deterioration of fractional shortening (FS) by 31.0±3.6%]. Injection of NRV CMs or biopolymer alone significantly ($p<0.01$) altered this remodeling process (manifested by a slight increase in FS (3.1±6.6% and 0.5±5.3%, respectively). Co-injection of the biopolymer and NRV CMs resulted in a significant increase in the cell-graft area (by 144%) and eventually to the best functional outcome, with FS improving by 26.3±6.6%. Finally, feasibility studies demonstrated the ability of the biopolymer to act as an effective cell-carrier of hESC-CMs and to significantly alter the remodeling process (FS improved from 18.9±1.7% to 20.0±1.5%, $p<0.05$). **Conclusions**: We describe a novel injectable in-situ-forming hydrogel that functions as an effective cardiomyocyte carrier and adds to the effect of the grafted cells to prevent unfavorable post-infarction cardiac remodeling.

PS 234 A Combined Gene and Cell Therapy Approach for Restoration of Cardiac Conduction.

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Aim: To test the feasibility of a novel strategy for cardiac conduction repair utilizing genetically engineered cells designed to form biological "conducting-cables". **Introduction**: Abnormal cardiac electrical conduction may underlie both bradyarrhythmias and reentrant tachyarrhythmias. However, no practical way exist for restoring or improving conduction in areas of conduction slowing or block. Therefore, restoration of conduction may represent an important antiarrhythmic target. **Methods**: An in-vitro model of conduction block was established using spatially-separated, spontaneouslycontracting, non-synchronized, human embryonic stem cell-derived cardiomyocyte - clusters. Immunostaining, dye-transfer, intracellular recordings (patch clamp), and multielectrode array (MEA) studies were performed to evaluate the ability of genetically-engineered HEK293 cells, expressing the SCN5A-encoded Na+ channel, to couple with cultured cardiomyocytes and to synchronize their electrical activity. Further studies were focused on extending the maximal coupling distance and on enhancing conduction velocity along the "conducting-cables" by co-expressing the inward-rectifier potassium channel (Kir 2.1 channel) in the SCN5A- HEK293 cells. A unique MEA plate consisting of larger recording area (256 electrodes) was utilized for testing this concept. **Results**: Connexin-43 immunostaining and Calcein dye-transfer experiments confirmed the formation of functional gap junctions between the engineered cells and neighboring cardiomyocytes. MEA and intracellular recordings were performed to assess the ability of the engineered cells to restore conduction in the co-cultures and to synchronize contractions among the beating clusters. Synchronization was defined by establishment of fixed local activation time differences between the cardiomyocyte-clusters and convergence of their spontaneous activation cycle-lengths. Non-transfected control cells were able to induce synchronization between cardiomyocyte-clusters separated by distances up to 300µm. In contrast, the Na+ channel-expressing cells synchronized contractions between clusters separated by up to 1000µm. Finally, we demonstrated that engineered cells expressing both Na+ and K+ channels extended the maximal coupling distance up to 5.5mm (the longest distance studied), which is 5.5 fold more distant than the maximal coupling distance facilitated by the cells expressing only the sodium channels. The synchronization occurred immediately after establishing co-culture between the engineered cells and the hESC-CMS- clusters and the conduction along the "cables" improved considerably. **Conclusions**: Genetically-engineered nonmyocytes, transfected to express Na+ and K+ channels, can form biological "conducting-cables" bridging and coupling spatially-separated cardiomyocyte-clusters in an in vitro model of conduction block. This proof-of-concept study describes a novel combined cell and gene therapy approach for restoration of cardiac conduction and might be useful for the development of therapeutic strategies for both brady- and tachy-arrhythmias.

PS 238 THE EFFECTS OF PROTAMINE SULPHATE ON THE DIFFERENT TYPES OF ACTIVATION OF THE ISOLATED RAT UTERUS.

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Aim: The aim of this experiment was to examine the relaxing effect of Protamine sulphate (PS) on the isolated uteri of rats and we tried to determine how this process developed, in spontaneous and calcium-induced activity, and also acetylcholine-induced activity, as well as the role of glibenclamide in antagonizing the effect of PS in spontaneous and calcium-induced activity. **Introduction**: Protamine sulphate is a mixture of polyamins, and it is used to counteract the anticoagulant effect of heparin. It has been found that PS has a relaxing effect in rats' uteri with spontaneous and calciuminduced activity as well as it decreases contractility induced by acetylcholine. There are several possible mechanisms that could cause the relaxation of muscle of uterus via the action of different types of receptors and signaling processes. They can involve K+ channels, a Ca2+-mediated effect, or some other receptor-mediated processes, but it has not proved any of these mechanisms yet. **Methods**: In our experiment we used uteri isolated by normotensive female Wister rats. The uteri were incubated for about 30 minutes in waterbath in De Jalon's solution at 37°C and aerated with 95% O2 and 5% CO2. Isometric contractions were recorded using an isometric force transducer. The preload of the preparation was approximately 1 g. In these conditions, some uteri immediately showed spontaneous contractions. Some uteri did not show spontaneous contractions, so they had to be stimulated by calcium or acetylcholine. When uteri achieved stable contractions, we put increasing doses of PS into the water bath. We examined the relaxing effects of protamine sulphate on the isolated rats uteri. We also used glibencamide 10mM because of its characteristics. Glibencamide is known as one of the most selective blockers of KATP channel, but it can also block voltage-sensitive pottassium (Kv) channels in concentrations higher than 10 mM. We used it in order to analyse its role as pottassium channels blocker in inhibiting effects of protamine sulphate. **Results**: We found out that increasing doses of PS (µg/ml: 10, 20, 50, 100, 150, 200, 300, 600) had a relaxing effect on uteri muscles in rats. Lower doses (µg/ml: 10, 20) caused no effect, but higher doses (µg/ml: 150, 200, 300, 600) were more effective than the lower ones, causing the complete relaxation. That effect was more expressed in spontaneous and calcium-induced contractions than in acetylcholineinduced activity. We also found out that glibencamide did not show any inhibiting effect on PS, both in spontaneous and calcium-induced activity. **Conclusions**: Our results showed that PS had a dose-dependent relaxing effect on uteri muscles. Mechanism of action is likely to be realized in KATP channel independent way.

PS 247 Different mechanism for diastolic dysfunction in diabetes and chronic pressure-overload.

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Aim: To study the myocardial morphological and functional changes induced by diabetes mellitus (DM) and/or pressure-overload (PO). **Introduction**: Diastolic heart failure (DHF) accounts for ~50% of heart failure cases and represents a growing problem in industrialized nations. Its pathophysiology is ascribed to impaired relaxation or increased myocardial stiffness. Often, chronic PO and DM lead to DHF. Moreover, hypertension is more prevalent among the diabetic population and exacerbates the extent of diabetic cardiomyopathy. Even so, functional and structural cardiac consequences of combined PO and DM are still unclear. **Methods**: Pressure-overload was performed

in Wistar-male rats by supra-renal aortic banding. After six-weeks, diabetes was induced by streptozotocin (65 mg/kg, ip) resulting in four groups: SHAM, banding (BA), diabetic (DM) and diabetic-banding (DB). Six weeks later, hemodynamic study was performed to evaluate cardiac performance. Samples were collected for histology, molecular studies and force measurement in isolated skinned cardiomyocytes. **Results**: Chronic PO increased LV hypertrophy (cardiomyocyte diameter: BA 22.0±0.4µm, SHAM 18.2±0.3µm), myofilament active force (Factive: BA 25.7±3.6kN/m2, SHAM 18.6±1.4kN/m2) and Ca2+ sensitivity (BA 5.56±0.02, SHAM 5.50±0.02) as well as phosphorylation of myofilamentary protein (MLC-2, MyBP-C) and insulin signaling pathways (Erk, Akt). At the extracellular matrix level, interstitial fibrosis (BA 10.8±0.9%, SHAM 5.3±0.6%) and pro-MMP-2 e MMP-9 activity were increased. In vivo, contractility (LV peak systolic pressure (LV-PES) and maximal LV wall stress (LV-Wstress)) was increased and both LV-PES and LV-Wstress correlated positively with Factive, while relaxation was impaired (τ; BA 14.0±0.9ms, SHAM 12.9±0.4ms). DM increased cardiomyocyte diameter (DM 21.4±0.4µm, DB 20.6±0.4µm), the expression of inflammatory (TNF-α) and apoptosis markers (Bax/Bcl-2). At the extracellular matrix level, diabetic animals displayed augmented myocardial fibrosis (DM 13.9±1.8%, DB 13.8±0.8%) and advanced glycation end-products (AGEs) deposition: 4.9±0.6score/mm2, 5.1±0.4score/mm2. In vivo, these abnormalities resulted in increased stiffness confirmed by the higher values of LV end diastolic pressure (LV-PED, DM 7.0±1.2mmHg, DB 6.7±0.7mmHg, SHAM 5.3±0.4mmHg) and end-diastolic pressure-volume relation (EDPVR, DM 0.59±0.18, DB 0.83±0.17, SHAM 0.41±0.10). Furthermore, diabetic animals displayed lower contractility (end-systolic pressure-volume relation (ESPVR), expression of MHC-α/MHCbeta and, as expected, lower activation of insulin signaling pathways (Akt phosphorylation levels). The association of DM and PO induced further pulmonary congestion (Lungs/body-weight: DB 5.23±0.21g/kg, SHAM 3.80±0.14g/kg) as this group combined overload-induced relaxation abnormalities and diabetes-induced stiffness. **Conclusions**: DM and PO led to distinct diastolic dysfunction phenotypes: while diabetes promoted myocardial stiffening, pressure overload impaired relaxation. The association of these damages accelerates the progression of DHF progression in diabetic-banded animals.

PS 255 Mycobacterial Thymic Immune Response.

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Aim: Investigate the characteristics of the immune response against mycobacteria within the thymus: Characterize the cytokine and chemokine profiles of infected thymi compared to those established in peripheral infected organs like the spleen and lung; Evaluate to what extent recent thymic emigrants and/or re-circulating mature T cells are responsible for the immune response against mycobateria within infected thymi. **Introduction**: Our laboratory has recently shown that, in the mouse model, mycobacteria are able to infect the thymus, the organ responsible for T lymphocyte differentiation, rendering newly differentiated T cells tolerant in responding to mycobacterial antigens. The bacterial load in the thymus slowly progresses, reaching a stagnation at more advanced periods of infection [16weeks post-infection (wpi)], while in the spleen it occurs after 4weeks. In the spleen, this stagnation is clearly associated to the appearance of IFN-γ secreting T cells, and so we suspected the involvement of these cells in the thymus as well. Knowing that newly generated T cells in the adult mice are not fully differentiated and that T cells that differentiate within an M. avium-infected thymus are tolerant to the invading pathogen, we suspected the involvement of mature T cells which are re-circulating from peripheral organs back to the thymus, in the thymic mycobacterial infection. **Methods**: Wild type and RAG-GFP mice were infected i.v. with 106 CFU M. avium. Expression of specific genes was assessed through qRT-PCR. Using the transgenic RAG-GFP mice, that allow the discrimination of newly generated T cells (GFP+) from re-circulating T cells (GFP-), we assessed the number, specificity and IFN-γ secreting ability of both these populations in the thymus. To analyze the specificity of re-circulating T cells in the thymus, cells were incubated with tetramers for the mycobacterial epitope antigen 85 (Ag85). **Results**: Coinciding with the stabilization of the bacterial load in the thymus is an increased expression of IFN-γ at 16wpi, in infected mice in comparison to uninfected, followed by an increase in iNOS expression, a marker of macrophagic activation, at 24wpi. Increased levels of the Th1 recruiting chemokines IP-10, MIG and MIP-1beta was detected in the thymus from 16wpi in comparison to non-infected mice. Although the number of re-circulating cells was not increased by infection, this pool was enriched with specific mycobacterial T cells. We were able to detect an increased number of Ag85-specific T cells within the pool of re-circulating T cells from 16wpi on. **Conclusions**: The data from this work presents evidence of an ongoing immune response in the thymus, which occurs at later time points and with a distinct activation profile from that in the spleen. The thymus recruits mycobacterial-specific T cells from the periphery, which appear to be major producers of IFN-γ upon stimulation with mycobacterial antigens. Consequently, this work suggests a role for re-circulation: cells that re-circulate back to the thymus might be part of a simple surveillance routine.

PS 256 A role for regulatory CD4+ T cells in the immune system reconstitution of HIV-infected patients under anti-retroviral therapy.

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Aim: The present work aims at understanding if the number or characteristics of the Treg cells are associated with the quality of the immune system reconstitution in a transversal study with HIV+ patients under HAART. Specifically, we aim to investigate how the proportion of Treg correlates with * the level of CD4+ T cell reconstitution; * the activation status of CD4+ T cells; * other characteristics of the patients like Nadir, age, duration of therapy. **Introduction**: Protection against infection is fundamental which is mostly mediated by the immune system. However, this system is subject to failure that might result in immunodeficiency. HIV infection is characterized by a progressive loss of CD4+ T cells, which has a significant impact on the efficiency of the immune /response. The highly active antiretroviral therapy (HAART) has reduced the morbidity and mortality associated with HIV infection by decreasing the viral load and increasing CD4+ T cell counts. Regulatory T (Treg) cells play a very important role in the immune system homeostasis, avoiding excessive activation during certain immune responses to infection and reducing the activation of auto-reactive T cells. These cells express the HIV co-receptor CCR5 and are highly susceptible to HIV infection and replication. Since Treg cells are crucial for the homeostasis of the immune system, the correct recovery of these cells during immune reconstitution due to HAART might have an important impact on this process. **Methods**: The aim of this work is to evaluate 60 HIV-infected adult individuals that are currently under HAART. Any patient interrupting therapy is excluded from the study. The clinicians collect all relevant information for each patient, namely age, sex, the latest CD4+ counts and HIV viral load, years in treatment, other existing pathologies and clinical signs of health/disease. Different blood cell populations are analyzed according to specific markers for each population by flow cytometry analysis: neutrophils (CD15), TCRαbeta lymphocytes (TCRαbeta), T cells (CD3), B cells (CD19), CD4+ versus CD8+ T cells, naïve (CD45RA) versus memory (CD45RO) cells, activated cells (CD69 and HLA-DR), recent thymic emigrants (CD31 and PTK7) and Treg cells (CD127lowCD25high-Foxp3+). **Results**: Our preliminary results suggest that an increased proportion of Treg cells is associated with a poor recovery of CD4+ effector T cells, and these Treg levels are negatively related to the Nadir value. **Conclusions**: Our results show a possible role for Treg cells in the maintenance of the homeostasis of the immune system by regulating the expansion and proliferation of CD4+ T cells.

PS 257 Function muscular evaluation in response to α-adrenergic stimulation in Diabetes and/or pressure Overload.

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Introduction: Both Diabetes mellitus (DM) and chronic pressure-overload due to hypertension are major causes of heart failure. Several studies suggest that the association of diabetes and hypertension results in more pronounced myocardial histological, ultrastructural and mechanical damages than each condition per se. Under cardiac stress conditions, such as hypertension or DM, the increased sympathetic activity and subsequent α-adrenergic (α-AR) stimulation is an important physiologic mechanism to enhance cardiac performance during augmented circulatory demands. The aim of this study was to investigate the role of myocardial α-adrenergic stimulation in pressure overload, diabetes mellitus and their association. **Methods**: Pressure-overload was established by suprarenal aortic banding in male Wistar rats. Six weeks later, diabetes was induced by streptozotocin (STZ, 65mg/kg, intraperitoneally), resulting in four groups: SHAM, banded (BA), diabetic (DM), and diabetic-banded (DM-BA). On the 12th week, left ventricular (LV) function was assessed in vitro by papillary muscle's performance at baseline and in response to increasing concentrations of phenylephrine (PHE; Dose A: 100mM; Dose B: 30mM; Dose C: 10mM; Dose DEF: 3mM) in the presence of a beta-adrenergic antagonist (nadolol, 0,01mM). **Results**: After 12 weeks, increasing concentrations of PHE decreased contractility in SHAM, BA, DM-BA groups (PS: Sham:-32,6±5,96%; BA:-17,7±8,07%; DM-BA:5,45±5,30%; dL/dtmax: Sham:-26,2±5,90%; BA:-15,0±8,34%; DM-BA:-5,6±5,02%; AT: Sham:-34,4±5,95%; BA:-18,9±7,80%; DM-BA:-9,3±5,92%; dT/dtmax: Sham:-31,7±6,48%; BA:-14,7±8,62%; DM-BA:-7,4±5,53%). Moreover, PHE showed a negative lusitropism as observed by the analyzed relaxation parameters (dL/dtmin: Sham:-37,1±9,87%; BA:-17,7±10,52%; DM-BA:-9,5±6,88%; dT/dtmin: Sham:-23,7±10,42%; BA:-17,3±8,30%; DMBA:-11,5±6,13%) (Table 1). Interestingly, only DM group displayed an increased in contractility and relaxation (PS: 17,3±10,52%; dL/dtmax: 13,3±8,70%; AT: 18,7±13,35%; dT/dtmax: 17,7±10,95%; dL/dtmin: 35,1±20,31%; dT/dtmin: 7,0±23,83%). **Conclusions**: Myocardial α-adrenergic stimulation with PHE improved contractility and relaxation only in diabetic animals. We conclude that this effect represent a compensatory response to the decreased betaadrenergic stimulation.

PS 261 IL-10 plays a role in the several behavioral dimension of depression.

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Aim: Study anxious-like, hedonic and cognitive behavior in mice lacking IL-10 expression (IL-10-/-) known to present depressive-like behavior. Evaluate the cytokine profile, in the periphery and brain regions, of these mice and correlate with behavioral alterations. **Introduction**: The role of pro-inflammatory cytokines in depression has been addressed by the scientific community during the last decades. In fact, patients with depression present increased serum levels of a sub-set of those cytokines. However, recent studies demonstrate that anti-inflammatory cytokines, namely IL-10, also play a role in this pathology. In fact, some studies show that depressed patients presented decreased IL-10 levels in the serum, while antidepressant treatment led to their increase. Accordingly, mice lacking IL-10 expression present depressive-like behavior that can be reversed with IL-10 administration. Symptoms like anxiety, anhedonia and cognitive impairment are usually present in depressed patients. Thus in this study we evaluated if these behavioral alterations were also present in mice lacking IL-10 expression. Moreover the mechanisms underlying the behavioral alterations present in IL10-/- mice are unknown. Since alterations in cytokine milieu are associated with depressive-like behavior we studied the cytokine profile of IL-10-/- in the periphery and also in the central nervous system. **Methods**: Females Balb/c IL10-/- and wild-type (WT), 3 months old, were use to study the behavior phenotype and the cytokine profile. Anxious-like behavior was evaluated in the Novelty-Suppressed Feeding (NSF). Hedonic behavior was studied in the burrowing test. To assess cognitive performance mice were submitted to Barnes Maze (BM) and Novel object Recognition (NOR) tests. The expression levels of several pro and anti-inflammatory cytokines were quantified by qPCR in the spleen (periphery) and several brain regions. **Results**: We observed that female IL-10-/- mice present an increased latency time in the NSF when compared to WT animals. The burrowing behavior was decreased in IL-10-/-. Furthermore, a decreased recognition index in the NOR and an increased latency time on the BM were observed in IL10-/- mice in comparison to controls. The study of cytokine profile in brain regions and periphery showed that IL10-/- mice present some alterations in the cytokine milieu. **Conclusions**: In addition to depressive-like behavior, female IL-10-/- mice also present anxious-like behavior, anhedonia and cognitive impairment, the same behavioral dimensions frequently altered in depressed patients. Interestingly all this behavioral alterations are associated with an imbalance in the cytokine expression in the brain and also in the periphery. These findings suggest that IL-10 could be a target molecule for the development of new antidepressant therapies.

PS 271 Ghrelin expression in the rat's eye.

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Aim: The purpose of this study was to evaluate ghrelin's ocular tissue distribution in the adult rat's eye and to confirm previously reported data, describing the identification of ghrelin's mRNA in the iris posterior epithelium and in the ciliary body non pigmented epithelium. **Introduction**: Ghrelin is an acylated 28 aminoacid peptide that was first discovered in the rat's gastric mucosa in 1999 by Kojima et al.. This peptide is the endogenous ligand of the somatosecretagogue's receptor (GHSR-1a), promoting growth hormone's release from the hypothalamus. Recently, it has been described that ghrelin induces the relaxation of iris's sphincter muscle independently from GHSR-1a, nitric oxide and calcium dependent potassium channels and dependently on prostaglandins release. Ghrelin also relaxes the iris' dilator muscle, being this process mediated by the GHSR-1a. Ghrelin's mRNA was also identified in the iris posterior epithelium and in the ciliary body non pigmented epithelium. **Methods**: Adult Wistar rats were sacrificed through an intraperitoneal injection of sodium pentobarbital and both eyes were immediately enucleated and processed for cryostat sections and indirect immunofluorescence protocol. Slides were incubated with anti-ghrelin, anti-histone H3 (positive control) and with 2% NGS (negative control) at 4ćC for 48 hours and then with secondary antibody containing a fluorescent tag during 1 hour. After incubation slides were examined under a fluorescence microscope. **Results**: We observed immunolocalization of ghrelin in the rat's ciliary body epithelium. Its major label appeared in the inner part of that epithelium facing the stroma of the ciliary processes. This peptide showed no expression in the posterior segment, namely in the retinal pigmented epithelium. **Conclusions**: Ghrelin is locally produced in the eye, mainly in the ciliary processes. Based on these findings, we can conclude that this peptide may play a role as a local regulator of the aqueous humor dynamics and ciliary muscle kinetics.

SURGERY *Session*

PS 115 Simultaneous pancreas-kidney transplantation vs. kidney transplantation in type 1 diabetic patients: which one provides a better quality of life?.

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Aim: The goal of this study was to compare quality of life between simultaneous pancreas-kidney transplant (SPKT) and kidney transplant (KT) among type 1 diabetic patients. **Introduction:** Long-term complications of Diabetes Mellitus (DM) are responsible for the majority of all morbidity and mortality associated with the disease. Terminal diabetic nephropathy is more and more dealt with a simultaneous pancreas-kidney transplant (SPKT) in type 1 DM patients instead of kidney transplant (KT), with a reduction in morbidity and mortality. **Methods:** A cross-sectional study was carried out in 49 patients in the SPKT group and 33 patients in the KT group. Quality of life was assessed with the validated SF-36 questionnaire. **Results:** The SPKT group presented better values in all domains, though only the Physical Function (80,17% vs. 68,33%, p=0,027) and General Health (43,88% vs. 32,58%, p=0,009) variables have yielded statistically significant results. **Conclusions:** The SPKT group presented statistically superior results in some domains of quality of life when compared to KT. Larger studies are needed in order to confirm this trend.

PS 132 The influence of gender and body mass index on eruption time of permanent teeth in children aged 6.

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Aim: The aim of this study is to determine the time of eruption of permanent teeth, if there is a change in the time of eruption and if it depends on gender and body mass index (BMI). **Introduction:** Tooth eruption is the developmental process responsible for moving teeth from its crypt position through the alveolar process into the oral cavity to its final position of occlusion with antagonist. The factors which affect the organism as a whole are reflected also on the growth and eruption of teeth. Overweight children have low growth hormon serum levels, but they have high leptin levels which may play role in earlier onset of puberty and development in girls or later in boys. Fat tissue has aromatase enzymes that convert testosterone to estrogen which slows down boys development. Reduced energy and protein intake can delay eruption. **Methods:** The research included 126 children, 65 girls and 61 boys, aged 6, in the city of Novi Sad and its surroundings. To evaluate nutritional status, weight and hight were measured, the BMI was calculated and percentile distribution was performed using the percentile curve. Dental examination was performed. The statistical significance was determined with statistical methods of descriptive and comparative analysis. **Results:** The mean value of existing permanent teeth for the entire group is 5,03 while by gender is significantly higher ($p<0.05$) for females. Underweight girls have significantly fewer teeth than overweight girls ($p<0.05$) and boys with normal weight have significantly more teeth than overweight boys ($p<0.05$). Children with low BMI have significantly fewer permanent teeth ($p<0.05$). **Conclusions:** Based on these results it can be concluded that dental age in 6 years old children is 5,03 teeth, that there is a change in the time of eruption of permanent teeth and that it depends on gender and BMI.

PS 152 THE EFFICIENCY OF LOW LEVEL LASER THERAPY IN THE TREATMENT OF THE MOUTH BURNING SYNDROME IN DIABETICS.

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Aim: To analyse the efficiency of low level laser treatment in diabetics burning mouth syndrome. **Introduction:** Low-power lasers are used in dentistry for treatment numerous oral painfull conditions and diseases. The biostimulative effects, especially analgesic, antiinflammatory and antiedemous effect are confirmed. The numerous changes in the oral cavity such as dry mucous membranes, burning mouth and tongue, decreased saliva flow rate, Candidiasis are diagnosed in diabetic patients. **Methods:** The study was conducted on 20 patients diagnosed as diabetes mellitus as well as Burning mouth syndrome. Patients were divided into two groups: Group I consisted of 10 patients who were treated with low-power laser and the group II consisted of 10 patients who were treated with therapeutic preparations (Dactanol Gel + Amp Lemod). LLLT was applied, with a Scorpion Dental Optima apparatus (wavelength 635 nm, strength 25mV, exposure time 120 s), in 5 daily sessions. Visual-Analogue Scale for pain was used for measuring oral discomforts in diabetics. **Results:** After the first session the statistically significant difference between the average value of pain between the groups was established. Namely, the patients treated by LLLT had significantly lower mean value of pain than those treated by Dactanol gelom+Amp Lemod ($p<0,05$). After II, and III sessions, the average value of pain decreased in both groups, but the decrease was more pronounced in patients treated with laser. In the end, the level of statistical significance of differences in mean of pain between the groups was ($p<0.001$). **Conclusions:** LLLT is beneficial as an adjunctive treatment modality for treating symptoms of mouth burning syndrome in diabetic patients.

PS 157 Automatic pre-bended customized bars for Nuss operation using chest CTscan.

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Aim: Study all patients that underwent Pectus Excavatum (PE) corrective surgery at the Pediatric Surgery department in Hospital de São João-Porto between 2000 and 2010 and evaluate the modifiable variables that affect the surgical results in which integrates i3DExcavatum System. **Introduction:** PE is the most common thoracic malformation occurring between 1:400 and 1:1000 births and more frequently in males. The surgical repair has progressed over the past 20 years due to improvements of the Nuss technique that aims to place a metal bar in the retrosternal space where it remains 2-3 years. We developed a new technology - i3DExcavatum System - that complements the surgical procedure allowing the automatic and personalized bar bending based in 3D CT scan previously to surgery. **Methods:** Through clinical process consultation, data regarding the surgical correction of PE between 2000 and 2010 was collected. To compare the internal references used for automatic bending with the external costal grade references used for manual-bending process, the distance between the skin and costal margin in the CT horizontal plan was measured including the deepest point of the sternum at midaxillaries lines and under the nipples. The lengths over the skin and above the external surface of the costal grade in this plan and the plan for insertion of the prosthesis were measured. To study the evolution of surgical correction influence area, the anterior chest surface pre-surgical and post-surgical 3D reconstructions were comparatively analyzed. **Results:** There were significant decreases ($p<0.001$) of 33 minutes in duration of surgery, 22 minutes in duration of anesthesia and 2.5 days in length of postsurgical stay in automatic modelling group. The complication rate is lower in the group of automatic modelling (31.6% vs. 8.3%, $p<0.05$). The skin-to-costal distances reached values over 20 mm at mid-axillaries lines and under the breasts mainly in females. Significant differences were detected between the skin and the costal lines in both genders. Despite deformity correction occurs similarly in all directions of the anterior chest wall, diverging measures and outliers were found. **Conclusions:** These results are encouraging and confirm the advantages of metal bar automatic modelling. i3DExcavatum System overcomes the time consuming and

boring step of manual bending, allowing creation of a smooth bar completely adapted to the patients characteristics and minimizing the errors induced by a manual bending process based in the skin surface.

PS 160 Treatment of femoral head osteonecrosis following IV drug: total hip arthroplasty versus core decompression.

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Aim: The main purpose of current study was to compare the functional outcomes after THA and CD in patients with femoral head osteonecrosis following long-term Tamjizak and Norjizak usage. **Introduction**: Femoral head osteonecrosis is a common problem following long-term corticosteroid usage. Tamjizak and Norjizak are two forms of IV drugs in Iran which contain corticosteroids. Currently, total hip arthroplasty (THA) and core decompression (CD) are two methods for treating femoral head osteonecrosis. **Methods**: The population in this clinical trial study consisted of 30 patients with femoral head osteonecrosis following-term Tamjizak and Norjizak usage aged 35.53±9.42 years (17-55). There were 25 males and 5 females. Mean time of IV drug injection was 3.2±1.6 years (1-5 years). Patients were divided into 2 groups: 15 underwent THA and 15 CD. The mean follow up was 14 months (6 months to 2 years). Functional hip score was used to assess the functional status of the patients Pre- and postoperatively. The score questions pain, mobility, and ability to walk. Each of these has 6 points. The greater the score the outcome will be better. Outcomes can be classified as very good, good, medium, fair, and poor. Finally, data were analyzed using paired and independent samples t-test. **Results**: The mean score was 7.8±1.9 preoperatively and 15.7±1 postoperatively in THA group and 7.4±1.6 and 14.33±1.2 in CD group. Paired t-test showed that there was a statistically meaningful difference between pre- and postoperative scores in 2 groups (p=0.00). The postoperative score of the THA group was significantly greater than the other (p=0.002). **Conclusions**: Compared to CD, current study showed superior functional outcomes with THA in the treatment of patients suffered from femoral head osteonecrosis following long-term corticosteroid usage.

PS 174 THE USE OF MODERN METHODS OF ENTEROSCOPY IN PREOPERATIVE DIAGNOSIS AND TREATMENT OF SMALL BOWEL TUMORS.

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Aim: To estimate the possibilities of videocapsule endoscopy (VCE) and balloon-assisted enteroscopy in preoperative diagnosis and minimally invasive treatment of the small bowel tumors. **Introduction**: From V.2003 to V.2011 we observed 56 pts. with definitely established or strongly suspected diagnosis of SBT. Small bowel tumors were diagnosed in 28 (50,0%) pts. (m-16, f-12, ranged from 18 to 76 years, mean age 54,7±13,9 years) by using VCE (Given Imaging, Olympus, OMOM) and flexible enteroscopy (FE) (single- and double balloon, push enteroscopy or laparoscopically-assisted balloon enteroscopy). At this period 12 (21,4%) pts. were admitted to the hospital and operated urgently (without previous examination) for small bowel obstruction/peritonitis or intestinal bleeding caused by angioleiomyoma (1), malignant carcinoid (4), adenocarcinoma (2), lymphoma (1), sarcoma (2) and melanoma metastasis (2). During complex examination in 16 (28,1%) pts. the diagnosis of SBT wasn`t confirmed. **Methods**: From V.2003 to V.2011 we observed 56 pts. with definitely established or strongly suspected diagnosis of SBT. Small bowel tumors were diagnosed in 28 (50,0%) pts. (m-16, f-12, ranged from 18 to 76 years, mean age 54,7±13,9 years) by using VCE (Given Imaging, Olympus, OMOM) and flexible enteroscopy (FE) (single- and double balloon, push enteroscopy or laparoscopically-assisted balloon enteroscopy). At this period 12 (21,4%) pts. were admitted to the hospital and operated urgently (without previous examination) for small bowel obstruction/peritonitis or intestinal bleeding caused by angioleiomyoma (1), malignant carcinoid (4), adenocarcinoma (2), lymphoma (1), sarcoma (2) and melanoma metastasis (2). During complex examination in 16 (28,1%) pts. the diagnosis of SBT wasn`t confirmed. **Results**: From 28 pts with SBT in 15 pts we performed VCE which defined indications to FE and radiological methods (in some cases) for diagnosis improvement in 13 pts (with subsequent surgery in 6) and directly to surgery in 2 pts. During FE in 19 pts. we were able to evaluate the tumor, to provide biopsy or to perform SBT removal. Histologically there were: hyperplastic polyp - 5, tubular adenoma - 3, Peutz-Jegher's hamartoma - 5, leomyoma - 2, carcinoid - 2, adenocarcinoma - 4, GIST - 4, undifferentiated carcinoma - 1, B-cell lymphoma -1, metastatic lesion -1. Conservative treatment (including chemotherapy) have been applied in 6 (21,4%) pts. Endoscopic interventions were performed in 10 (35,7%) pts.: polypectomy by electroexcision (9) - 19 tumors were removed, the biggest one - 60mm; mucosal resection (1) - in FA P case, 3 adenomas were removed, carcinoid was removed by a polypectomy snare. There were three complications - small bowel intussusception in a patient with Peutz-Jegher's syndrome during VCE, the patient was urgently operated; capsule retention in a patient with adenocarcinoma, that was solved by resection of the affected bowel; bleeding in 1 patient after polypectomy in ileum that was stopped using argon plasma coagulation. Surgical operations were performed in 12 (42,9%) pts.: partial small bowel resection of tumor via mini-laparotomy access after previous laparoscopy (3) and conventional laparotomy (9) for adenocarcinoma - 4, GIST - 4, leomyoma - 2, large Peutz-Jegher's polyp complicated by acute intussusception during VCE - 1, melanoma metastasis - 1. Nor intra- neither postoperative complications or deaths have been revealed in all these patients. **Conclusions**: Diagnostic possibilities of VCE and balloon-assisted enteroscopy along with biopsy helps to select the right treatment policy: 57,1% pts with SBT needs conservative treatment (with endoscopic removal in 35,7% of cases) while surgery is necessary in 42,9% mainly malignant cases. Due to the novel methods of deep enteroscopy (VCE and balloon assisted enteroscopy) SBT are being diagnosed more often at the earlier stages with greater accuracy while no complications are developed.

PS 176 Improvement of cardiovascular markers after Roux-en-Y gastric bypass in female patients.

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Aim: The purpose of our study was to estimate the influence of Roux-en-Y gastric bypass on markers of cardiovascular events in female patients. **Introduction**: Obesity is strongly linked to cardiovascular diseases, the leading cause of mortality in Poland. Bariatric operations significaty reduce weight and treat obesity. We assume that in addition to weight reduction, bariatric surgery affects several cardiovascular markers. **Methods**: We retrospectively analysed the data of 22 female patients operated on from 2008 to 2009 (Roux-en-Y gastric bypass) . Patients were evaluated before operation and in 3, 6, 12 months. We measured biochemical cardiovascular risk biomarkers: total cholesterol, high-density lipoprotein (HDL), low-denisty lipoprotein (LDL), trigliceryde, total cholesterol/HDL ratio and fibrinogen. The data were analysed using Statistica 9.0PL. **Results**: The mean age of the 22 patients was 41 years, the mean BMI was 49 (±6) kg/m2, 32% were diabetic and 59% were hypertensive. Significant improvement occurred in the cardiovascular biomarkers: TC, HDL, LDL, HDL/TC ratio, fibrinogen and trigliceryde level ($p<0.05$). Total cholesterol decreased from 171,1 (±28,4) mg/dL to 137,4 (±27,1) mg/dL at 12 months ($p<0,05$) ; HDL level increased from 43,1 (±10,8) mg/dL to 53,6 (±11,2) mg/dL at 12 months ($p<0,05$) ; LDL level decreased from 98,3 (±21,6) mg/dL to 66,9 (±23,6) mg/dL at 12 months ($p<0,05$). ; Total cholesterol/HDL ratio decreased from 4,1 (±0,9) to 2,6 (±0,7) at 12 months($p<0,05$); fibrinogen level decreased from 469,2 (±112,7) mg/dL to 383,6 (±85,3) mg/dL at 12 months($p<0,05$); trigliceryde level decreased from 151,3 (±37,6) mg/dL to 83,1 (±30,6) mg/dL at 12 months ($p<0,05$). **Conclusions**: The findings of the study have proved the influence of bariatric operations on cardiovascular markers in female patients. The improvment of all investigated factors has been shown after bariatric operation with statistic significance in total cholesterol, HDL, LDL,fibrinogen level and TC/HDL ratio.

PS 222 Improvement in Type 2 Diabetes Mellitus after Roux-en-Y Gastric Bypass in Morbidly Obese Patients.

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Aim: The aim of this study was to assess the influence of Roux-en-Y gastric bypass on diabetic parameters in diabetic morbidly obese patients. **Introduction**: Obesity is a problem closely related to type 2 diabetes mellitus. There are growing evidences that bariatric procedures such as Roux-en-Y Gastric Bypass (RYGB) lead to improvement or even complete resolution of type 2 diabetes mellitus (T2DM) in morbidly obese patients. **Methods**: Forty one patients underwent RYGB from 2008 to 2009, nine of them were diabetic (22%). Data of diabetic patients were analyzed retrospectively. Body mass index (BMI), fasting plasma glucose level, fasting insulin level and HbA1c were measured before and 12 months after surgery. HbA1c level lover than < 6.5% was defined as well controlled diabetes. **Results**: Mean BMI changed from 46.3±6.4kg/m2 to 31.4±7.9 kg/m2 in 0, 12 months period (p<0,05). Mean fasting plasma glucose level decreased from 124,3 ±34.8mg/dl before operation to 84.9 ±9.9 mg/dl after operation (p<0,05). Mean HbA1c level decreased from 7.1 ±0.8mg/dl before operation to 5.61 ±0.72mg/dl after operation (p<0,05). Mean fasting insulin level decreased from 15.3±10.9 uIU/ml before operation to 4.49±4.13 uIU/ml after operation (p<0.05). Before operation 2 of 9(22.2%) patients had HbA1c level lover than 6.5%. Twelve months after surgery 8 of 9 (88.9%) patients had HbA1c level lover than 6.5%. **Conclusions**: Diabetic parameters were improved 12 months after RYGB operation in morbid obese patients with type 2 diabetes. Percentage of patients with well controlled diabetes increased after operation.

PS 246 Fetal Middle Cerebral and umbilical arteries blood flow velocities in normal and diabetic associated pregnancies.

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Aim: To assess the Doppler parameters in the umbilical and fetal Middle Cerebral arteries in women with Gestational diabetes mellitus (GDM) and normal pregnancies. **Introduction**: Gestational diabetes mellitus (GDM) is one of the most current complications in pregnancies; which has an incidence as high as 13% in high-risk ethnic groups.GDM associated gestations may be susceptible for both maternal and fetal complications, which is approved by some studies. Several studies showed that maternal-placental blood flow might have changes caused by hyperglycemia during the pregnancy; As a compensatory mechanism to the decrease of placental blood flow, redistribution of blood flow from peripheral vessels to the brain was occurred,which can be well defined by Doppler Ultrasound measurements of Umbilical artery(UA) and Middle cerebral arteries(MCA) of the fetuses. Nowadays fetal cerebral blood flow velocities assessment has become a suggested way of evaluation of high-risk pregnancies. Although some studies have been done on evaluating the pregnancy complications such as Intra Uterine Growth Restriction (IUGR) and pre-eclampsia, and they have shown significant changes in the Doppler parameters of the fetal MCA; there isn't sufficient evidence about the effect of GDM on the fetuses. **Methods**: A cross-sectional study was performed on 66 pregnant women, including 33 women with GDM and the others without it, in Akbar-Abadi University Hospital in Tehran, Iran during 2010-2011. Peak systolic and diastolic velocities, pulsatiliy index(PI), resistance index(RI) and systolic/diastolic ratio(S/D) were recorded in Umbilical artery(UA) as well as both right and left Fetal Middle Cerebral Arteries(MCAs) for every recruited pregnant women by means of Doppler ultrasonography. **Results**: The mean gestational age at the time of examination was 34.45(SD=2.62) weeks in GDM-positives and 34.63(SD=3.23) in GDM-negatives (P=0.218). Although all of the measured Doppler parameters had higher values in GDM-positives, but the differences were not significant between two groups of study; except for the left fetal MCA-PI which was significantly higher in GDM associated group [2.07(SD=0.07) vs. 1.85(SD=0.74), P=0.03]. **Conclusions**: Evaluating other pregnancy complications including Intra Uterine Growth Restriction (IUGR) and preeclampsia, some studies have shown significant changes in the blood flow velocity of the fetal MCA. Our study is one of the first to confirm that gestational diabetes could also correlate with the increased pulsatility index of the fetal MCA. This result could be due to the brain-sparing phenomenon; and as it was demonstrated that hyperglycemia affect the diabetic adult vessels, it might also involve fetal vessels in GDM associated pregnancies.

PS 249 MINIMALLY INVASIVE SURGERY IN THE TREATMENT OF BILIARY TRACT LITHIASIS.

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Aim: Evaluation the frequency of laparoscopic cholecystectomy (LC) compared to conventional (OC), length of hospital stay and cost-benefit procedures, as well as the distribution of patients according to sex and age structure, in our material. **Introduction**: Minimally invasive surgery in the treatment of biliary tract lithiasis, or laparoscopic cholecystectomy, based on the classic surgical principles with new technology solutions, includes planned and emergency surgery. **Methods**: This retrospective analysis included 618 patients, with the diagnosis of biliary tract lithiasis, male and female, age over 18 years old, who were hospitalized and treated at the Clinics for Abdominal, Endocrine and Transplantation Surgery at the Clinical Center of Vojvodina, in the period from 01.01. to 31.12.2010. Laparoscopic cholecystectomy was performed in all patients who were scheduled for elective surgery, unless they didn`t want this approach. In emergency operations, the selection of approach was on the treating surgeon, with the consent of the patient. **Results**: Results show a higher frequency of LC-367 (59%) compared to OC-251 (41%). Length of hospital stay for patients operated LC is significantly shorter and is 2 days, while the OC is 7days, which is statistically significant (p <0.05). Cost-benefit procedures LC compared to OC, shows a high level of statistical significance (p <0.005). LC frequency is higher in women 71%, and represents a statistically significant difference (p <0.05). The patients are from the same age group, which means that there are no restrictions on the age structure of patients. **Conclusions**: Length of hospital stay LC is significantly shorter and allows faster return normal life activities, average of 7 days. Laparoscopic cholecystectomy represents effective and economical way to treatment of biliary tract lithiasis.