1. **IMPACT OF RANTES, MCP-1 and IL-8 IN MAST CELLS**

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Chemokines are cytokines with chemotactic properties on inflammatory cells and other cell types. RANTES, MCP-1 and related molecules, constitute the C-C class of chemokine supergene family and a group of cytokines produced by hematopoietic cells, while IL-8 constitute the C-X-C class. The roles of most of these chemokines are not well known, although members of the chemokine family are inflammatory agents. The C-C chemokine plays a role in regulating Th-cell cytokine production and leukocyte trafficking. In this study we clearly show that RANTES and MCP-1 are mediators of acute inflammatory responses. Our report describes additional biological activities for RANTES, MCP-1, and IL-8, suggesting that these chemokines play a fundamental role in histamine and serotonin generation and cell function in mast cells. *J Biol Reg Homeost Ag 2010; 24:1-5*

2. BAX GENE SILENCING: A POTENTIAL INTERVENTION IN ALUMINUM-INDUCED NEURAL CELL DEATH

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There is a vast amount of evidence indicating that Bax plays a major role in the development,
maintenance, and survival of neurons and neuron-supporting cells such as glial cells. The high potency of Bax small interfering RNA (siRNA), as shown by many experimental studies, makes it a rational candidate as a co-therapeutic agent in apoptotic cell death. To investigate whether Bax RNA interference (RNAi) may serve as a potential intervention in neural cell death induced by aluminum, we herein established aluminum (Al)-treated gliatoma (C6) cells as a model for evaluating neurotoxic injury on normal glia. Using the cell model, we undertook a different approach by inducing glial cell death with Al and then using *Bax* gene RNAi to suppress glial cell death. Combining cell viability assays and expression analyses by quantitative real-time PCR (qRT-PCR) and immunocytochemistry, we selected and validated the optimal siRNA from 3 candidate siRNAs for the *Bax* gene. Sequenced reduction of neural cell death was determined with flow cytometry. Our data identified siRNA1 as the most effective siRNA. The optimal concentration of the transfection agent was 20 nM and the optimal incubation period was 72 h. The transfection and knockdown efficiencies were 95 percent and 62 percent, respectively, which closely correlated with Bax protein expression and also the cell apoptosis intervention. Taken together, Bax is essential for apoptosis induced by aluminum. Inactivation of the *Bax* gene could be an effective strategy for delaying the onset of apoptosis induced by Al. Our results reveal promising therapeutic potential for *Bax* gene silencing in Al-induced neurodegeneration. *J Biol Reg Homeost Ag 2010; 24:6-17*

3. Guanosine-induced decrease in side population of lung cancer cells: lack of correlation with ABCG2 expression

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Cancers contain a side population (SP), a subset of cells that is greatly enriched in stem cells and which contains malignant progenitors. SP cells are characterised by high efflux capability for Hoechst 33342 dye and for anti-cancer therapeutic agents through transporters; ABCG2 (ATP-binding cassette transporter G2) is currently most closely associated with the SP phenotype. Guanosine is an important intercellular signalling molecule; it stimulates stem cell proliferation *in vivo* and affects cholesterol efflux *in vitro* through activation of ABCG transporter (ABCG1), raising the possibility that it might also affect ABCG2 and hence the SP. We examined the effects of guanosine on the SP of A549 lung cancer cells. Fluorescence-activated cell sorting (FACS) revealed that exposure to 10

microM guanosine significantly decreased the proportion of SP cells after 48 hours but not after 6 hours. In contrast, Western blot analysis showed that 10 microM guanosine significantly decreased ABCG2 expression after 6 hours, but not after 48 hours. These data demonstrate that guanosine affects both the proportion of SP cells and ABCG2 transporters, but the lack of correlation between ABCG2 expression and the SP phenotype indicates that transporters other than ABCG2 are involved in maintaining the SP phenotype in A549 lung cancer cells. *J Biol Reg Homeost Ag 2010; 24:19-25*.

4. The interaction of humic substances with the human prion protein fragment 90-231 affects its protease K resistance and cell internalization

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In this paper we analyzed the determinants and the structural effects of the interaction of human prion protein fragment 90-231 (HuPrP) with humic substances, (HS) including humic (HA) and fulvic (FA) acids, natural refractory organic polyanions widely diffused in soils and waters. We show that this interaction is mainly driven by non-specific electrostatic attraction involving regions situated within alpha-helix A and beta-sheet S1 of human PrP. FA binding to HuPrP altered its ability to acquire some PrPSc-like characteristics induced by the mild thermal denaturation of the peptide (1 h at 53 degrees C). In particular, in the presence of FA, HuPrP shows a reduced amount of beta-sheet content (as demonstrated by the reduced
binding of thioflavin T), an increased sensitivity to protease K and an inhibition of the entering in the fibrillogenic pathway. FA/HuPrP interaction caused the aggregation of the peptide in unstructured macrocomplexes, as demonstrated by the altered electrophoretic migration in semi-denaturing detergent-agarose gel assay. Importantly, in the presence of FA the rate of internalization of HuPrP in human neuroblastoma cells was significantly reduced as compared to that of the beta-structured peptide. Therefore, HS inhibited the acquisition of PrPSc-like structural properties that, in turn, are responsible for HuPrP intracellular accumulation and lead to neuronal death. Important implications of these data are that HuPrP-HS complexes, being unable to be internalized in living cells may represent a molecular mechanism for the reduced transmission of prion transmission from HS-rich soil also in the presence of contamination from infected animals. *J Biol Reg Homeost Ag 2010; 24:27-39*.

5. Protective effect of a poly-phytocompound on early stage nephropathy secondary to experimentally-induced diabetes

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Diabetic nephropathy (DN) is a severe and life-threatening complication of long-standing diabetes. As one of the main causes of end-stage renal disease, the prevention and treatment of DN in early stage, and the slowing down of DN progression are of utmost importance and are topics of several ongoing research studies. Nutraceuticals endowed with antioxidant-anti-inflammatory properties may offer an opportunity of integrative treatment for this condition. Male Wistar rats were randomly assigned to two groups. One group of rats (diabetic group) received a single tail-vein injection of STZ compound (50 mg/kg) under light anaesthesia. A protective dose of 0.5 ml of 5% dextrose was given intraperitoneally 30 min before the administration of STZ. One diabetic group was fed a normal pellet diet (group A) while group B was fed the diet added with DTS (panax pseudoginseng, eucommia ulmoides), (Kyotsu Jigyo, Tokyo, Japan) in the proportion of 50/25 (percent weight/weight), at the dose of 50 mg/kg/day throughout the experimental period. At the end of 8 weeks, 24-hour urine was collected for the measurement of the albumin concentration: blood samples were collected for serum biochemistry and the rats were sacrificed for kidney measurement of oxidative stress and histomorphological features. Nephrin and Macrophage Chemoattractant Protein-1 (MCP-1) gene expression were also assessed by fluorescence real-time quantitative PCR after RNA extraction and cDNA synthesis. STZ-treated animals showed significantly increased in lipid peroxidation in the kidney and in proteinuria. DTS supplementation did not affect plasma glucose but significantly decreased malonyldialdehyde (MDA) plasma level and the overall redox parameters together with a partial mitigation of proteinuria. Histological analysis showed also that DTS significantly reduced the glomerular volume together with glomerulosclerosis and interstitial fibrosis score (p less than 0.05), the latter two being correlated to proteinuria (p less than 0.05). DTS supplementation also enabled a reduction of diabetes-induced decrease of nephrin mRNA expression and a 67 percent reduction of MCP-1 mRNA up-regulation (p less than 0.01). Taken altogether, these data show that, besides the mandatory control of glycemia, intervention with a nutraceutical with antioxidant and anti-inflammatory properties may have beneficial effects when integrated in the mainstream of the therapeutic regimen. *J Biol Reg Homeost Ag 2010; 24:41-49*.

6. Osteoinduction properties of different growth factors on cells from non-union patients: *in vitro* study for clinical application

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This report compares the effect of rhBMPs and PRG on cells derived from human non-union sites. Treatment of non-union continues to be a challenging task for the trauma surgeon often resulting in unsatisfactory results and long-term morbidity. Over the past two decades, the possibility to use growth factors in bone regeneration has been investigated. In this study we compared the *in vitro* capability of two recombinant human bone morphogenetic proteins (rhBMP-2 and rhBMP-7) and activated plateletrich plasma (PRG) to stimulate proliferation and/or differentiation of cells derived from non-union patients. Cells derived from the lesion sites, osteoblasts and mesenchymal stem cells (MSCs) derived from other bone sites of the same patients were used. Treatment with rhBMP-7 or rhBMP-2 showed an improvement in the expression of osteoblastic markers (osteonectin and osteocalcin) in cells derived from human non-union sites. This enhancement was more marked in MSCs, while no significant changes were observed in osteoblast cultures. The PRG treatment produced in all analysed samples a considerable increase in cell proliferation without affecting cell differentiation. On the basis of our results, for an effective biological treatment of non-unions, small amounts of autologous bone marrow (MSCs) are necessary in the lesion site in order to provide both growth factors and a sufficient number of responsive cells. Finally, our results prove that sequential timing administration of PRG and rhBMPs may be used in new therapeutic strategy. *J Biol Reg Homeost Ag 2010; 24:51-62*.

7. Female reproductive dysfunction during ageing: role of methylglyoxal in the formation of advanced glycation endproducts in ovaries of reproductively-aged mice

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Reproductive dysfunction with ageing has been so far extensively characterized in terms of depletion of ovarian follicles and reduced ability to produce gametes competent for fertilization. Nevertheless, molecular mechanisms underlying this process are still poorly understood. In the present study we addressed the hypothesis that methylglyoxal (MG), a major precursor of Advanced Glycation Endproducts (AGE), may contribute to molecular damage occurring during ovarian ageing. Our results showed that the biochemical activity of glyoxalase 1, the main component of the MG scavenging system, is significantly decreased in ovaries from reproductively-aged mice in comparison with the young group. This effect was associated with decreased expression at protein and RNA level of this enzyme and increased intraovarian level of MG. MG-arginine adducts argpyrimidine as detected with a specific
antibody was found to accumulate with ageing in specific ovarian compartments. Separation of ovarian proteins by 2D gels and Western blotting revealed an approximate 30-fold increase in the extent of protein glycation in aged ovaries along with the appearance of eight argpyrimidine modified proteins exclusive for the aged group. In conclusion, the present results show that impaired MG detoxification causing relevant damage to the ovarian proteome might be one of the mechanisms underlying reproductive ageing and/or ageing-like ovarian diseases. *J Biol Reg Homeost Ag 2010; 24:63-72*.

8. Selective adenosine A2a receptor agonists reduce the apoptosis in an experimental model of spinal cord trauma

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Adenosine is an important regulator of inflammatory mechanisms. Functional studies indicate a protective effect of adenosine A2A receptor agonists in spinal cord injury (SCI). The basic molecular mechanisms accounting for their protective effects from spinal cord injury have to be fully elucidated. The aim of this study is to evaluate *in vivo* protection by two selective A2A receptor agonists, 2-[p-(2-carboxyethyl)phenylethylamino]-50-ethylcarboxamidoadenosine (CGS 21680, 100 microg/kg) and (4-[3-(6-amino-9-(5-cyclopropylcarbamoyl-3,4-dihydroxytetrahydro-furan-2-yl)-9H-purin-2-yl)prop-2-ynyl] piperidine-1-carboxylic acid methyl ester) (ATL 313, 3 microg/kg) on the degree of apoptosis, in the experimental model of spinal cord injury. Spinal cord trauma was induced by the application of vascular clips (force of 24 g) to the dura via a four-level T5-T8 laminectomy. Spinal cord trauma in mice was characterised by edema, neutrophilic

infiltration and apoptosis. ATL 313, administered by subcutaneously implanted osmotic minipumps after SCI, clearly reduced motor deficit for up to 19 days after operation. The selective A2A receptor agonists ATL 313 and CGS 21680 administered after SCI, reduced tissue damage, TUNEL staining, cytokine (TNF-alpha) expression, Bax, Fas-L and Caspase-3 expression, Annexin-V staining, while increasing Bcl-2 expression. In conclusion, our results demonstrate that treatment with adenosine A2A receptor agonists prevents the apoptotic process that is an important step of secondary damage after SCI. *J Biol Reg Homeost Ag 2010; 24:73-86*.

9. Plasma adiponectin and leptin concentrations in professional rugby players

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Adipose tissue synthesizes and secretes a number of cytokine hormones, defined adipokines, which have emerged as critical regulators of several metabolic functions, including energy homeostasis, insulin action and lipid metabolism. The present study is aimed at assessing the relationship between plasma concentrations of leptin and adiponectin and body composition in a cohort of 38 male professional rugby players (age: 22-35 years). Anthropometric evaluation included body mass index (BMI, range: 23.4-35.1 kg/m2) and whole body bioelectric impedance to determine absolute fat-free mass (FFM), absolute fat mass (FAT), relative percentage of fat mass (FAT%) and fat-free mass (FFM%). FAT% ranged from
15 to 34%, corresponding to a FAT of 11.5-38.7 kg, whereas FFM range was 62.1-83.5 kg. Plasma leptin range was 1.2-4.3 ng/mL and adiponectin range was 2.0-16.6 μg/mL. Plasma leptin and adiponectin concentrations and their ratio did not correlate with BMI, nor with FAT, FAT%, FFM and FFM%, even after correction for BMI. The findings of this study suggest that in professional rugby players some additional factors, like neuroendocrine adaptations, other than adipose mass play a relevant role in the determination of adipokine levels, which in this group appear to be rather independent of body composition. *J Biol Reg Homeost Ag 2010; 24:87-91*.

10. Effectiveness of a cold dessert, with or without the addition of a mixture of digestive herbs, in subjects with functional dyspepsia

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Functional dyspepsia represents a clinical condition of pain and/or persistent or recurrent discomfort that concerns a large portion of the healthy population. It has already been shown that some herbs (*Melissa Officinalis, Cynara scolymus*) can have favorable effects on digestion. The principal aim of this study is to determine whether the ingestion of Gran Soleil dessert, with or without herbs, after meals can be beneficial to health in subjects suffering from functional dyspepsia. For this purpose, thirty subjects with functional dyspepsia were enrolled and were asked to consume Gran Soleil with or without herbs; these subjects reported the course of their symptoms on VAS scale, during the basal period and after the ingestion of Gran Soleil with and without herbs. It has been shown that the ingestion of Gran Soleil without herbs can induce a reduction both in the number of events connected to a dyspeptic syndrome and in their intensity; moreover the assumption of Gran Soleil with the addition of herbs helped to intensify this effect. *J Biol Reg Homeost Ag 2010; 24:93-98*.

11. Eosinophilic bronchiolitis indicating eosinophilic airway disease with overexpression of carcinoembryonic antigen in sinus and bronchiole: case report

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The present case showed eosinophilic bronchiolitis and sinusitis with an overexpression of carcinoembryonic antigen (CEA) in lung and sinus and an elevation of serum CEA level, both of which were improved by oral steroid therapy. A 54-year-old asthmatic woman had developed a shortness of breath on exertion, and the chest X-ray revealed diffuse centrilobular shadows. Her serum CEA level had increased gradually. Eosinophil infiltration and overexpression of CEA were demonstrated in both the lung and sinus by immunohistochemistry. Both the lung and sinus lesions, and the serum CEA level were improved by oral steroid therapy. No evidence of tumor was found by extensive examination. From this case, eosinophilic bronchiolitis was considered to be an airway disease like "eosinophilic sinobronchiolitis" through the common pathophysiology of CEA, and serum CEA level was a good marker of disease condition. *J Biol Reg Homeost Ag 2010; 24:99-102*.

12. Visual analogue scale assessment of respiration might be a surrogate for spirometry in allergic rhinitis

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Allergic rhinitis (AR) may be frequently associated with or precede asthma. Bronchial involvement in AR is usually detected by spirometry, however, spirometers are rarely available. The aim of this study is to verify the suitability of the use of visual analogue scales (VAS) as a surrogate for screening spirometry in assessing respiration in AR patients. One hundred twenty patients with allergic rhinitis were studied. VAS for respiration assessment and spirometry were performed in all patients. There was a significant, though weak, relationship between VAS assessment of respiration and FEV1 ($p=0.0076$; $r=0.244$). In conclusion, this preliminary study suggests the use of VAS as screening to assess the respiration of patients with allergic rhinitis who may be candidates for spirometry. *J Biol Reg Homeost Ag 2010; 24:103-105*.