

EDITORIAL

COMPARISON OF BENEFICIAL ACTIONS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS TO FLAVONOIDS

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Inflammation is involved in increasing number of diseases necessitating the development of new, effective and safe treatments. Non steroidal anti-inflammatory drugs (NSAIDs) have been helpful in many instances, but they only inhibit cyclooxygenase (COX), but not the generation or actions of cytokines. Instead, some natural flavonoids have multiple anti-inflammatory effects, including COX inhibition, and a much safer profile. Increasing evidence indicates that inflammation plays a critical role in the pathogenesis of many diseases that also involve mast cells (1-3) (Table I). Consequently, the need for new, effective and safe anti-inflammatory drugs is all the more urgent. Corticosteroids are quite potent, but have many adverse effects such as increased risk of infections, osteoporosis, glaucoma and depression (4-8). Biological agents such anti-TNF are useful in certain conditions, such as rheumatoid arthritis and psoriasis, but has been associated with increased risk of infection and leukemia.

THE TRANSCRIPTIONAL REGULATORS, THE IMMUNE SYSTEM AND THE CIRCADIAN CLOCK

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The immune system function oscillates with a 24-hour period driving circadian rhythmicity of immune responses. A circadian timing system comprising central and peripheral oscillators entrains body rhythmicity of physiology and behavior to environmental cues by means of humoral signals and autonomic neural outputs. In every single cell an oscillator goes ticking through a molecular clock operated by transcriptional/translational feedback loops driven by the rhythmic expression of circadian genes. This clock gene machinery steers daily oscillations in the regulation of immune cell activity, driving the periodicity in immune system function. The transcriptional networks that regulate temporal variation in gene expression in immunocompetent cells and tissues respond to diverse physiological clues, addressing well-timed adjustments of transcription and translation processes. Nuclear receptors comprise a unique class of transcriptional regulators that are capable of gauging hormones, metabolites, endobiotics and xenobiotics, linking ligand sensing to transcriptional responses in various cell types through switching between coactivator and corepressor recruitment. The expression of coregulators is highly responsive to physiological signals, and plays an important role in the control of rhythmic patterns of gene expression, optimizing the switch between nycthemeral patterns, and synchronizing circadian rhythmicity with changing physiological demands across the light-dark cycle. The nuclear receptors and transcription factors expressed in the immune components contribute to the cross-talk between the circadian timing system, the clock gene machinery and the immune system, influencing transcriptional activities and directing cell-type specific gene expression programs linked to innate and adaptive immune responses.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

OBESTATIN INHIBITS LIPOGENESIS AND GLUCOSE UPTAKE IN ISOLATED PRIMARY RAT ADIPOCYTES

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The first two authors contributed equally to this work.

Ghrelin and obestatin are encoded by the preproghrelin gene and originate from post-translational processing of the preproghrelin peptide. Obestatin is mainly present in the stomach, but its action is focused on appetite inhibition in opposition to ghrelin function. Recently, it has been presented that obestatin may regulate adipocyte metabolism and influence fat content. However, obestatin action is still poorly understood. Therefore, we aimed to investigate obestatin function on adipocyte metabolism in the rat. We studied changes in the mRNA expression of active and inactive isoforms of obestatin receptors. In addition, we analyzed influence of obestatin on lipogenesis, lipolysis and glucose transport in isolated adipocytes. Moreover, we also performed analysis of obestatin action on lipolysis in differentiated rat preadipocytes with silenced obestatin receptor. We found significantly higher expression of the obestatin receptor *Gpr39-1a* active form at an mRNA level following adipocytes incubation with obestatin. We did not observe expression changes in the inactive form of obestatin receptor *Gpr39-1b*. Additionally, we found significant changes in *Gpr39-1a* expression following obestatin receptor silencing in cells incubated with obestatin in comparison to control. Obestatin inhibited both, basal and insulin-stimulated lipogenesis and glucose transport in adipocytes. Furthermore, obestatin potentiated adrenalin-stimulated lipolysis. We also found reduced glycerol release following obestatin incubation in adipocytes with silenced *Gpr39* gene. Our results indicate that obestatin acts via the GPR39 receptor in isolated adipocytes, and that through this mechanism obestatin influences lipid accumulation, glucose uptake and lipolysis.

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EFFECT OF LOW TEMPERATURE CULTIVATION ON INSULIN SECRETORY OF HUMAN PANCREATIC ISLETS

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The experiment compared the physiological function (insulin secretory capacity) and membrane integrity of human adult pancreatic islets incubated in culture at 37°C and 24°C. Pancreatic tissue was digested with Collagenase XI, using a non-automated method. Cultures were incubated at 37°C and 24°C. Secretory capacity of the islets is determined by measuring of the stimulation index (SI) on the 1st, 3rd and 7th day of cultivation. Membrane integrity of the islets was determined by dithizone staining. Both groups of examined cultures show a slight increase in SI during the incubation. However islets incubated at 24°C show higher SI values than those incubated at 37°C on the 1st, 3rd and 7th day of incubation. And on the first day of incubation, this difference was statistically significant ($p < 0.05$). Islets incubated at 37°C showed preservation of membrane integrity, the islets are regular spherical shape, while those incubated at 24°C lose such an organization. During the seven-day cultivation, islets incubated at a standard temperature of 37°C show less preserve physiological functions in relation to cultures incubated at 24°C, but islets incubated at 37°C show more regular morphological forms.

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EFFECTIVENESS OF OMALIZUMAB IN NON-ALLERGIC SEVERE ASTHMA

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Omalizumab is an effective drug for allergic asthma. The purpose of this study was to evaluate the effectiveness and tolerance of this drug in non-allergic GINA step V asthma patients. This study was single-centre, prospective, open-label, observational, naturalistic. Non-allergic asthma patients requiring a mean dose of oral prednisolone of at least 5 mg/day during ≥ 1 year or an accumulated oral corticosteroid dose/year ≥ 1500 mg were enrolled. At entry and the end of the 12-month follow-up we measured blood eosinophilia and IgE concentration; at every monthly visit a forced spirometry and exhaled fraction of nitric oxide (NO) were carried out. The subjects were seven adult patients (5 female), age range 37-63 years, with the following mean values: IgE: 226.7 ± 176 IU/mL; FVC $74 \pm 18\%$; FEV₁ $57 \pm 11\%$; NO: 21.2 ± 7 ppb. The study was approved by the IRB of the hospital. One patient decided to stop treatment after 12 weeks and was excluded from the evaluation. We did not observe changes in eosinophil count, spirometry or NO values. Three patients considered responders did not need prednisolone during the follow-up. The mean daily dose of prednisolone fell from 6.6 ± 8.1 mg/day at entry to 1.5 ± 2.3 mg/day ($p < 0.16$) at the end of follow-up. The mean monthly accumulated dose fell from 92 ± 112 to 12 ± 26 mg/month ($p = 0.26$). Total blood IgE increased 1.93-fold. Side effects were only local: treatment tolerance was excellent; three out of six patients seemed to slightly benefit from anti-IgE treatment; to date there is no evidence strong enough to systematically prescribe omalizumab in non-allergic asthma patients.

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**PROTECTIVE EFFECT OF OLEANOLIC ACID AGAINST β CELL DYSFUNCTION
AND MITOCHONDRIAL APOPTOSIS:
CRUCIAL ROLE OF ERK-NRF2 SIGNALING PATHWAY**

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The first three authors contributed equally to this work

Pancreatic β cell dysfunction is a hallmark of diabetes. Our previous results have shown that oleanolic acid (OA) has anti-diabetic potential. However, there is little literature reporting the effect of OA on β cell dysfunction. The present study was designed to investigate the protective effect of OA against lipotoxicity and the underlying mechanisms. Lepr (db/db) diabetic mice were subjected to fasting blood glucose measurement, intraperitoneal glucose tolerance test after the administration of OA for two weeks. Histopathological observation was conducted by HE staining and transmission electron microscopy assay. Pancreatic islets were isolated from db/db diabetic mice and C57BL/6J mice. Palmitic acid (PA) was used to induce lipotoxicity *in vitro*. Apoptosis was evaluated in pancreatic islets in diabetic mice and in isolated pancreatic islets and β -TC3 cells by TUNEL assay. Cellular ATP content, mitochondrial function and redox balance were examined. Phosphorylation of c-Jun NH2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) and the activation of nuclear erythroid factor 2 p45-related factor 2 (Nrf2) signaling were evaluated by western blotting. In db/db mice, OA significantly protects β cell function against lipotoxicity, evidenced by inhibition of apoptosis and improvement of glucose tolerance. In cells, OA administration may protect against PA-induced apoptosis and decrease of GSIS, in which process the activation of Nrf2 is essential. Once Nrf2 is activated, OA could induce GCLc expression, promote the production of GSH, and thus inhibit JNK phosphorylation and solid the antioxidant defense of mitochondria, leading to the inhibition of mitochondrial apoptosis. ERK signaling pathway is responsible for OA-induced activation of Nrf2 and the protective effect of OA. Overall, our study enhances the understanding of the protective effect of OA on β cell and provides clues for further studies on the underlying mechanisms.

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COMPARATIVE EVALUATION OF GASTRIC GHRELIN CELLS AND LEVELS OF HORMONE IN THE SERUM OF HEALTHY WOMEN AND MEN

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Due to difficulties in obtaining human material, most of the data concerning the site of occurrence and synthesis of ghrelin are based on animal studies. There are only few reports describing ghrelin-containing cells in the human digestive tract, based on the limited human material obtained during surgery or biopsy. The aim of this study was to compare and evaluate the distribution and morphology of ghrelin cells in the stomach and the levels of hormone in the serum of healthy men and women. The study included 18 subjects with normal gastric mucosa (12 men and 6 women). Immunohistochemistry was performed using rabbit anti-ghrelin (human) antiserum. Ghrelin level in serum was measured by ELISA. The total number of ghrelin positive cells was greater in the stomach of women than men. Ghrelin-immunoreactive cells were more elongated and larger in the stomach of women. The serum ghrelin level was higher in men than in women. Ghrelin concentration in serum correlates negatively with body mass index and weight in both genders, whereas the correlation between ghrelin level and age was positive in women and negative in men. The number of cells containing ghrelin in the stomach does not reflect the serum hormone levels. The differences in gastric ghrelin cells and ghrelin levels in serum between women and men, indicate that secretion of hormone can be under control sex hormones or other unknown factors.

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JAGGED-1–HES-1 SIGNALING INHIBITS THE DIFFERENTIATION OF TH17 CELLS VIA ROR γ t

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Notch signaling plays an important role in differentiation of T cells. However, little is known as to action of it in differentiation of Th17 cell subset. In this study, a soluble Jagged-1/Fc chimera protein (Jagged-1) was directly used to activate Jagged-1–Notch signaling, while Hes-1-targeting siRNA was used to knock down *Hes-1* gene to investigate effect of Jagged-1–Hes-1 signaling on the differentiation of CD4⁺ T cells into Th17 cells. The results showed that Jagged-1 could promote the expression of Hes-1 and Deltex-1 mRNAs and the expression of NICD, Hes-1 and Deltex-1 proteins, which might be significantly blocked by DAPT, a specific inhibitor of Notch signaling. Jagged-1–Hes-1 signaling resulted in the markedly decreased in situ expression of ROR γ t in the CD4⁺ T cells induced by IL-6 plus TGF- β . Flow cytometric analysis showed the reduction of IL-17 production in CD4⁺ T cells by Jagged-1, but the enhancement of it by Hes-1-targeting siRNA. The level of IL-10 produced by the treated cells was also enhanced, whereas the expression of IL-17 was prominently attenuated, which could be offset by anti-Jagged-1 antibody or DAPT. The results indicate that Jagged-1–Hes-1 signaling can suppress the skewing of CD4⁺ T cells toward Th17 cells via ROR γ t, for which Hes-1 may be crucial.

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IMMUNE RESPONSES TO TETANUS VACCINATION IN ITALIAN HEALTHY SUBJECTS AND CHILDREN WITH RECURRENT INFECTIONS

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The ability of vaccine antigen to generate protection is a challenge that cannot be restricted to the antibody response; however, the contribution of T cell-mediated mechanisms has not been extensively analyzed. Age and administration to specific categories of patients, i.e. children with recurrent infections (RI), are some of the factors that might affect the vaccine immune response. We investigated the humoral and cellular response to tetanus toxoid (TT) vaccine in 104 healthy children (HC), 11 newborns and 22 healthy adults to characterize the status of immunity according to age and compared it to 118 RI children. Humoral and cellular responses varied in both groups according to age and doses of TT administered. The prevalence of antibody and cellular response was similar in both cohorts (HC 88% and 82% versus RI 86% and 85%), however, TT antibody values were significantly higher in 12-18 months old RI children compared to HC (median: 5 IU/ml vs 1.10 IU/ml) ($p = 0.02$). The lack of an efficient immune response was observed in 12-15% of children from both cohorts. Our data showed that specific antibodies were responsible for early protection, whereas cell-mediated mechanisms may contribute to the generation of long-term immunity after an appropriate vaccine recall. The occurrence of higher TT antibody values in 12-18 months old RI children deserves additional research to determine whether they are caused by different infectious agents and/or by other environmental factors. Clarification of this issue is important for categorizing patients into an optimal vaccine policy.

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CURCUMIN INDUCES APOPTOSIS IN BREAST CANCER CELL LINES AND DELAYS THE GROWTH OF MAMMARY TUMORS IN NEU TRANSGENIC MICE

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Breast cancer is a leading cancer in women and despite the benefits of the current therapies a significant number of patients with this tumor is at risk of relapse. Some of the alterations taking place in breast cancer cells are currently exploited by molecularly targeted drugs. Different drugs have been developed which target a single molecule but, given that the tumor originates from the dysregulation of many genes, there is the need to find new drugs that have more than one molecular target. Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] (CUR), a polyphenolic compound found in the spice turmeric, is a pleiotropic molecule able to interact with a variety of molecular targets and has antitumor, anti-inflammatory, antioxidant, immunomodulatory and antimicrobial activities. Here we demonstrate that CUR inhibits the growth of breast cancer cell lines in a dose dependent manner, with IC₅₀ values in the micromolar range, and induces an increase in the percentage of cells in sub-G₀ phase, representing the apoptotic cell population. The activation of apoptosis was confirmed by PARP-1 cleavage and by the increased ratio between the pro-apoptotic Bax and the anti-apoptotic Bcl-2 protein. In addition, in CUR-treated cells the activity of ERK1/ERK2 MAP kinases was down-regulated. The cytotoxic effects of CUR were observed in breast cancer cells expressing either high or low levels of ErbB2/neu. The *in vivo* antitumor activity of CUR was tested in BALB-*neuT* mice transgenic for the neu oncogene, which develop atypical hyperplasia of the mammary gland at 6 weeks of age and invasive carcinoma at 16 weeks of age. CUR, administered to mice both early and in an advanced stage of mammary carcinogenesis, induced a significant prolongation of tumor-free survival and a reduction of tumor multiplicity. In addition, CUR administration was safe, since no modification of hematological and clinical chemistry parameters could be observed in BALB-*neuT* and BALB/c mice treated with this compound for several weeks. These findings support further studies on the therapeutic potential of CUR in combination with standard therapies in breast cancer patients.

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p63 EXPRESSION IN LARYNGEAL SQUAMOUS CELL CARCINOMA IS RELATED TO TUMOR EXTENSION, HISTOLOGIC GRADE, LYMPH NODE INVOLVEMENT AND CLINICAL STAGE

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To analyse the relationship of the immunohistochemical p63 expression with tumoral extent, histologic grade, lymph node involvement and clinical stage in laryngeal squamous cell carcinoma (LSCC), a series of 81 patients with primary LSCC treated by primary surgery was retrospectively evaluated. Immunohistochemistry was performed on formalin-fixed and paraffin-embedded tissue blocks from surgical samples. Clinicopathologic data were correlated with the p63 staining results. Differences in p63 immunoreactivity between the different groups were compared using both parametric analysis of variance (ANOVA) and non-parametric Kruskal-Wallis test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the R statistical package. We found a statistically significant association between p63 protein expression and increase of tumor extension (T1 vs T3), of histological grading, of level of lymph node involvement (N0 vs N1 and N2), and clinical stage (I vs IV). Our findings suggest that abnormal expression of p63 may be involved in the early phases of laryngeal tumorigenesis and this oncoprotein might become a useful predictor of clinical outcome.

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THREE NOVEL HUMAN SPORADIC MELANOMA CELL LINES: SIGNALING PATHWAYS CONTROLLED BY MC1R, BRAF AND β -CATENIN

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We studied the behaviour of three novel human sporadic melanoma cell lines (hmel1, hmel9, hmel11) extracted from tumors with different degrees of malignancy, concerning the cell signalling pathways controlled by MC1R, BRAF, NRAS and β -catenins. The novel cell lines were compared to metastatic cell lines (HBL, LND1), wild type (wt) for MC1R and BRAF genes, that have been extensively characterised and were used as control. All the novel cell lines have silent or no MC1R mutations even though MC1R signalling is severely impaired. Conversely, they harbour BRAF mutations at the V600 residue. These mutations determine a constitutive ERK phosphorylation in all the three cell lines. Our new melanoma cell lines were BRAF mutated in hetero- and homozygosis, even with a wild type MC1R, and unresponsive to NDP-MSH treatment. Quantity and subcellular localization of β -catenin were analyzed in both novel and control cell lines. In HBL and LND1 there were high levels of β -catenin distributed in the cytoplasm/nucleus, while in the novel melanoma cell lines β -catenins were less abundant and seemed to be located at the plasma membrane/cytoplasm and absent in the nucleus. We sequenced β -catenin cDNA for all the melanoma cell lines, and found mutations in HBL, LND1 and hmel1, while hmel9 and hmel11 were wt. We found that β -catenin levels were not influenced by the RAS/RAF/MAPK pathway because inhibition with PD98059 (a MEK inhibitor) did not produce any effect on β -catenin stability and/or localization.

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DIFFERENTIAL ROLE OF EGF AND bFGF IN HUMAN GBM-TIC PROLIFERATION: RELATIONSHIP TO EGFR-TYROSINE KINASE INHIBITOR SENSITIVITY

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Glioblastoma multiforme (GBM) is among the most devastating human tumors being rapidly fatal despite aggressive surgery, radiation and chemotherapies. It is characterized by extensive dissemination of tumor cells within the brain that hinders complete surgical resection. GBM tumor initiating-cells (TICs) are a rare subpopulation of cells responsible for tumor development, growth, invasiveness and recurrence after chemotherapy. TICs from human GBM can be selected in vitro using the same conditions permissive for the growth of normal neural cells, of which share some features including marker expression, self-renewal capacity, long-term proliferation, and ability to differentiate into neuronal and glial cells. EGFR overexpression and its constitutive activation is one of the most important signaling alteration identified in GBM, and its pharmacological targeting represents an attractive therapeutic goal. We previously demonstrated that human GBM TICs have different sensitivity to the EGFR kinase inhibitors erlotinib and gefitinib, depending on the differential modulation of downstream signaling cascades. In this work we investigated the mechanisms of resistance to erlotinib in two human GBM TIC cultures, analyzing EGF and bFGF individual contribution to proliferation, clonogenicity, and migration. We demonstrated the presence of a small cell subpopulation whose proliferation is supported by EGF and a larger one mainly dependent on bFGF. Thus, insensitivity to EGFR kinase inhibitors as far as TIC proliferation results from a predominant FGFR activation that hides the inhibitory effects induced on EGFR signaling. Conversely, EGF and bFGF induced cell migration with similar efficacy. In addition, unlike neural stem/progenitors cells, the removal of chondroitin sulphate proteoglycans from cell surface was unable to discern EGF- and bFGF-dependent subpopulations in GBM TICs.

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COULD PAST CHLAMYDIAL VASCULAR INFECTION PROMOTE THE DISSEMINATION OF *CHLAMYDIA PNEUMONIAE* TO THE BRAIN?

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Chlamydia pneumoniae, a pathogen responsible for respiratory tract infections, has been associated with atherosclerosis which, along with hypertension, hyperlipidemia, cardiovascular and/or cerebrovascular ischemia and stroke, is a risk factor for chronic neurological disorders. Several studies have demonstrated the ability of *C. pneumoniae* to disseminate from lungs to arteries through peripheral blood mononuclear cells. Once inside the vascular tissue, *C. pneumoniae* infection may disseminate via peripheral monocytes to the brain over the intact blood-brain barrier, and contribute to the development of chronic neurological disorders. The aim of our study was to evaluate whether past *C. pneumoniae* vascular infection may promote the dissemination of this microorganism to the brain, therefore we investigated the presence of *C. pneumoniae* in post-mortem brain tissue specimens of patients with past chlamydial vascular infection. Seventy six post-mortem brain tissue specimens from 19 patients with past chlamydial vascular infection were investigated for the presence of *C. pneumoniae* by immunohistochemistry, polymerase chain reaction, *in situ* polymerase chain reaction and *in situ* reverse transcription polymerase chain reaction. As control, 28 brain tissue specimens were taken from 7 age and sex matched subjects without chlamydial infection. *C. pneumoniae* was detected in 16 (84.2%) out of 19 patients with chlamydial vascular infection whereas it was not detected in control subjects (p=0.0002). In conclusion, the main result of our study is the evidence that a chlamydial vascular infection can disseminate to the brain. It will be important for current and future researches to perform large-scale prospective studies on cardiovascular patients with chlamydial vascular infection in order to evaluate the long-term pathological alterations of the brain.

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BONE MARROW CONCENTRATED CELL TRANSPLANTATION: RATIONALE FOR ITS USE IN THE TREATMENT OF HUMAN OSTEOCHONDRAL LESIONS

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Bone marrow is one of the best characterized stem cell microenvironments that contains Mesenchymal Stem Cells (MSCs), a rare population of non-hematopoietic stromal cells. MSCs have been indicated as a new option for regenerative medicine because of their ability to differentiate into various lineages such as bone, cartilage and adipose tissue. However, isolation procedures are crucial for the functional activity of the transplanted cells. The use of concentrated bone marrow cells (BMCs) enables a cell population surrounded by its microenvironment (niche) to be implanted while avoiding all the complications related to the *in vitro* culture. The cells of the niche are able to regulate stem cell behavior through direct physical contact and secreting paracrine factors. The aim of this study was to characterize BMCs *in vitro* to evaluate their ability to differentiate toward mature cells and try to understand whether there are differences in the chondrogenic and osteogenic potential of cells from patients of different ages. Mononuclear Cells (MNCs) isolated by Ficoll were used as control. Both cell populations were grown in monolayers and differentiated with specific factors and analyzed by histological and molecular biology assays to evaluate the expression of some specific extracellular matrix molecules. The present investigations revealed the ability of BMCs to act as isolated cells. They are able to form colonies and differentiate toward chondrogenic and osteogenic lineages, the latter pathway appearing to be influenced by donor age. The results obtained by this study support the use of BMCs in clinical practice for the repair of osteochondral damage, which might be particularly useful for the “one-step” procedure allowing cells to be directly implanted in operating room.

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EFFECT OF AUTOLOGOUS PLATELET-RICH PLASMA ON DISTRACTION OSTEOGENESIS IN THE MANDIBLE OF RABBITS: A MORPHOLOGIC AND MORPHOMETRIC APPROACH

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Distraction osteogenesis of the jaw is a common surgical practice in the treatment of pediatric craniofacial deformities. Autologous platelet rich plasma (PRP) has been used to increase the healing potential of bones in humans during distraction osteogenesis. This article aims to study the morphometric and morphologic parameters resulting from the effect of PRP on bone healing after mandibular distraction in rabbits. Right mandibular distraction was performed in 12 rabbits divided equally into 2 groups. PRP and physiological saline were injected, according to a defined protocol, in the callus following distraction of the experimental and control groups respectively. The rabbits were sacrificed after a consolidation period of 45 days and the mandibles were surgically removed. Bone mineral density, radiographic analysis, mechanical properties and histological features of the lengthened bones were assessed using radiographic examination, dual X-ray absorptiometry, biomechanical testing and histology. Results showed that the regenerate bone density, the amount of trabeculation in addition to the bone mineral density and mineral content, as measured by absorptiometry, were better with PRP but not significantly different between groups. Two radiographs revealed a more consistent healing in the experimental mandibles compared with erratic outcomes in corresponding controls. Two of the latter could not be subjected to any mechanical testing because the mandibular parts, connected with fibrous tissue, were separated. Consequently, the biomechanical test depicted greater maximal loads in the experimental group. The histological studies exhibited more ossification and less connective tissue fibers in the experimental group. PRP accelerated healing of mandibles in rabbits following distraction and improved their biomechanical properties. These findings have significant clinical implications on reducing the period of consolidation of the mandibles which may not be immobilized like other bones for long periods of time.

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ERYTHROPOIETIN DOES NOT AFFECT TNF AND IL-6 PRODUCTION DIRECTLY

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The tissue-protective action of erythropoietin (EPO) in animal models is often associated with reduced inflammation. However, there are many contrasting reports of the effect of EPO on the production of inflammatory cytokines induced by lipopolysaccharide (LPS) *in vitro*, with different papers reporting an inhibition, an upregulation, or a lack of effect. Negative results are likely underestimated by a publication bias. As EPO has anti-inflammatory actions in models associated with tissue injury, we hypothesized that EPO could specifically inhibit the induction of inflammatory cytokines by danger signals associated with cell death, and investigated its effect on the induction of IL-6 or TNF by high-mobility group-box 1 protein (HMGB1) or by necrotic cells. We did not observe any significant effect of EPO in these models; neither EPO affected the response induced by TLR agonists different from LPS, or by extracellular ATP-mediated activation of the inflammasome. We conclude that the inhibition of inflammation by EPO is likely to be an indirect effect, secondary to its tissue-protective activity, or that it requires a prior priming induced by the injury.

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MECHANISMS OF OCULAR NEUROPROTECTION BY ANTIOXIDANT MOLECULES IN ANIMAL MODELS

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This work was conducted to evaluate the efficacy of a treatment on retinal ganglion cells (RGC) and on astrocytes of the optic nerve of glaucomatous eyes, using a combination of α -lipoic acid (ALA) and superoxide dismutase (SOD). Thirty-two male Wistar rats were fed with a diet supplemented with ALA, SOD, ALA and SOD or with no product for 8 weeks. Ocular hypertension was induced with 2% methylcellulose (MTC) and then rats were sacrificed. TUNEL assay showed a marked fluorescence in the ganglion cells and astrocytes of MTC-treated rats evidencing induction of apoptosis. In contrast, sections of eyes pretreated with ALA and SOD showed a lack of fluorescence quite similar to that of the controls. Similarly, eyes sections from rats pre-treated with ALA and SOD showed reduced differential expression of inducible nitric oxide synthase (iNOS) and of caspase-3 in compared to normally-fed/MTC-inoculated cases. An increase of ALA and SOD exerts an antiapoptotic effect and protects against oxidative stress and hence against the structural remodelling of the RGCs and astrocytes of the optic nerve in the presence of an ischemic and pressure stress.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

ALTERATIONS OF ROS PATHWAYS IN SCLERODERMA BEGIN AT STEM CELL LEVEL

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Scleroderma is a chronic systemic autoimmune disease (primarily of the skin) characterized by fibrosis (or hardening), vascular alterations and autoantibodies production. There are currently no effective therapies against this devastating and often lethal disorder. Despite the interest for the immunomodulatory effects of mesenchymal stem cells (MSCs) in autoimmune diseases, the role of MSCs in scleroderma is still unknown. A pivotal role in scleroderma onset is played by oxidative stress associated with the accumulation of great amounts of reactive oxygen species (ROS). This study depicts some phenotypic and functional features of MSCs isolated from the skin of healthy and scleroderma patients; the ROS production and accumulation, the expression of ERK1/2 and the effects of the stimulation with PDGF, were analyzed in MSCs; results were compared to those observed in primary fibroblasts (Fbs) isolated from the same subjects. We found that the pro-oxidant environment exerted by scleroderma affects MSCs, which are still able to counteract the ROS accumulation by improving the antioxidant defenses. On the contrary, scleroderma fibroblasts show a disruption of these mechanisms, with consequent ROS increase and the activation of the cascade triggered by scleroderma auto-antibodies against PDGFR.

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LETTER TO THE EDITOR

INSULIN RESISTANCE IN FIRST-TRIMESTER PREGNANT WOMEN WITH PRE-PREGNANT GLUCOSE TOLERANCE AND HISTORY OF RECURRENT SPONTANEOUS ABORTION

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The first two authors contributed equally to the manuscript

Insulin resistance (IR) has been reported to play an important role in recurrent spontaneous abortion (RSA) among patients with polycystic ovary syndrome (PCOS). However, scant materials exist regarding the independent effect of IR on RSA. The aim of this study is to investigate the status of IR in first trimester pregnant patients with normal pre-pregnant glucose tolerance and history of RSA. This two-center case-control study enrolled totally 626 first trimester pregnant women including 161 patients with a history of recurrent spontaneous abortion, who were pre-pregnantly glucose-tolerant according to oral glucose tolerance test (OGTT), and 465 women with no history of abnormal pregnancies of any kind. Clinical, biochemical and hormonal parameters were simultaneously measured in all participants. Serum β -HCG, estradiol, progesterone, fasting plasma glucose and fasting plasma insulin levels, as well, the calculated homeostasis model assessment of insulin resistance index (HOMA-IR), fasting plasma glucose/insulin ratio(G/I) and pregnancy outcome were analyzed and compared. Serum β -HCG and progesterone were found to be significantly lower in RSA group compared to controls. Subjects in RSA group were found to have higher HOMA-IR and lower G/I ratio than those in control group. Serum β -HCG and progesterone were negatively correlated with HOMA-IR, and positively with G/I ratio even after adjustment for BMI. The spontaneous abortion rate within first trimester pregnancy of RSA patients was significantly higher than that in controls. In conclusion, woman with recurrent spontaneous abortion and normal pre-pregnant glucose metabolism tends to be more insulin resistant during first trimester pregnancy than healthy controls, no matter whether she has PCOS or not. Insulin resistance might be one of the direct causes that lead to recurrent abortion.

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LETTER TO THE EDITOR

IN VIVO LIVER EXPRESSION OF TLR2, TLR3 AND TLR7 IN CHRONIC HEPATITIS CG. TARANTINO¹, A. DI CRISTINA¹, R. PIPITONE¹, P.L. ALMASIO¹,
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The role of innate immune response mediated by Toll-like receptors in HCV infection, is not yet well understood and there is a lack of data regarding liver tissue expression of these molecules in chronic hepatitis C (CHC). Our study is aimed to investigate *ex vivo*, liver expression of TLR2, TLR3 and TLR7, which are more involved in the immune-pathogenesis of CHC, and to explore possible correlations with features of disease. We obtained liver biopsies and collected peripheral blood mononuclear cells (PBMC) from 23 consecutive patients with CHC and from 6 patients of control, without liver disease, undergoing surgery for cholecystectomy. The levels of TLRs mRNA in the samples were determined using a real-time reverse transcription quantitative PCR (RT-qPCR). We found a significant high expression of TLR3 in the liver of CHC patients respect to controls (also higher than expression in the PBMC). Conversely no differences emerged in the TLR2 and TLR7 levels between cases and controls. Also we found a correlation of TLR2 and TLR7 levels with the grade of necro-inflammation in the liver. Furthermore TLR7 hepatic levels resulted related to a more advanced stage of liver fibrosis. Ours is the first study to provide data on tissue expression of TLRs during chronic hepatitis C and we believe that it could lead to a better understanding of the role of these molecules in the HCV-mediated liver damage.

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LETTER TO THE EDITOR

ULTRASTRUCTURAL AGE-RELATED CHANGES IN THE SENSORY CORPUSCLES OF THE HUMAN GENITAL SKIN

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In human genital skin the majority of superficial sensory corpuscles is represented by glomerular corpuscles. These corpuscles show an own morphology. In this report the ultra-structure of the sensitive corpuscle in the penis skin of the younger and older subjects was compared, showing that the genital skin of the older humans contains more simple complexes than the younger ones. Our findings support the view that the age-related changes that can be observed in human glomerular genital corpuscles are consistent with an increase of the simple complexes and a strong decrease of the poly-lamellar one in the older people. These findings demonstrate that human genital corpuscles underwent age-related changes. Moreover our morphological findings can be correlated in relation to the clinical evolution of the sensitivity in the genital skin.

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LETTER TO THE EDITOR

EVALUATION OF PATTERNS AND APPROPRIATE GASTRO PROTECTION IN ALBANIAN PRIMARY HEALTH CARE USERSS. KELLICI¹, A. DIBRA², X.H. DRACINI², L. DEDA³, M. FIDA³ and G. BURAZERI⁴

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Our aim was to assess the prevalence of gastro protection in the Albanian population using non-steroidal anti-inflammatory drugs (NSAIDs). A cross-sectional study, conducted in November-December 2011 in Albania, included 610 NSAIDs users (236 men and 374 women) who visited pharmacies to receive their NSAID medication. A structured questionnaire was administered to all participants including information on age, sex, educational status, pathology being treated with NSAID, presence of gastrointestinal ulcer or related complications, duration of NSAIDs therapy, type of drug used, and gastro protection therapy. Almost all participants (N=599) received NSAIDs to treat rheumatic and/or musculoskeletal disorders. Of these, 475 individuals were on chronic therapy with high daily doses of NSAIDs. Concomitant gastro protective therapy was found in 184 individuals (30% of the overall sample). Women and the more educated individuals received more gastro protection than men and the low educated counterparts, respectively (33.4% in women vs 25% in men; 47% in highly educated vs 18% in low educated). Appropriate use of gastro protective therapy for NSAID users needs to be promptly implemented in Albania, as its inappropriate use raises ethical and economic concerns. Prescriptions should follow clear guidelines for prevention of gastrointestinal damage following NSAIDs therapy among persons at high risk.

LETTER TO THE EDITOR

IMMUNOMODULATING ACTIVITY OF PIDOTIMOD IN
CHILDREN WITH DOWN SYNDROME

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Acute respiratory tract infections (ARTIs) are the most frequent illnesses in pediatric age, frequently experienced in children with Down Syndrome (DS) due to the associated immune defects of both specific and non-specific immunity. Pidotimod, a synthetic immunostimulant, was shown to reduce the rates of ARTIs in children with DS, however the mechanisms associated with this effect is currently unknown. We analyzed immune parameters in DS children who received the seasonal 2011–2012 virosomal-adjuvanted influenza vaccine. Eighteen children aged 3–10 years (mean age 7.1±2.6 years) were randomly assigned (1:1 ratio) to receive Pidotimod 400 mg, administered orally once a day for 90 days or placebo. At the recruitment (T0) all children received a single dose of virosomal-adjuvanted influenza vaccine (Flu). Blood samples were collected at T0 and 3 months after the recruitment (T3) in order to evaluate innate and adaptative immune responses pathway. Flu-specific IgG1 and IgG3 levels in plasma samples were determined at pre-vaccination (T0), and 1 (T1) and 3 months (T3) post-vaccination. The use of Pidotimod was associated with the upregulation of a number of genes involved in the activation of innate immune responses and in antimicrobial activity. Interestingly the ratio of Flu-specific IgG1/IgG3 was skewed in pidotimod-treated individuals, suggesting a preferential activation of complement-dependent effector mechanisms. Although preliminary these data suggest that Pidotimod can potentiate the beneficial effect of immunization, possibly resulting in a stronger activity of both innate and adaptive immune responses.

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LETTER TO THE EDITOR

INTERACTIONS AMONG PRO-INFLAMMATORY CYTOKINES, IGF SYSTEM AND THYROID FUNCTION IN PRE-PUBERTAL OBESE SUBJECTS

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On behalf of the Italian study groups on growth, obesity, and thyroid disorders in childhood

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Obesity is a state of chronic inflammation. Data on IGF system are often discrepant, and their relationships with mediators of inflammation are unknown. Furthermore, changes in thyroid function have been reported. We aimed at investigating the changes in these systems, and verify any relationships among cytokines, IGF system, thyroid function and insulin-insensitivity. Fifty obese pre-pubertal children, and 55 normal-weight subjects comparable for age and sex were enrolled. Serum IGF-I, IGF-II, IGFBP-1, IGFBP-2, IGFBP-3, IL-6 and TNF- α were assayed. In obese children insulin, TSH and FT4 were measured also, and the HOMA-IR index was calculated. Increased IGF-II, IL-6 and TNF- α , and decreased IGFBP-1 and IGFBP-2 concentrations were found in obese compared to normal-weight children. The IGF-I/IGFBP-3 molar ratio was also reduced in the obese subjects. In the obese children with high HOMA-IR index, IGFBP-1 and -2 serum concentrations were significantly decreased compared with those with normal insulin sensitivity, and in the obese subjects with increased TSH, IGFBP-2 concentrations were lower, and IGFBP-3 levels were higher compared to their counterparts with normal TSH levels. Among the significant correlations, BMISDS was correlated with IGF-II, and TSH. IGF-II concentrations showed a positive relationship with IL-6. TSH was correlated with IGFBP-2 also. The data showed interactions among IL-6, IGF system, insulin sensitivity, and thyroid function with changes being related to the degree of obesity. Chronic inflammation in obese children was confirmed. Some of the changes in the IGF system could be a consequence of insulin resistance and could account also for later complications in obese subjects.

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LETTER TO THE EDITOR

**INFLUENCE OF NIGHT-SHIFT AND NAPPING AT WORK ON URINARY MELATONIN,
17- β -ESTRADIOL AND CLOCK GENE EXPRESSION IN
PREMENOPAUSAL NURSES**

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Night-workers experience disruption of the sleep-wake cycle and light at night which may increase breast cancer risk by suppressing the nocturnal melatonin surge, resulting in higher levels of circulating estrogens. Night-work may also deregulate peripheral clock genes which have been found to be altered in breast cancer. This study investigated urinary 6-sulfatoxymelatonin (aMT6s), serum 17- β -estradiol levels in premenopausal shift nurses at the end of the night-shift compared to a control group of daytime nurses. Peripheral clock gene expression in lymphocytes were also investigated. All participants were sampled in the follicular phase of the menstrual cycle. The effect of nurses' ability to take a short nap during the night-shift was also explored. The shift-work group had significantly lower aMT6s levels than daytime nurses independently of a nap. Night-shift napping significantly influences 17- β -estradiol levels resulting in higher outcomes in nurses who do not take a nap compared to napping group and daytime workers. Peripheral clock genes expression investigated was not significantly different among the groups. Our findings suggest that shift nurses experience changes in aMT6s levels after a night-shift. Napping habits influence 17- β -estradiol levels at the end of a night-shift. These findings might be related to the increased cancer risk reported in night-shift workers and suggest that a short nap during night-shifts may exert a positive effect.

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LETTER TO THE EDITOR

**ANTIPROLIFERATIVE EFFECTS OF 5-FLUOROURACIL AND OXALIPLATIN
IN COLON CANCER CELL LINES: COMPARISON OF THREE DIFFERENT
CYTOTOXICITY ASSAYS**

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Adjuvant therapy in colorectal cancer has evolved to become the standard of care, whereas the tumor capability of activating effective mechanisms of defence against both chemical and physical cytotoxic agents represents a serious obstacle to the successful therapy of human tumors. Therefore, the possibility to have an assay useful to measure the drug sensitivity of tumor cells has a great importance. A number of cytotoxicity assays are currently available, each of them using a specific approach to detect different aspects of cell viability, such as cell integrity, proliferation and metabolic functions. The purpose of this study is to compare, under identical experimental conditions, three common cytotoxicity assays (ATP-lite, MTT and CCK-8 assays) in the assessment of the anti-proliferative effects of 5-fluorouracil (5-FU) and oxaliplatin (OHP) on three colon cancer cell lines (WiDr, SW620 and HT-29). Regarding 5-FU, the three assays were found to be significantly correlated with a moderate or high correlation coefficient, whereas in the case of OHP we found different outcomes among the assays. Our study demonstrates that the CCK-8 is the most sensitive assay for detecting changes of cell viability, suggesting that the viability measured in cells after drug exposure depends on several parameters like the drug used, the biological characteristics of the target cell and the specific approach employed by the method to detect distinct cell growth and metabolic functions.

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