Inflammatory bowel diseases (IBD) are a group of pathological conditions characterized by chronic inflammation of the gastrointestinal tract, including Crohn’s disease and ulcerative colitis. To date, imaging of IBD is based on several radiological techniques such as barium studies, magnetic resonance imaging, and computed tomography (CT). Endoscopy is the gold standard for the assessment of the large bowel and proximal small intestine in patients with IBD allowing the biopsy of the visualized bowel. Positron emission tomography (PET) and PET/CT with Fluorine-18-fluoro-2-deoxy-D-glucose (FDG) is a functional imaging method used to detect abnormalities in glucose metabolism in a variety of disorders. FDG accumulates mainly in tumours, but increased uptake and retention has been shown also in lesions with a high concentration of inflammatory cells, such as granulocytes and activated macrophages. Recent literature data demonstrate that FDG-PET and PET/CT may be useful noninvasive tools for identifying and localizing active IBD. In patients with an established diagnosis of IBD, FDG-PET and PET/CT may provide information about disease activity, location and extent of the disease within the intestinal tract, allowing early recognition of disease relapse and possible complications. Furthermore, these techniques may play a role in assessing the treatment response to medical therapy in patients with IBD.
Different studies have demonstrated the efficacy of extremely low frequency electromagnetic fields (ELF EMFs) in the treatment of pain. In particular, the positive effects of ELF EMFs seem to depend on their respective codes, such as frequency, intensity and waveform, even if the exact mechanism of interaction is still debated. The most commonly used for extremely low frequency magnetotherapy is a 100Hz sinusoidal field (ELF) with a mean of induction of few Gauss. This article reviews the therapeutic application of a musically modulated electromagnetic field (TAMMEF), a new-generation of electromagnetic field used for extremely low frequency magnetotherapy characterized by variable frequencies, intensities and waveforms. Both clinical and experimental studies, performed by authors of the present review, have demonstrated the efficacy of ELF and the new TAMMEF systems in several musculoskeletal disorders such as osteoarthritis, rheumatoid arthritis, carpal tunnel syndrome, shoulder periarthritis and cervical spondylosis. Moreover, it has been demonstrated that ELF and TAMMEF systems are not only effective, but also safe, from clinical and experimental point of view. In fact, clinical trials did not reported any undesired side effect, while in vitro studies showed that ELF EMFs did not induce uncontrolled cell proliferation, did not affect cell viability and did not induce apoptosis. With their efficacy and safety, ELF and even more the new TAMMEF systems represent a valid complementary or alternative treatment to standard pharmacological therapies in reducing both pain and inflammation of patients affected by musculoskeletal disorders.
Klebsiella pneumoniae is a common hospital-acquired pathogen, causing urinary tract infections, nosocomial pneumoniae, and intra-abdominal infections. K. pneumoniae is also a potential community acquired pathogen. The aims of this study are to determine epidemiology of ESBL-producing K. pneumoniae and K. oxytoca in Iran during different seasons, to determine the prevalence of blaTEM, SHV and CTX-M responsible for ESBL production among ESBL-producing K. pneumoniae and K. oxytoca in the different wards and hospitals in Iran. Klebsiella spp producing ESBLs were identified by phenotypic and genotyping methods. The findings in this study revealed that 36.5%, 51.7% and 45.6% of K. pneumoniae were producing ESBLs in Ilam, Milad and Emam Reza hospitals, respectively. The results revealed blaSHV was responsible for ESBLs production. The analysis showed significant difference of ESBLs production by K. pneumoniae in winter (53%) in comparison to the other seasons $P \leq 0.01$. The results also showed a significant difference in blaSHV that was the dominant gene responsible for ESBL production $P \leq 0.049$ but no significant difference was observed between blaTEM and blaSHV. The results showed that the highest ESBL production was found in K. oxytoca isolated from patients in Emam Reza Hospital and the lowest frequency of ESBL production was found among K. oxytoca in Ilam hospital. BlaSHV was found as dominant gene responsible for ESBLs production by K. pneumoniae and K. oxytoca, followed by blaCTX-M.
PLASMA AND ADIPOSE TISSUE LEVELS OF SELECTED GROWTH/INHIBITORY FACTORS, PROTEOLYTIC ENZYMES AND SPHINGOSINE-1-PHOSPHATE IN HUMANS

W. BŁOGOWSKI¹,², K. SERWIN², D. SAŁATA³, M. BUDKOWSKA³, B. DOŁĘGOWSKA³, M. ŁOKAJ⁴, P. PROWANS⁴ and T. STARZYŃSKA¹

Departments of ¹Gastroenterology, ²Physiology, ³Laboratory Diagnostics and Molecular Medicine, and ⁴Plastic, Endocrine and General Surgery, Pomeranian Medical University in Szczecin, Poland

Received June 21, 2012 – Accepted August 31, 2012

Recent studies have shown that adipose tissue (AT), while implicated in orchestrating the sophisticated process termed “immunometabolism,” may also serve as a potential niche for various bone marrow-derived (stem) cells. However, at present, the direct biochemical and immunomodulatory composition of the human AT environment has not been studied. Several substances that might play a crucial role in regulating stem cell migration and/or homing to AT, have been implicated, namely, hepatocyte/vascular endothelial growth factor (VEGF/HGF), leukemia inhibitory factor (LIF), and sphingosine-1-phosphate (S1P). Therefore, we examined and compared the AT concentrations of these substances between plasma, subcutaneous, and omental AT samples derived from 35 generally healthy subjects. VEGF, HGF, LIF, and metalloproteinases (MMP)-2 and MMP9 levels were measured using ELISA, and S1P concentrations were analyzed using reverse-phase high performance liquid chromatography. We found that AT levels of analyzed growth/inhibitory factors were generally comparable (VEGF and LIF) or even higher (HGF) than the corresponding levels in the peripheral blood, particularly in overweight/obese subjects. In subcutaneous AT, significantly lower VEGF and LIF concentrations were observed, and these were accompanied by higher MMP levels. No depot-specific differences in S1P concentrations were found in all examined groups. Moreover, we established several associations between analyzed molecular substances and body composition, BMI, or adiposity index of the examined patients. In conclusion, our study revealed that human AT possesses relatively high levels of selected growth/inhibitory factors and of chemoattractants involved in the regulation of stem cell trafficking, and these factors are associated with the metabolic status of an individual. Further studies are needed to clearly establish the role of these factors in the regulation of bone marrow-derived (stem) cell homeostasis and homing in human AT.
IS PENTRAOXIN-3 STRONGER THAN C-REACTIVE PROTEIN TO DETERMINE INFLAMMATION IN PERITONEAL DIALYSIS PATIENTS?

M. GURSU, S. OZTURK, Z. AYDIN, S. KARADAG, Y. DOVENTAS, M. KOLDAS, S. UZUN, A. SUMNU and R. KAZANCIOGLU

1Haseki Training and Research Hospital, Department of Nephrology, Istanbul, Turkey; 2Haseki Training and Research Hospital, Department of Biochemistry, Istanbul, Turkey; 3Bezmialem Vakif University, Medical Faculty, Department of Nephrology, Istanbul, Turkey

Received April 22, 2012 – Accepted July 25, 2012

Pentraxin–3 (PTX-3) is the prototype of long pentraxins and is produced by many tissues and organs including vascular endothelial cells in response to pro-inflammatory signals. It is thought to be an independent indicator of disease activity. We analyzed the correlation of PTX-3 with other markers of inflammation in peritoneal dialysis (PD) patients. Non-diabetic patients on chronic PD program who meet the dialysis adequacy criteria and who had no active infectious/inflammatory disease were included. Demographic and clinical parameters were recorded as well as hsCRP, fibrinogen, interleukin–6 (IL-6) and PTX-3 levels; and the correlation between them were studied. Twenty-five patients (mean age: 45.7±12.5 years; female/male ratio: 16/9) were included. Mean PTX-3 level was 2.16±2.76ng/ml. PTX-3 was found to be correlated positively with only IL-6 among inflammatory markers (r=0.827; p<0.001) but not with hsCRP. With linear regression model, IL-6 was the only independent determinant of PTX-3 levels. PTX-3 may be a more valuable marker of inflammation than CRP in patients on PD.
TARGET IDENTIFICATION AND VALIDATION OF
(+)2-(1-HYDROXYL-4-OXOCYCLOHEXYL) ETHYL CAFFEATE,
AN ANTI-INFLAMMATORY NATURAL PRODUCT

L. LI1,2, H-W. ZENG1, F. LIU1, J-G. ZHANG4, R-C. YUE1, W-Q. LU3, X. YUAN1,
W-X. DAI1, H. YUAN4, Q-Y. SUN1, J. HUANG3, H-L. LI3, Y-S. LF, L. SHAN1,a
and W-D. ZHANG1,4,b

1Department of Natural Product Chemistry, Second Military Medical University, Shanghai, PR China;
2Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, Liaoning Province, PR China;
3School of Pharmacy, East China University of Science and Technology, Shanghai, PR China;
4Department of Natural Product Chemistry, School of Pharmacy, Shanghai Jiaotong University, Shanghai, PR China

Received July 16, 2012 – Accepted October 23, 2012

The first two authors contributed equally to this work

(+)-2-(1-hydroxy-4-oxocyclohexyl) ethyl caffeate (HOEC) was isolated from Incarvillea mairei
var. granditlora (Wehrhahn) Grierson. The plants of the Incarvillea genus have long been used as folk
medicines for the treatment of inflammation-related diseases in China. 5-Lipoygenase (5-LOX), a key
enzyme in the arachidonic acid (AA) cascade, was identified as a potential target of HOEC by a pull-
down assay, and then extensively validated by biosensor-based affinity detection, enzyme-based activity
assays, cell-based AA metabolite analysis and computer-aided AA network simulation. Further in vivo
studies of AA-induced ear oedema, ovalbumin (OVA)-induced lung inflammation and collagen-induced
arthritis demonstrated the anti-inflammatory potency and validated the therapeutic target of HOEC.
This work revealed that HOEC acted as an anti-inflammatory agent targeting 5-LOX, which not only
confirmed the key role of 5-LOX in inflammation but also provided a paradigm for the exploration of
natural product mechanisms of action.
THE INFLAMMATORY CHEMOKINE CXCL10 MODULATES SYNAPTIC PLASTICITY AND NEURONAL ACTIVITY IN THE HIPPOCAMPUS

J.N. KODANGATTIL¹, G. MÖDDEL², M. MÜLLER³, W. WEBER⁴ and A. GORJI¹,⁵,⁶

¹Institute of Physiology I, University of Münster, Münster, Germany; ²Klinik für Epileptologie, Universitätsklinikum Bonn, Bonn, Germany; ³Klinik und Poliklinik für Neurologie, Universitätsklinikum Bonn, Bonn, Germany; ⁴Institute of Animal Physiology, University of Münster, Münster, Germany; ⁵Shefa Neuroscience Research Center, Tehran, Iran; ⁶Razavi Neuroscience Research Center, Mashhad, Iran

Received June 18, 2012 – Accepted September 18, 2012

Chemokines, a family member of cytokines, have been shown to play a major role in central nervous system inflammation. Among other chemokines, CXCR3 and its ligand CXCL10 are involved in the pathophysiology of several neuroinflammatory conditions. Most of these conditions are also associated with an increased incidence of seizure or epilepsy. Using age-matched wild-type (WT), as well as CXCR3-receptor-deficient (CXCR3-KO) mice, the present study aimed to investigate the effect of the chemokine CXCL10 and its receptor CXCR3 on synaptic plasticity as well as neuronal activities in hippocampal brain slices. Using field potential and intracellular recordings, the effect of exogenous CXCL10 on tetanus-induced long-term potentiation (LTP) as well as the neuronal spike activity was evaluated in hippocampal CA1 area. Exogenous application of CXCL10 enhanced LTP in WT mice, whereas it exerted no significant effect on CXCR3-KO mice. During intracellular recordings of spontaneous spike activity, exogenous application of CXCL10 significantly enhanced the amplitude, duration, and after-hyperpolarization of action potentials in slices obtained from WT mice compared to CXCR3-KO mice. In addition, CXCR3-KO mice exhibited a lower GABAᵦ-mediated excitation in hippocampal CA1 neurons compared to WT mice. These data show that the inflammatory chemokine CXCL10, probably via its receptor CXCR3, modulates neuronal activity and synaptic plasticity in the hippocampus. CXCL10 may be involved in seizures observed during neuroinflammatory diseases such as meningitis and encephalitis.
“Slow” and “Fast” C3 complement variants (C3S and C3F) result from a g.304C>G polymorphism that changes arginine to glycine at position 102. C3 variants are associated with complement-mediated diseases and outcome in transplantation. In this work C3 genotyping is achieved by a Real Time PCR – High Resolution Melting (RT-PCR-HRM) optimized method. In an analysis of 49 subjects, 10.2% were C3FF, 36.7% were C3SF and 53.1% were C3SS. Allelic frequencies (70% for C3S and 30% for C3F) were in Hardy-Weinberg equilibrium and similar to those published previously. When comparing RT-PCR-HRM with the currently used Tetraprimer-Amplification Refractory Mutation System PCR (T-ARMS-PCR), coincidence was 93.8%. The procedure shown here includes a single primer pair and low DNA amount per reaction. Detection of C3 variants by RT-PCR-HRM is accurate, easy, fast and low cost, and it may be the method of choice for C3 genotyping.
INDOLE-3-CARBINOL EXERTS SEX-SPECIFIC EFFECTS IN MURINE COLITIS

J.M. BENSON, C.A. BEAMER, B.P. SEAVER and D.M. SHEPHERD

Department of Biomedical and Pharmaceutical Sciences and Center for Environmental Health Sciences, University of Montana, Missoula, MT, USA

Received November 7, 2011 – Accepted August, 2012

Due to the severe adverse effects that can accompany conventional therapies for Crohn’s disease, the search for natural complementary therapies has increased dramatically in recent years. Indole-3-carbinol (I3C), a constituent of cruciferous vegetables, possesses anti-inflammatory properties; however, its effects on intestinal inflammation have yet to be evaluated. To test the hypothesis that I3C dampens intestinal inflammation, C57Bl/6 mice were treated with I3C and exposed to 2,4,6-trinitrobenzenesulfonic acid (TNBS) to induce colitis. Several parameters of disease severity and inflammation were subsequently evaluated. I3C dampened the disease severity, as indicated by decreased body weight loss and decreased severity of clinical signs. Interestingly, this effect was observed in female but not male mice, which displayed a trend towards exacerbated colitis. Differential effects were observed in the profiles of cytokine production, as the production of pro-inflammatory cytokines was increased in males. The sex-specific effect of I3C in TNBS-induced colitis is a novel finding and warrants further investigation since this is a common dietary compound and is also available commercially.
PROTECTIVE EFFECTS OF MESENCHYMAL STEM CELLS WITH TRANSIENT OVEREXPRESSION OF HMGB1 ON BALLOON-INDUCED CAROTID ARTERY INJURY

S-H. NIU, L-G. JIAN and L-H. ZHANG

Department of Cardiology, the Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China

Received April 20, 2012 – Accepted September 25, 2012

Mesenchymal stem cells (MSC) play a crucial role in endothelial repair after artery injury. The high mobility group box 1 (HMGB1) is a key modulator of the homing of MSC to impaired artery and endothelialization. This study was aimed to determine whether balloon-induced carotid artery injury could be improved by transplantation with MSC modified by HMGB1. MSC were infected by adenoviral serotype 5 encoding recombinant green fluorescent protein (GFP) gene and HMGB1 (ad5GFP-HMGB1). The expression of HMGB1, vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA) was detected in MSC using Real-time PCR, Western blot and semi-quantitative immunohistochemical assays. In vivo, reendothelialization was examined in rats subjected to carotid artery injury. The homing of MSC was observed under fluorescence microscopy, and the levels of serum tumor necrosis factor-α (TNF-α) and C-reactive protein (CRP) was assessed by ELISA assay. As a result, compared with the MSC group, the expression of HMGB1, VEGF and PCNA was markedly increased, vascular reendothelialization was accelerated, and the levels of serum TNF-α and CRP were decreased in group ad5GFP and ad5GFP-HMGB1. Transplantation of MSC infected with adGFP-HMGB1 strengthened the MSC effect. Taken together, modification of HMGB1 can enhance the protective effects of MSC on balloon-induced carotid artery injury through up-regulation of VEGF and PCNA expression and inhibition of the inflammatory response. HMGB1 in MSC may represent a novel therapeutic target for the treatment of endothelial repair.
C-reactive protein (CRP) has a prognostic role in cardiovascular and pulmonary diseases. Recent data suggest its pro-inflammatory effects in atherosclerotic lesion formation. This raises the hypothesis of whether or not CRP has pro-inflammatory effects on pulmonary vasculature by inducing the production of endothelin-1 (ET)-1, a potent vasoconstrictor and proliferative cytokine, and expression of adhesion molecules which could culminate in inflammatory cell recruitment and vascular injury.

Human pulmonary artery endothelial cells (HPAECs) were cultured and incubated with 25µg/ml of human recombinant CRP and with interleukin (IL)-1β 10ng/ml, a well-known activator of endothelial cells, which served as a positive control for 24 hours. Expression of vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 was assessed by flow cytometry. Secretion of ET-1 from HPAECs was also evaluated. In this study we show that incubation of HPAECs with human recombinant CRP for 24 hours induced a significant increase in ICAM-1 expression (from 610 to 6553 mean fluorescence intensity, p < 0.005) and VCAM-1 expression (from 212 to 303 mean fluorescence intensity, p < 0.05), as compared to control. Adhesion molecule induction was similar to that observed in endothelial cells activated with IL-1β. Likewise, CRP potentiated the ET-1 production by HPAECs. The levels of ET-1 were significantly higher at 24 hours (control 19.94±3 vs CRP 46.54±18 pg/ml, p < 0.05).

In conclusion, this study makes a novel observation that CRP induces expression of adhesion molecules and secretion of ET-1 in HPAECs. Our study provides the first evidence that CRP exerts direct pro-inflammatory effects on pulmonary artery endothelial cells.
OVEREXPRESSION OF RECOMBINANT LIPASE FROM BURKHOLDERIA CEPACIA IN ESCHERICHIA COLI

M. RAFTARI¹, S. GHAFOURIAN²,³, N. SADEGHIFARD³, F. ABU BAKAR¹, N. SAARI¹ and Z. SEKAWI²

¹Faculty of Food Science and Technology, University Putra Malaysia, Serdang, Selangor, Malaysia; ²Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, University Putra Malaysia, Serdang, Selangor, Malaysia; ³Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran

Received April 22, 2012 – Accepted August 3, 2012

This study attempts to clone and express the extracellular lipase from Burkholderia cepacia in Escherichia coli using pET system as well as to determine the enzyme activity of recombinant lipase. The extracted DNA from B. cepacia was used as a template for amplifying lipase gene, and then the lipase gene was subcloned into pET-32a and subsequently transformed into E. coli BL21. Media assay and SDS-PAGE were carried out to analyse the results. Nucleotide sequencing of the DNA insert from the clone revealed that the lipase activity corresponded to an open reading frame consisting of 1092 bp coding for a 37.5-kDa protein. The successful expression of lipase was confirmed by obtaining blue color colonies on Nile Blue Sulphate Agar and big band at 37.5-kD size on SDS-PAGE. The enzyme activity assay also showed the high lipase activity around 590 μg lipase ml⁻¹ culture 30 min⁻¹ of recombinant E. coli BL21. The specific lipolytic activity of the recombinant lipase was 185 U/mL which is around 35-fold higher than the native baseline. The findings suggest that the crude recombinant lipase has potential application in digestion of lipids and fatty acids. In conclusion, the results of the current study showed a lipase gene encoding an enzyme with non-specific hydrolysis activity, which could be applied as lipase biosensor for digestion of lipids in food and medicine as well as oil-contamination treatment.
GENE EXPRESSION PROFILING ASSOCIATED WITH THE PROGRESSION OF CLASSIC KAPOSI’S SARCOMA

E. GUTTMAN-YASSKY1,2,3, A. CHIRICOZZI1,3,4, J. JACOB-HIRSCH5, S. TINTLE3, A. KHATCHERIAN3, N. AMARIGLIO5, G. RECHAVI5, J.G. KRUEGER5, S.P. NISTICÒ4, R. BERGMAN6 and R. SARID1

1Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel; 2Department of Dermatology, Weill-Cornell Medical College, Cornell University, New York, USA; 3Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, USA; 4Department of Dermatology, University of Rome Tor Vergata; 5Institute of Hematology and Sheba Cancer Research Center Tel Hashomer Sackler School of Medicine, Tel Aviv University, Israel; 6Department of Dermatology, Rambam Medical Center and the Technion, Haifa, Israel

Received July 6, 2012 – Accepted October 25, 2012

Although Kaposi’s sarcoma (KS) gene expression profile is closer to lymphatic (LEC) rather than blood vascular endothelial cells (BEC), uncertainty still surrounds the cellular origin of KS. To follow KS progression from early to late (nodular) stage, and characterize the molecular fingerprinting associated with each stage, gene arrays were used to compare gene expression profile of 9 skin samples of classic KS (4 Early, 2 Mixed, and 3 Nodular CKS samples) to 4 normal samples. Results for selected genes were validated by Real-time (RT) PCR and immunohistochemistry. Genes regulating immune and defense responses, angiogenesis, apoptosis and proliferation were differentially expressed in different KS stages compared to normal skin. Hierarchical clustering separated normal skin from KS with a clear gradient from early to nodular KS lesions. The gene expression level of endothelium markers, metalloproteinases, angiogenic factors and chemokines, gradually increased from normal through all KS stages. The expression of LEC genes highly increased from early to nodular KS. In the initiation phase we noticed a higher expression of growth factors, as compared to progressive stages. LEC and BEC markers co-exist in “KS expression signature”, although the LEC signature prevailed. Our results also show a complex environment of inflammatory cells and chemokines during KS evolution. A pathogenic hypothesis where cellular hyperproliferation is driven by local expression of chemokines and growth factors without clonal expansion of cells is suggested.
Current approaches to control asthma do not involve direct assessment of airway inflammation. The aim of this study is to assess whether the therapeutic adjustments of steroid treatment according to a stepwise algorithm based on sputum Eosinophils (sEos) and fractioned exhaled Nitric Oxide (FeNO) were effective in maintaining the stability of a group of stable asthmatic patients during a twelve-month follow-up. Fourteen asthmatic patients, treated for asthma according to a previously published protocol, were enrolled in the study. The patients underwent clinical evaluation, pulmonary function tests, measuring of airway hyperresponsiveness to methacholine, and determination of FeNO and sEos at visit 1. These procedures were repeated after 6 and 12 months (Visits 2 and 3, respectively). Symptoms score gradually improved during the study (p=0.008), no changes were observed in the frequency of clinical asthma exacerbations or in airway hyperresponsiveness to methacholine. At the end of the study both sEos and FeNO were significantly improved (p=0.011 and p=0.003, respectively) and at visit 3 the median steroid dose was reduced (p=0.039) in accordance with the improving of symptoms score, FeNO and sEos values. A direct relationship was observed between the difference of FeNO values and the difference of sEos registered between visits 1 and 2 (r²=609, p<0.001) and between visits 2 and 3 (r²=646, p<0.001). In conclusion, long-term titration of asthma inhaled steroid treatment based on sEos and FeNO values was able to provide long-term clinical stability and improvement to the asthmatic patients studied, without significant increases in the steroid dose.
BALKAN ENDEMIC NEPHROPATHY RISK ASSOCIATES TO THE hs1.2 Ig ENHANCER POLYMORPHISM

D. FREZZA¹, E. SERONE¹, S. LOLLI¹, R. CIANCI², P. D’ADDABBO³, C. MATTIOLI¹, V. GIAMBRA⁴, N. PAVLOVIC⁵, V. DJORDJEVIC⁵, S. KOSTIC⁵, F. PANDOLFI² and E. KOSTIC⁵

¹Department of Biology “Enrico Calef”, University of Roma Tor Vergata, Rome, Italy; ²Institute of Internal Medicine, Catholic University of Sacred Heart, Rome, Italy; ³Department of Biology, University of Bari, Bari, Italy; ⁴Terry Fox Laboratory, BC Cancer Agency, Vancouver, Canada; ⁵Clinic for Nephrology, Clinical Center Nis, University of Nis, Serbia

Received April 30, 2012 – Accepted July 20, 2012

Balkan Endemic Nephropathy (BEN) is a kidney degenerative disease with a high incidence in the valleys of the Danube and tributary rivers. Many studies describe it as a multifactorial disease. Environmental as well immuno-inflammatory and genetic cofactors have been suggested to trigger the onset of the disease. Recently, high levels of C-reactive protein were demonstrated in BEN patients. We performed this study to evaluate the possible correlation of BEN with the polymorphism of the Ig heavy chain 3’Regulatory Region enhancer hs1.2 that is related to changes of consensus for trans activators binding within the DNA sequence and probably consequent autoimmune and inflammatory diseases. Therefore, we studied three cohorts: 1) 111 control subjects, 2) 95 BEN patients in dialysis therapy and 3) 133 components of a large family “J” in the same geographical area. The allelic frequencies of hs1.2 of BEN patients and family “J” components had similar decrease frequency of allele *1 and increase of allele *2 in respect to the controls. This trend suggests the association of allele *1 as a protective and allele *2 as a risk component for the disease. The presence of a consensus sequence for NF-κb in the allele *2 may link the polymorphism to the inflammatory activity of BEN. This study supports the presence of an inflammatory pathway in BEN through the involvement of polymorphic enhancer hs1.2 influencing differently binding complexes and consequently the 3D structure of 3’ Regulatory Region of IgH. Our work is the first study that clearly links BEN to a gene involved in the regulation of immune response.
INFLUENCE OF POLYMORPHISM -308 G/A OF THE TNF-α GENE ON HIGH MOBILITY GROUP BOX-1 PROTEIN IN RHEUMATOID AND SPONDYLO-ARTHRITIS PATIENTS

A. BITTO1, F. POLITO2, G. BAGNATO3, R. TALOTTI2, M. ATTERITANO2, N. IRRERA1, R. IENTILE1, N. FERLAZZO2, D. CACCAMO1, G. BAGNATO2, A. CALIRP4, F. SQUADRITO3 and D. ALTAVILLA1

1Department of Clinical and Experimental Medicine and Pharmacology, Section of Pharmacology, University of Messina, Italy; 2Department of Biochemical, Physiological and Nutritional Sciences, University of Messina, Italy; 3Department of Internal Medicine, Rheumatology Unit, University of Messina, Italy

Received September 27, 2011 — Accepted June 1, 2012

Single nucleotide polymorphism (SNP) in the human Tumor Necrosis Factor-α (TNF-α) gene promoter, the -308 G/A variant, has been associated with increased TNF-α levels that may amplify the severity of rheumatoid arthritis (RA) and a poor responsiveness to TNF-α blockade therapy. High mobility group box protein (HMGB-1) is a pro-inflammatory cytokine that plays a pivotal role in the pathogenesis of RA and may be an original target of therapy. The aim of this study is to investigate whether the -308 G/A variant of the TNF-α gene is associated with altered expression of HMGB-1. A total of 110 consecutive patients with rheumatoid arthritis and spondylo-arthritis (analysing spondyloarthritis, psoriatic arthritis and spondyloarthritis associated with inflammatory bowel disease) referring to the Rheumatology Unit of Messina University Hospital were enrolled. Patients were genotyped for the -308 TNF-α gene promoter polymorphism. Clinical status was also assessed. HMGB-1 and TNF-α mRNA (Real Time PCR) from total blood and plasma HMGB-1 (Western Blot analysis) and TNF-α (ELISA) protein were also evaluated. Irrespective of the underlying disease, patients carrying the G/A genotype showed enhanced HMGB-1 and TNF-α mRNA levels and increased circulating concentration of the inflammatory cytokines when compared to patients with G/G genotype. The data suggest that subjects carrying the TNF-α -308G/A genotype have enhanced expression of HMGB-1 protein that may explain, at least in part, the increased severity of the disease.
Chronic and persistent inflammatory processes in bones may lead to severe erosions with consequent functional impairment sometimes requiring amputation of the limb. To explore the relationship between inflammation and bone erosion, biopsies of patients with osteomyelitis due to arterial occlusive disease or to diabetes mellitus were examined (n=31). Histologically, inflammation and bone erosion were confirmed. In the eroded bones the number of osteoclasts correlated with the abundance of infiltrated polymorphonuclear neutrophils (PMN), which were highly activated as shown by expression of MHC class II. For functional characterisation of the infiltrating PMN, patients with implant-associated osteomyelitis, a condition associated with persistent bacterial infection and bone destruction, were recruited. The cells were recovered from infected sites and examined ex vivo. These PMN expressed MHC class II and produced interleukin (IL)-8, a further indication of PMN activation. To assess a possible link between infiltrating PMN and bone erosion, we tested the effect of IL-8 on osteoclast generation in vitro. CD14+ monocytes derived from the peripheral blood of healthy individuals were cultivated with monocyte colony stimulating factor (M-CSF) and IL-8. Within 3 days, a translocation of the transcription factor NFATc1 into the nucleus was seen, and by 10 to 20 days multinucleated cells with typical osteoclast morphology appeared that expressed tartrate-resistant acid phosphatase (TRAP) and cathepsin K. Moreover, the cells were able to resorb bone, proving that IL-8 was able to induce the differentiation of monocytes to osteoclasts. Because IL-8 is a major cytokine produced by activated PMN, we propose that in the course of persistent infection infiltrating PMN contribute to induction of osteoclast formation, thus providing a link between inflammation and bone erosion.
This study was designed to evaluate whether a weight-bearing exercise training played 3 times a week can have benefits on bone mineral density and neuromuscular function in women with a diagnosis of osteopenia. The study enrolled 22 women aged between 45 and 65, with densitometric diagnosis of osteopenia. The participants were randomly assigned to a group of exercise (n=11) and a control group (n=11). The exercise program lasted for 45 min and consisted of a combination of strength exercises that seek to cause a mechanical osteoblastic stimulus by use of gravity, body weight, fall with anti-gravity reaction, in combination with exercises for the improvement of balance and coordination. The outcome measures used to assess the result on bone mass are the bone Mineralometric DEXA method for femoral head-neck region and lumbar spine and biochemical markers of bone turnover (resorption and neoformation) and for the evaluation of neuromuscular function was chosen to use surface electromyography (sEMG) as an indicator of overall activity and speed activation of lumbar paravertebral muscles and of the lower limbs antigravity muscles, stabilometric analysis and 6’ Walk Test. In addition each person enrolled was given EuroQol and ICF core set of osteoporosis, respectively, to assess the quality of life, as well as activity limitations and participation restrictions associated. In the exercise group, mean values and changes in average rates for the balance, muscle strength, walking ability and quality of life, mean bone mineral content and bone turnover markers, corresponding to the assessments made at 0 (before rehabilitation intervention) and Time 1 (program ended), showed a statistically significant improvement. The results of this study demonstrate that a group rehabilitation program of exercises based on gravitational load, aimed to improve muscle strength and trophism, coordination and balance, can provide advantages of unquestionable importance, not only on the slope of increase bone mass of neuromuscular function and reducing risk of falling, but on health in general.
No data are present in the literature regarding chemokine (CXC motif) ligand (CXCL)9 and CXCL11 circulating levels in cryoglobulinemia associated with hepatitis C (MC+HCV), in presence/absence of autoimmune thyroiditis (AT). Serum CXCL9 and CXCL11 have been measured in 38 MC+HCV patients without AT (MCo), 38 MC+HCV patients with AT (MC+AT), and in matched controls without (control 1) or with thyroiditis (control 2). Serum CXCL9 and CXCL11 were significantly higher: in control 2 than control 1 (p<0.05); in MCo than control 1 and control 2 (p<0.001, for both); in MC+AT than control 1 and control 2 (p<0.0001, for both), and than MCo (p=0.01, for both). Our study demonstrates markedly high serum levels of CXCL9 and CXCL11 in patients with MC+HCV compared to healthy controls; in MC+HCV patients increased CXCL9 and CXCL11 levels were significantly associated with the presence of AT. Moreover, a strong relation between circulating CXCL9 and CXCL11 in MC+HCV has been shown.
Oral Squamous Cell Carcinoma (OSCC) is the sixth most frequent malignant tumour. There is some evidence that tongue cancer has a higher local failure rate and poorer prognosis than other anatomical sites in the oral cavity. We used tongue squamous cell carcinoma cell lines harbouring mutated p53/p16 as tongue cancer models to study the influences exerted by p53 and p16 genes on the expression of micro RNAs (miRNAs). The study was performed on microarray chips harbouring 298 miRNA sequences. OSCC cell lines used in this study were SCC-4, SCC-15 and SCC-25, all three carrying mutated/hypermethylated p53/p16. The expression values normalized to healthy control of 298 miRNAs were obtained for each cell line. MiRNA 196b was found hyperexpressed in the three cell lines. MiRNAs 19b-1, 21, 27a, 30d, 134, 339, 379 and 465 were found altered in two out of three cell lines. miRNAs found altered in one cell line out of three were: 7b, 23a, 25, 30c, 30e-3p, 107, 125b, 124a, 214, 216, 325 and 384. A literature review for each miRNA found significant was undertaken. Some miRNAs have a well-known role in oral cancer, some have been put in correlation with other cancers/diseases, others are found significant for the first time. These early results in tongue cancer cell lines harbouring mutation of p16/p53 need further analyses to understand whether this variation of miRNA levels are directly influenced by the malfunction of these proteins or if, vice-versa, altered miRNA levels influence the function of p16 and p53.
Infertility is a problem afflicting about 1/6 couples, and in 40% of cases this is primarily due to the male. Male infertility is a multifactorial pathology and it seems mainly related to sperm motility or sperm number. However, a diagnosis of infertility is frequently not followed by a precise explanation of its cause, reflecting our poor understanding of the spermatogenesis-related regulatory mechanisms and gene expression profiles. Therefore, this study was design to investigate the relative gene expression of a specific gene profile in ejaculate spermatozoa of men affected by infertility. This profile included 13 mitochondrial gene encoding subunits of respiratory chain and 7 nuclear sperm motility-related genes.

We used values of progressive sperm motility (PR) to separate subjects affected by infertility into two groups, showing PR values higher (H group) or lower (L group) than the mean of the sample, and to classify fertile men (control group). We did not obtain a statistically significant difference in nuclear gene expression patterns in spermatozoa among these three groups. On the other hand, we observed an over-expression in 11/13 tested mitochondrial genes in the population of infertile males with altered sperm motility compared to the control group. This over-expression led us to speculate that there is an abnormal mRNA transcription of these 11 subunits, that impaired the normal energy supply ensuring sperm motility. Regarding the under-expression of 2/13 tested mitochondrial genes, we could assume that the spermatozoa mtDNA has accumulated mutations involving these two genes (CYB and ND4L).

In conclusion, our results will provide useful information for the development of molecular diagnostic tools for clinical assessment of sperm health. However, further investigation into other sperm-related genes is needed to establish their roles in male fertility.
The present study was aimed to evaluate the effect of introducing administration of the proteolytic enzyme serratiopeptidase in the combined mechanical-antibiotic treatment of periimplantitis (PI). Two randomized groups of 64 adults with a diagnosis of PI were studied over a 6-month period. All patients were treated with a combined mechanical and antibiotic protocol for 15 days. The experimental group (EG) was administered antibiotic and serratiopeptidase, while the control group was administered antibiotic alone. To evaluate the effects of the two treatment protocols, clinical and radiographic indices, the concentration of IL-1β, IL-6 and TNF-α in the gingival crevicular fluid, the amount of total bacterial DNA and the presence of specific bacteria were assessed at baseline and at 6 months from treatment. Success rates of combined treatments at 6 months were 96.9% and 78.1% for the EG and CG respectively \( (P \leq 0.01) \). Implants of the EG showed greater enhancement of clinical, microbiological and inflammatory parameters as compared to those of the CG. Microbiological analyses showed that resistance to combined therapy was constantly associated with the isolation of bacterial species that are not common periodontal pathogens (mainly \textit{S.aureus} and \textit{P.aeruginosa}). The data demonstrate that the addition of serratiopeptidase to combined mechanical-antibiotic treatment protocols of periimplantitis significantly improves outcomes and suggest that serratiopeptidase acts at different levels during the healing process.
Mast cells are important not only in allergic reactions, but also in inflammation and are involved in a variety of responses including the immediate release of potent inflammatory mediators after activation by cross-linking of FcεRI molecules. Prostaglandin D2 (PGD2) is a major cyclooxygenase metabolite of arachidonic acid produced by mast cells and it is released following allergen challenge in allergic diseases. IL-33 is an inflammatory cytokine which is critically involved in the regulation of in vitro and in vivo cyclooxygenase production, providing a potential therapeutic target for inflammatory disorders.

In this study, using human derived umbelical cord blood mast cells, we show that IL-33 (50 ng/ml), and calcium ionophore A 23187 (0.5 μg/ml), compound 48/80 (10⁻⁵ M) or anti-IgE (10 μg/ml), enhanced the production of PGD2 and this effect was inhibited by indomethacin. However, IL-33 was unable to induce tryptase release in these cells. These effects confirm the inflammatory property of IL-33 by stimulating PGD2 but not tryptase in human mast cells. The inhibitory effect of this new cytokine may have a potential therapeutic response in allergic and inflammatory diseases.
The purpose of this study is to evaluate the effects of rehabilitative approach using MJS and dynamic antigravity postural system (SPAD) with extracorporeal shockwave therapy (ESWT) on rotator cuff syndrome associated tendon supraspinatus and infraspinatus medium tear (1-3 cm) of the tendons. In the last few years, ESWT has been proposed as an elective treatment in somatic diseases with encouraging short-term results. For this study the authors enrolled 108 patients who underwent 3 treatments with ESWT associated with 24 rehabilitation sessions over 3 weeks. Outcome measures were the VAS for pain and the Constant Murley Scale. The outcomes were measured pre-training, post-training and at 2, 4, 6 month follow-ups. Additional follow-up evaluation sessions were performed every year for 5 years by a telephone interview to evaluate changes in pain and function and the efficacy of treatment. Our study shows that the therapeutic efficacy of rehabilitative approach with ESWT in the rotator cuff syndrome with medium tears persists over time and significantly improves the patient’s quality of life. The results obtained are certainly to be attributed to the biological mechanisms that ESWT are able to engage in tissues of the rotator cuff. The results seen at the conclusion of the treatment were maintained over the following years, thanks to the use of MJS and SPAD. In our opinion a conservative treatment with extracorporeal shock-wave (ESW), dynamic antigravity postural system (SPAD) and multi joint system (MJS) should be considered as an alternative and effective treatment for rotator cuff syndromes with medium tears.
Inflammatory tenosynovitis is an inflammation that involves the tendons and synovial sheaths caused by minor trauma repeated for a long period of time. This inflammatory disease may be involved in the onset of tunnel carpal syndrome (CTS), because of the thickening of the tendon sheath that may produce an increase in the carpal canal pressure and damage of the median nerve in the wrist. Recent studies suggest that in patients with CTS pathological changes occur in the subsynovial connective tissue, such as vascular proliferation and non-inflammatory synovial fibrosis. However, little is known about the pathological mechanism of tenosynovial thickening. The aim of this study is to evaluate the potential role of vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE\textsubscript{2}) and trasforming growth factor-beta (TGF-\textbeta) in the modifications of connective synovial tissue of CTS specimens in order to determine whether these factors play a role in the development of this disease. Ten specimens from patients with CTS and four control tissues (cadavers) were analyzed by immunohistochemistry using specific antibodies against these growth factors. A temporary increase in the production of these molecules was found in cells within the vessels and synovial lining during the intermediate phase of the syndrome, when the histology of the tenosynovium changes from oedematous to fibrotic. Our data confirm a close correlation between the expression of PGE\textsubscript{2} and VEGF. Recent histological examinations have shown a marked increase in vascular proliferation and reduction of fibroblast density in specimens from CTS patients during the intermediate phase. Our study indicates that the expression of TGF-\textbeta in fibroblasts and vascular endothelial cells of synovial connective tissues of CTS patients was significantly higher than in those of controls. These findings suggest that angiogenesis appears to take place as a part of a regenerative reaction that results in fibrosis. We believe that VEGF, TGF-\textbeta and PGE\textsubscript{2} may be potential therapeutic targets in the treatment of this disease although proof of this evidence requires further studies.
The aim of the study was to show that the addition of extracorporeal shockwave therapy (ESWT) may significantly improve beneficial effects of eccentric training together with high efficiency focused acoustic waves for jumper’s knee. We speculate that such an effect may be due to increased mechanotransduction effects on affected tissues. We assessed changes in pain and function in 42 male football players (aged 18-34 years) after a treatment protocol consisting of 1 session with focused ESWT per week combined with 3 physiotherapy sessions per week, for 3 consecutive weeks. While treatment protocol was administered, ordinary activities, but not playing football were permitted. Their condition was evaluated before treatment, at the end of the rehabilitation period (3 weeks) and at 2 months, 4 months and 6 months after the end of treatment by clinical examination, instrumental analysis and VAS for pain assessment. Functional ability related to symptoms was assessed with VISA score. At the end of 2005, 2006, 2007, 2008 and 2009 we carried out a telephone interview to investigate changes in pain and function and the efficacy of the treatment over time. Follow-up controls showed a reduction of average VAS score; after 6 months, tendons showed a structure closer to normal at ultrasonographic investigation. At the last telephone interview in 2009 many patients reported to consider ESWT as an effective treatment and described a significant improvement in their functional abilities, a significant reduction in drug consumption and 88% of subjects continued to play agonistic football. In conclusion, our results showed that, through the addition of ESWT, the effects of the classic vibration and eccentric training combination were improved compared to those found in our experience without ESWT. Although a control group was not included in the study (vibration and eccentric training without ESWT), results show a promising improvement and justify future prospective studies with a control group and more case series.
LETTER TO THE EDITOR

DISPERSE YELLOW DYE: AN EMERGING PROFESSIONAL SENSITIZER IN CONTACT ALLERGY DERMATITIS


1U.O.C. Dermatology, NESMOS Department, Faculty of Medicine and Psychology, University of Rome “Sapienza”, Rome, Italy; 2U.O.C. Plastic Surgery, Faculty of Medicine and Psychology, University of Rome “Sapienza”, Rome, Italy; 3Faculty of Medicine, University of Towson (Maryland), USA

Received February 16, 2012 – Accepted September 18, 2012

Disperse dyes are well known as common sensitizers in contact allergy dermatitis. Disperse yellow 3 is usually adopted in the textile industry for dying synthetic fibers, but is also used in hair dyes and for colouring plastic materials. We describe three cases of two males and one female patient, respectively a painter, an actor and a nursery-school teacher, who presented contact allergy dermatitis to disperse yellow 3 dye.
LETTER TO THE EDITOR

PSEUDOXANTHOMA ELASTICUM AND LIGHT-CHAIN AMYLOIDOSIS

M. CARLESIMO¹, C. ABRUZZESE¹, G. CORTESI¹, A.M. CICCONE², C. POGGI², M. LOMBARDI³, A. MOSCETTI⁴, G. LA VERDE⁴ and E. MARI⁵

¹NESMOS, U.O.C. Dermatology, Sant’Andrea Hospital University of Rome “Sapienza”, Faculty of Medicine and Psychology, Rome, Italy; ²U.O.C. Thorax Surgery, Sant’Andrea Hospital University of Rome “Sapienza”, Faculty of Medicine and Psychology, Rome, Italy; ³U.O.C. Histopathology, Sant’Andrea Hospital University of Rome “Sapienza”, Faculty of Medicine and Psychology, Rome, Italy; ⁴U.O.C. Ematology Sant’Andrea Hospital University of Rome “Sapienza”, Faculty of Medicine and Psychology, Rome, Italy; ⁵U.O.C. Clinica Dermatologica Dipartimento di Medicina Interna e Specialità Mediche University of Rome “Sapienza”, Rome, Italy

Received April 13, 2012 – Accepted September 21, 2012

Pseudoxanthoma elasticum is a heritable disorder of connective tissue characterized by cutaneous, vascular and ocular changes that result from the accumulation of fragmented elastic fibres. Even though the etiopathogenesis is not still completely understood, in recent years in literature some Authors have considered pseudoxanthoma elasticum as a metabolic disorder. We present the case of a 45-year-old man affected by pseudoxanthoma elasticum and light-chain amyloidosis and we discuss the possible reasons that led to this association.
LETTER TO THE EDITOR

ANTIPROLIFERATIVE PROPERTIES OF THE SEROTONIN RECEPTOR ANTAGONIST ONDANSETRON CORRELATE WITH INCREASED NITRIC OXIDES RELEASE AND INDUCIBLE NITRIC OXIDE SYNTHASE ACTIVITY IN THE ACUTE LYMPHOBLASTIC LEUKEMIA CELL LINE REH

J. PRADA1,2, S. SHALAPOUR2, M. PFAU2, G. HENZE2 and K. SEEGER2

1Biomedical Research Center, Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum, Humboldt University of Berlin, Berlin, Germany; 2Department of Paediatric Oncology and Hematology, Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum, Berlin, Germany

Received July 4, 2012 – Accepted October 17, 2012

A recent report from our group described that the (serotonin receptor-3)-antagonist ondansetron exhibits antiproliferative effects in the B-cell precursor acute lymphoblastic leukemia (BCP-ALL) cell line REH. Furthermore, after each application of ondansetron to cultured REH cells, significant increases (+23%) in the concentration of nitric oxides (NO) were observed in the cell supernatants after 72 hours incubation in standard conditions, and this effect was found to correlate with the described antiproliferative activity. This feature was further confirmed by using mRNA dot blot hybridizations with a specific gene probe for the inducible NO-synthase (iNOS), yielding significant increases (+100%) of iNOS mRNA, which were found to widely correlate with the detected increases of NO release, and also with the previously described antiproliferative effects. The presented results are the first report on high specific pro-inflammatory features of a (serotonin receptor 3)-antagonist in a BCP-ALL cell line, which are associated with previously described antiproliferative properties.
LETTER TO THE EDITOR

SYNCHRONOUS ONSET OF SECONDARY RAYNAUD’S PHENOMENON IN MONOZYGOTIC TWINS

A. RICCIO, M.G. SANGIOLO and G. TARANTINO

Clinical and Experimental Medicine Department,
Medical School, Federico II University, Naples, Italy

Received March 27, 2012 – Accepted October 19, 2012

The heritability of primary Raynaud’s phenomenon has been reported in previous works. In this paper we describe the simultaneous onset of Raynaud’s phenomenon, rapidly evolved in acrocyanosis and diagnosed as secondary Raynaud’s phenomenon, observed in monozygotic twins. This case supports the role of genetic factors in the pathogenesis of such disorder. Moreover, the singular synchronism of its appearance is discussed.