1. The effects of COX-2 anti-inflammatory drugs on soft tissue healing: a review of the literature

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COX-2 specific inhibitors (coxibs) have become a popular treatment for musculoskeletal disorders given that the incidence of gastrointestinal side effects is lower with these drugs than with traditional non-steroidal anti-inflammatory drugs. The aim of this review is to discuss the results of animal studies investigating the role of coxibs in the healing of soft tissues. MEDLINE was searched (years 2001-2009) for studies analyzing the effect of coxibs on the healing of soft tissues. There are relatively few data in the literature suggesting that coxibs can impair soft tissue healing and the data existing have the limitation of having been generated in animal studies. In fact, the method of administration and the doses used make it difficult to translate these results to the clinical setting. Short-term use of coxibs following lesions to ligaments or tendons remains a prudent choice. Traditional anti-inflammatory drugs are a safer treatment for patients with a high cardiovascular risk. These drugs should, however, be evaluated carefully with regards to gastrointestinal events and their still poorly defined effect on tissue healing. J Biol Reg Homeost Ag 2010; 24: 107-114

2. Jaw osteonecrosis related to bisphosphonate for bone metastasis

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The efficacy of bisphosphonate in controlling skeletally-related event in cancer patients without a great number of adverse events has resulted in a widespread use of these medications in oncology. Zoledronic acid and pamidronate are the most common bisphosphonates intravenously administered as a preventive treatment of bone complications encountered in multiple myeloma, as well as a palliative treatment of bone metastases in a large variety of solid tumours including breast, prostate and lung cancers. However, in recent years a relationship has been established between these drugs and a new bone injury characterised by avascular necrosis of bone that was isolated to the jaws. This paper reviews the literature concerning the discovery of this disease, its clinical, radiological and histological manifestations; its pathogenesis, with a look at the treatment and future options in preventing this complication and in treating hypercalcemia and bone lytic lesions in solid tumours. J Biol Reg Homeost Ag 2010; 24: 115-121
3. The potential therapeutic role of potassium channel modulators in asthma and chronic obstructive pulmonary disease

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The involvement of a number of potassium channels has been reported in respiratory conditions such as asthma and chronic obstructive pulmonary disease (COPD), supporting the idea that potassium channel modulating agents may help control it. Experimental evidence and preclinical models suggest that ATP-dependent K+ (KATP) channel openers, big-conductance K+ (BKCa) channel openers, and intermediate-conductance K+ (IKCa) channel blockers may be the most effective agents for treating asthma and COPD. Modulation of potassium channels by these agents may produce beneficial effects such as bronchodilation, a reduction in airways hyperresponsiveness (AHR), a reduction in cough and mucus production and an inhibition in airway inflammation and remodelling. The aim of this paper is to investigate the role of K+ channel modulation in the pathogenesis, progression and exacerbation of asthma and COPD, and to review the evidence suggesting that K+ channel modulators may be a valuable treatment option for these respiratory diseases. J Biol Reg Homeost Ag 2010; 24: 123-130

4. IL-35, an anti-inflammatory cytokine which expands CD4+CD25+ Treg Cells


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Interleukin 12 (IL 12) p35/p40 is a heterodimeric cytokine which plays a critical role in inflammation, immunity and tissue proliferation, and also plays a relevant function in T helper (Th) cell polarization and Th1 T-cell differentiation. IL-12 family members, IL-12p70, IL-23, IL-27 and IL-35, play an important role in influencing helper T-cell differentiation. EBV-induced gene 3 can be associated with the p35 subunit of IL-12 to form the EBI3/p35 heterodimer, also called IL-35. It has been shown that IL-35 has biological activity and able to expand CD4+CD25+ Treg cells, suppress the proliferation of
CD4+CD25− effector cells and inhibit Th17 cell polarization. IL-35 has been shown to be constitutively expressed by regulatory T (Treg) cells CD4(+)CD25(+)-Foxp3(+) and suggested to contribute to their suppressive activity. IL-35 is a crucial mediator which provokes CD4+CD25+ T cell proliferation and IL-10 generation, another well-known anti-inflammatory cytokine, along with TGFβ cytokine. These studies suggest that IL-35, together with other successfully discovered cytokine inhibitors, represents a new potential therapeutic cytokine for chronic inflammation, autoimmunity and other immunological disorders. J Biol Reg Homeost Ag 2010; 24: 131-135

5. Novel interventions targeting on apoptosis and necrosis induced by aluminum chloride in neuroblastoma cells


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Aluminum chloride induces neuroblastoma cell (SH-SY5Y) death following in vitro exposure. The objective of this study is to define apoptosis and necrosis in an in vitro model system of SH-SY5Y cells, and to investigate appropriate defense mechanisms with caspase-3 small interference RNA (siRNA) and necrostatin-1 (Nec-1). SH-SY5Y cells were treated with aluminum chloride for 24 h, followed by analysis of cell death rates and alterations in morphology. The results show that aluminum chloride could induce cell death by a combination of apoptosis and necrosis. Treatment with caspase-3 siRNA resulted in inhibition of caspase-3 gene and protein expression, both indicative of apoptosis reduction. In addition, decrement of apoptotic rate was evident. Interestingly, treatment with caspase-3 siRNA could markedly up-regulate the expression of LC3-II, indicating a shift of cell death mode, from apoptosis to autophagy. Nec-1 treatment significantly affected necrosis induced by aluminum chloride, resulting in decreased necrotic rates and marked inhibition of LC3-II expression. Results showed for the first time that cell death induced by aluminum chloride could be rescued by caspase-3 siRNA and Nec-1 in SH-SY5Y cells, and co-administration of both produced an additive effect on reducing cell death. These data will pave the way for future studies investigating the prevention of cell death in Al neurotoxicity both in vivo and in vitro. J Biol Reg Homeost Ag 2010; 24: 137-148

6. Serum concentration of insulin-like growth factor-i, but not tumor necrosis factor-alpha, measured twelve months after stenting of the infarct-related artery, is associated with in-stent restenosis


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Percutaneous coronary intervention (PCI) has revolutionized the management of and outcomes in patients with ST-segment elevation myocardial infarction (STEMI). The role of insulin-like growth factor-I (IGF-I) and tumor necrosis factor-alpha (TNF- alpha) in restenosis has been intensively studied. We aimed to investigate the power of serum IGF-I and TNF- alpha concentrations to predict restenosis in patients who had previously undergone PCI for STEMI. Thirty-seven patients were enrolled in the study. Twelve months prior to the study they underwent successful PCI with stent placement for STEMI. The patients were divided into two groups: group 1 – patients with in-stent restenosis in the infarct-related artery (N=9); group 2 – patients without in-stent restenosis in the infarct-related artery (N=28). Baseline profile was similar in both groups. The mean diameter and length of placed stents were similar in both groups. Smaller minimal lumen diameter (MLD) and greater lumen loss (LL) were observed in group 1. Median IGF-I concentrations were substantially higher in patients with ISR compared to those without ISR (170 ng/mL vs 115 ng/mL, p=0.004). Strikingly, median TNF- alpha levels were lower in group 1 (2.4 pg/mL vs 4.1 pg/mL, p=0.05). Correlation analysis showed that serum IGF-I levels were significantly associated with diameter stenosis (R=0.29 p=0.05), LL (R=0.37 p=0.02), MLD (R= -0.38 p=0.03), and stent length (R=0.30 p=0.05). The cut-off value to predict restenosis for IGF-I was less than 158 ng/mL (sensitivity 55%, specificity 93%, positive predictive value 71 percent, negative predictive value 87 percent). IGF-I detected twelve months after stent placement during the acute phase of AMI may be a late determinant of restenosis. High concentrations of IGF-I could play a permissive role in the progression of NIH and subsequently restenosis. It seems that as far as TNF- alpha is concerned, diagnostic value remains inconclusive. J Biol Reg Homeost Ag 2010; 24: 149-156

7. A day trip to a forest park increases human natural killer activity and the expression of anti-cancer proteins in male subjects


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We previously reported that 2-night/3-day trips to forest parks enhanced human NK activity, the number of NK cells, and intracellular anti-cancer proteins in lymphocytes, and that this increased NK activity lasted for more than 7 days after the trip in both male and female subjects. In the present study, we investigated the effect of a day trip to a forest park on human NK activity in male subjects. Twelve healthy male subjects, aged 35-53 years, were selected after giving informed consent. The subjects experienced a day trip to a forest park in the suburbs of Tokyo. They walked for two hours in the morning and afternoon, respectively, in the forest park on Sunday. Blood and urine were sampled in the morning of the following day and 7 days after the trip, and the NK activity, numbers of NK and T cells, and granulysin, perforin, and granzyme A/Bexpressing lymphocytes, the concentration of cortisol in blood samples, and the concentration of adrenaline in urine were measured. Similar measurements were made before the trip on a weekend day as the control. Phytoncide concentrations in the forest were measured. The day trip to the forest park significantly increased NK activity and the numbers of CD16- and CD56- NK cells, perforin, granulysin, and granzyme A/B-expressing NK cells and significantly decreased CD4+ T cells, the concentrations of cortisol in the blood and adrenaline in urine. The increased NK activity
lasted for 7 days after the trip. Phytoncides, such as isoprene, alpha-pinene, and beta-pinene, were detected in the forest air. These findings indicate that the day trip to the forest park also increased the NK activity, number of NK cells, and levels of intracellular anti-cancer proteins, and that this effect lasted for at least 7 days after the trip. Phytoncides released from trees and decreased stress hormone levels may partially contribute to the increased NK activity. J Biol Reg Homeost Ag 2010; 24: 157-165

8. Osteogenic properties of human dental pulp stem cells


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Stem cells are a promising tool for bone tissue regeneration. Dental pulp stem cells (DPSCs) can be easily obtained even in human young adults. In this study we investigated the capability of DPSCs, to express the osteoblastic phenotype when cultured with osteogenic medium. DPSCs isolated from the dental pulp of impacted third molar teeth were cultured with appropriate medium to induce osteoblast differentiation. Using Western-Blot, RT-PCR and microarray analysis, we studied the expression of osteoblastic parameter, and by Von Kossa staining we evaluated the production of mineralized matrix nodules. The results were compared with controls represented by undifferentiated DPSCs. DPSCs, differentiated into osteoblast-like cells, express large amount of alkaline phosphatase (ALP), collagen I (Coll I), osteopontin (OPN) and osteocalcin (OCN), all these parameters characterizing the osteoblastic phenotype. Differentiated DPSCs express Runx2 and JunB, a member of the AP-1 complex; both the transcription factors are associated with osteoblast differentiation and skeletal morphogenesis. Moreover, DPSCs express insulin growth factor-binding protein 5 (IGFBP-5), one of the regulating proteins of IGFs function. Finally, DPSCs can form mineralized matrix nodules that are a feature exclusive to osteoblasts. DPSCs could represent a potential source of osteoblasts to be used for bone regeneration. J Biol Reg Homeost Ag 2010; 24: 167-175

9. Rupatadine improves nasal symptoms, airflow and inflammation in patients with persistent allergic rhinitis: a pilot study

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Nasal obstruction is the main symptom in patients with allergic rhinitis and may be measured
by rhinomanometry. Rupatadine is a new antihistamine with potential antiallergic activities. The aim of this pilot study is to evaluate nasal symptoms, nasal airflow and nasal mediators in patients with persistent allergic rhinitis, before and after treatment with rupatadine. Twenty patients with persistent allergic rhinitis were evaluated, 15 males and 5 females (mean age 35 ± 9.1 years), all of whom received rupatadine (10 mg/daily) for 3 weeks. Nasal and ocular symptoms (measured by VAS), rhinomanometry, and nasal mediators (ECP and tryptase) were assessed in all subjects before and after treatment. Rupatadine treatment induced significant symptom relief (both nasal and ocular, respectively p=0.005 and p=0.0004), including obstruction (p=0.0015) and significant increase of nasal airflow (p=0.0025). Moreover, there was a significant difference of nasal mediators. In conclusion, this pilot study demonstrates the effectiveness of rupatadine treatment in: i) improving nasal and ocular symptoms, ii) increasing nasal airflow, iii) exerting antiallergic activity in patients with persistent allergic rhinitis. These positive results could explain the effectiveness of rupatadine in the treatment of persistent allergic rhinitis, as reported in a previous study. Further controlled studies need to be conducted to confirm these preliminary findings. J Biol Reg Homeost Ag 2010; 24: 177-183

10. Comparative evaluation of genome-wide gene expression profiles in ruptured and unruptured human intracranial aneurysms

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Few studies have evaluated the over or the underexpression of genes directly in samples of aneurysmal wall and extracranial pericranial vascular tissue to investigate the genetic influence in formation and rupture of intracranial aneurysms. We present the results obtained using the DNA microarray technique analysis on sample tissues collected during surgery. We collected and analyzed 12 aneurismal and 9 peripheral arteries [superficial temporal (STA) and middle meningeal artery (MMA)] specimens from ruptured aneurysm group patients (13 cases), 10 aneurismal and 12 STA and MMA samples from unruptured aneurysm group patients (14 cases) and 5 STA and MMA artery specimens from control group patients (4 cases). Total RNA was isolated from samples and subjected to cDNA microarray analysis with the use of the human genome U133A GeneChip oligonucleotide microarray (Affymetrix, Santa Clara, CA), which allows to analyze a total number of 14,500 genes in the same time. For genes of interest, real-time RT-PCR was performed to confirm their expression level. Total RNA was isolated from samples and subjected to DNA microarray analysis with the use of the human genome U133A GeneChip oligonucleotide microarray, which allows to analyze a total number of 14,500 genes at the same time. For genes of interest, real-time RT-PCR was performed to confirm their expression level. Regarding ruptured aneurysms, genes were identified showing differential expressions (overexpressed or downregulated) pertaining to specific pathways, particularly those for the structural proteins of the extracellular matrix, members of matrix metalloproteinase (MMP) family (which resulted as being overexpressed) and genes involved in apoptotic phenomena. Particularly, real-time RT-PCR analysis confirmed the upregulation of MMP-2, MMP-9 and pro-apoptotic genes, such as Fas, Bax and Bid, and the downregulation of anti-apoptotic genes, such as Bcl-X(L) and Bcl-2. In a compared analyses of ruptured vs unruptured aneurysms, a different expression was also detected regarding gene coding the tissue inhibitor of matrix metalloproteinases 3 (TIMP-3), which appeared markedly downregulated in unruptured...
aneurysms, where its expression in unruptured aneurysms was similar to that observed in controls. Another gene differently expressed is nitric oxide synthase (iNOS), which appeared overexpressed in ruptured aneurysms when compared to unruptured aneurysms. Our study is the first, to our knowledge, that compares gene expression profiles (genoma-wide) in intracranial aneurysms. The results of our study suggest that the inhibitor of the metalloproteinase, the pathway of nitric oxide and the apoptotic process play a key-role in reducing the resistance of the arterial wall, that can result in formation and rupture of the intracranial aneurysms. *J Biol Reg Homeost Ag* 2010; 24: 185-195


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Multidrug resistance (MDR) in cancer cells is often caused by the high expression of the plasma membrane drug transporter P-glycoprotein (Pgp) associated with an elevated intracellular glutathione (GSH) content in various human tumors. Several chemosensitizers reverse MDR but have significant toxicities. Sedatives are often used to control anxiety and depression in cancer patients. In this *in vitro* study we investigated the effects of three plant derived sedatives such as apigenin (Api), fisetin (Fis), flavonoids and honokiol (Hnk) on Pgp activity and cellular GSH content in order to evaluate their potential use as chemosensitizing agents in anticancer chemotherapy. Human doxorubicin (doxo) resistant uterine sarcoma cells (MES-SA/Dx5) that overexpress Pgp, were treated with each sedative alone (10 µM) or in combination with different doxo concentrations (2-8 µM). We measured the intracellular accumulation and cytotoxicity of doxo (MTT assay), the cellular GSH content (GSH assay) and ROS production (DFC-DA assay), in comparison with verapamil (Ver), a specific inhibitor for Pgp, used as reference molecule. We found that exposure at 2 and 8 µM doxo concentrations in the presence of Api, Fis and Hnk enhanced significantly doxo accumulation by 29±3.3, 20±4.8, 24±6.6% and 14±1.7, 8.3±4.2, 10.7±3.1% respectively, when compared with doxo alone. These results were consistent with the increase of sensitivity towards doxo in MES-SA/Dx5, resulting in 1.7, 1.2, 1.4-fold and 1.2, 1.0 and 1.1-fold increases, respectively. Moreover, treatment with Api decreased markedly cellular GSH content (18%) and increased ROS production (>20%) on MES-SA/Dx5 cells, while a significant reduction in ROS levels was observed in Hnk and Fis treated cells, when compared to untreated control. Our *in vitro* findings provide a rationale for innovative clinical trials to assess the use of natural sedatives or their derivatives as potential adjuvants to anticancer treatment for overcoming multidrug resistance Pgp-mediated in cancer patients. *J Biol Reg Homeost Ag* 2010; 24:196-206.

12. Effects of palladium nanoparticles on the cytokine release from peripheral blood mononuclear cells of non-atopic women


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Multidrug resistance (MDR) in cancer cells is often caused by the high expression of the plasma membrane drug transporter P-glycoprotein (Pgp) associated with an elevated intracellular glutathione (GSH) content in various human tumors. Several chemosensitizers reverse MDR but have significant toxicities. Sedatives are often used to control anxiety and depression in cancer patients. In this *in vitro* study we investigated the effects of three plant derived sedatives such as apigenin (Api), fisetin (Fis), flavonoids and honokiol (Hnk) on Pgp activity and cellular GSH content in order to evaluate their potential use as chemosensitizing agents in anticancer chemotherapy. Human doxorubicin (doxo) resistant uterine sarcoma cells (MES-SA/Dx5) that overexpress Pgp, were treated with each sedative alone (10 µM) or in combination with different doxo concentrations (2-8 µM). We measured the intracellular accumulation and cytotoxicity of doxo (MTT assay), the cellular GSH content (GSH assay) and ROS production (DFC-DA assay), in comparison with verapamil (Ver), a specific inhibitor for Pgp, used as reference molecule. We found that exposure at 2 and 8 µM doxo concentrations in the presence of Api, Fis and Hnk enhanced significantly doxo accumulation by 29±3.3, 20±4.8, 24±6.6% and 14±1.7, 8.3±4.2, 10.7±3.1% respectively, when compared with doxo alone. These results were consistent with the increase of sensitivity towards doxo in MES-SA/Dx5, resulting in 1.7, 1.2, 1.4-fold and 1.2, 1.0 and 1.1-fold increases, respectively. Moreover, treatment with Api decreased markedly cellular GSH content (18%) and increased ROS production (>20%) on MES-SA/Dx5 cells, while a significant reduction in ROS levels was observed in Hnk and Fis treated cells, when compared to untreated control. Our *in vitro* findings provide a rationale for innovative clinical trials to assess the use of natural sedatives or their derivatives as potential adjuvants to anticancer treatment for overcoming multidrug resistance Pgp-mediated in cancer patients. *J Biol Reg Homeost Ag* 2010; 24:196-206.
The object of this study is to determine the cytokine release from PBMCs exposed to Pd model nanoparticles emitted from catalytic converters. PBMCs of 8 healthy non-atopic women were incubated in the presence of Pd nanoparticles (5-10 nm) or salt (potassium hexa-chloropalladate) $10^{-5}$ and $10^{-6}$ M. Release of cytokines in supernatant of PBMCs was then determined. In cultures without LPS, IL-10 and IL-17 release from PBMCs was inhibited by Pd salt, while Pd nanoparticles inhibited TNF-$\alpha$ and IL-17 release. In LPS-stimulated cultures, release of IFN-gamma, TNF-alpha, IL-10 and IL-17 was inhibited by Pd salt, whereas IFN-gamma release was enhanced and TNF-alpha and IL-17 release was inhibited by Pd nanoparticles. In conclusion, Pd salt inhibits cytokine release, whereas Pd nanoparticles exert modulatory effects enhancing the release of IFN-gamma, a Th1 cytokine typical of delayed allergic reactions. This result is interesting considering the increase of allergic contact dermatitis to Pd in people exposed to Pd nanoparticles in urban environments. *J Biol Reg Homeost Ag* 2010; 24: 207-214

13. **Objective and subjective assessment of digestion after ingestion of an iced dessert in healthy volunteers**

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The aim of our study is to assess, with objective measures, the impact on digestion of a coffee-flavoured iced dessert ingested at the end of a standardized meal; moreover, a subjective assessment, using a specific questionnaire, was carried out in order to compare objective and subjective data. Ten healthy male volunteers, after ENT and psychological assessment, underwent two scintigraphic evaluations (standardized meal without and with coffee-flavoured iced dessert) and, after the meal, filled in a specific questionnaire named “dynamic digestibility questionnaire”. In our sample the ingestion of the coffee-flavoured iced dessert seemed to improve the digestibility of a standardized meal: the difference between the curves of gastric emptying without and with iced dessert is statistically significant. These data are strongly confirmed by subjective assessment: the dynamic digestibility questionnaire (DDQ) showed a higher digestibility of a standardized meal with
the coffee-flavoured iced dessert in comparison to a normal meal. The current study represents a preliminary report on this topic with a small sample of healthy volunteers: further studies on larger population are requested in order to confirm all the encouraging results herein discussed. J Biol Reg Homeost Ag 2010; 24: 215-220

14. Soluble serum HLA-G in children with allergic rhinitis and asthma

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Allergic rhinitis (AR) and asthma are characterized by Th2 polarized immune response. Soluble HLA (sHLA) molecules play an immunomodulatory activity. It has been reported that both molecules are increased in sera of patients with pollen-induced allergic rhinitis studied outside the pollen season. To date, however, no study has investigated them in AR children. The aim of this preliminary study is to evaluate serum sHLA-G levels in children with AR or asthma patients and in a group of healthy controls. Forty-seven symptomatic allergic patients were enrolled. A group of 50 healthy subjects was considered as control. Serum sHLA-G levels were determined by the immunoenzymatic method. Children with AR had significantly higher levels of sHLA-G molecules than normal controls or children with allergic asthma. However, there is no difference between children with AR and asthmatics. In conclusion, the present study provides the preliminary evidence that serum sHLA-G molecules are significantly increased in children with AR. J Biol Reg Homeost Ag 2010; 24: 221-224

15. Pulmonary embolism with minimal d-dimer increase - disagreement between clinic and laboratory: case report

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Pulmonary embolism is still currently considered a very insidious disease and if not diagnosed and treated rapidly is lethal in almost 10% of all cases. Clinical and patient history data are essential for the diagnosis and evaluation of the clinical risk of pulmonary embolism. Pulmonary embolism, particularly during minor episodes, was primarily identified by abnormalities in D-dimer concentration during laboratory testing. Indeed, an increase in D-dimer plasma levels was consequently identified as a valid diagnostic element for pulmonary embolism and therefore, in the absence of D-dimer abnormalities, a tendency to exclude such diagnosis exists. This case report describes the importance of carrying out level II diagnostic investigations which may be particularly valid in patients with a minimal rise in D-dimer
levels and a clinical suspicion of a pulmonary embolism. This method allows for a quick diagnosis with early therapeutic measures which improve survival rates during the acute and critical phase. J Biol Reg Homeost Ag 2010; 24: 225-230