1. VEGF, substance P and stress, new aspects: a revisited study


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Mast cells play an essential role in diverse physiological and pathological processes, such as atherosclerosis, malignancy, asthma, pulmonary fibrosis and arthritis, directly interact with bacteria, and appear to play a vital role in host defense against pathogens. Mast cells could be recruited in the inflammatory site, by MCP-1, RANTES and SCF, to selectively secrete proinflammatory molecules; these could include growth factors, histamine, which is mitogenic (H1) and an immunosuppressant (H2), neovascularization agents, such as heparin, IL-8, and VEGF, as well as proteases that could permit new blood vessel formation.

Neurogenic inflammation involves vasodilation and plasma protein extravasation in response to neural stimulation. Upon stimulation, sensory neurons release Substance P and other neuropeptides and activate neurokinin-1 receptors leading to plasma protein extravasation from post-capillary venules. Substance P is a neuropeptide that is released from nerve endings in many tissues and plays an important role in immunological and inflammatory states, and it is also a mediator of tissue injury, asthma, arthritis, allergy and autoimmune diseases. SP-positive nerve fibers and mast cell contacts are increased by acute stress in mice leading to dermal mast cell degranulation. VEGF is produced by inflammatory cells. IL-33 is the newest inflammatory member of the IL-1 cytokine family and we show here that SP can induce VEGF secretion from mast cells and IL-33 augments the effect of SP in VEGF transcription and translation protein. J Biol Reg Homeost Ag 2010; 24: 229-237.

2. Tissue infiltrating lymphocytes: the role of cytokines in their growth and differentiation

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The second half of the XX century saw a continuous improvement in the understanding of
cellular immunology. The discovery of monoclonal antibodies permitted to identify several functional T-cell subpopulations, characterized by a specific pattern of cytokine secretion. According to their functions, cytokines have been divided into two main groups: pro- and anti-inflammatory. Cytokines are involved in several aspects of immunity and inflammation. Because of its importance in host defence, the cytokine system is redundant and therefore different cytokines may perform similar activities. Although cytokines and inflammatory processes have been studied widely in the peripheral blood, it is our opinion that the most important pathogenetic events occur at the tissue level, therefore the study of Tissue-infiltrating lymphocytes (TIL) is of foremost importance. In this review we therefore focus on the cytokine microenvironment; different local tissue cytokine-cocktails can modulate and regulate T-cell proliferation and differentiation. CD4+ T-cells are not characterized by irreversibly differentiated endpoints, but there is an evident plasticity of these cells with a large possibility of differentiation options. We will discuss the issue and give examples of the diseases where the study of TIL and their microenvironment are most significant, including tumors, primary immunodeficiencies, rheumatoid arthritis, inflammatory skin diseases and coronary disease. We also review the role of apoptosis and the environment of mucosal immunity. J Biol Reg Homeost Ag 2010; 24:239-249.

3. Current strategies for the treatment of autoimmune diseases

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Autoimmune disease therapy may be considered a puzzle under construction. Current treatments for autoimmune diseases include physical therapy, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, disease-modifying anti-inflammatory drugs (DMARDs), anticytokine therapies, inhibition of intracellular-signaling pathways, costimulation inhibition, biological inhibitors of T cell function, B-cell anergy and depletion, regulatory T cells, stem cell transplantation. New biologic drugs that target specific cells or cytokines involved in the early inflammatory response started because of their improved efficacy and limited toxicity. The hematopoietic stem cell transplantation represents a possible therapeutic strategy for autoimmune diseases resistant to available treatments. J Biol Reg Homeost Ag 2010; 24:251-259.

4. Meat-specific IgG and IgA antibodies coexist with IgE antibodies in sera from allergic patients: clinical association and modulation by exclusion diet

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IgE-mediated responses play a pivotal role in allergic patients with food intolerance.
However, the association of food-specific IgG and IgA antibodies with the clinical outcome of allergic patients is still a matter of controversy. In this study we investigate whether beef-specific IgG and IgA antibodies may coexist with beef-specific IgE antibodies in food-allergic patients and examined their clinical relevance in different allergic settings. Beef-specific IgE, IgG and IgA antibodies were determined by solid-phase enzymoimmunoassay (ELISA) in a population of allergic patients (N=125) classified into patients with asthma, skin disease or gastrointestinal disorders, as well as in control subjects (N=80). IgE antibodies specific for citric fruits, tomato, cow’s milk, chicken’s egg and wheat were also determined. Beef was the predominant allergenic food in the whole population, not only for IgE (57.6 percent; \(P\) less than 0.001), but also for IgG and IgA isotypes (53.6 percent and 34.0 percent, respectively, \(P\) less than 0.001). Beef-specific IgE, IgG and IgA antibodies increased significantly in sera from patients with asthma, gastrointestinal disorders and skin allergy compared to sera from control subjects (\(P\) less than 0.001). Remarkably, IgG and IgA isotypes were significantly detected, even in the absence of IgE, in the three allergic conditions. All allergic patients, including those showing only IgG and IgA antibodies, significantly ameliorated their symptoms, and their levels of beef-specific antibodies were considerably reduced in response to a cow meat exclusion diet. While patients with gastrointestinal or skin allergic diseases were capable of tolerating beef following an established period of diet exclusion, asthmatic patients experienced a relapse of symptoms and showed a considerable increase in IgE, IgG and IgA-specific antibodies when re-challenged with a beef-enriched diet. Thus, beef-specific IgG and IgA antibodies coexist with IgE antibodies in sera from allergic patients and are significantly associated with the clinical course of allergic disorders, particularly asthma. J Biol Reg Homeost Ag 2010; 24:261-271.

5. Specific inhibition of protein kinase C\(\beta\) expression by antisense RNA affects the activation of Jurkat T lymphoma cells

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Antisense RNA technology was employed to specifically inhibit the expression of the protein kinase Cbta (PKCbta) isoform in Jurkat cells, to explore its influence on the expression of surface antigens (CD69) and the cytokines interleukin-8 (IL-8), tumour necrosis factor (TNF)-alpha and beta, and to characterise its controversial involvement in the expression of IL-2 and its receptor (IL-2R). Transfection of cells with an antisense PKCbta construct (as-PKCbta-pREP3) significantly increased IL-2R/CD25 expression in phorbol 12-myristate 13-acetate (PMA)-stimulated as-PKCbta-pREP3 transfectants, in contrast to Jurkat cells transfected with a control as-PKCa-pREP3 plasmid. IL-2 production, in contrast, was strongly inhibited in both transfectant populations stimulated by PMA plus the calcium ionophore ionomycin. Three clones (asb1/asb2/asb3), selected from as-PKCbta-pREP3 transfectants, showed decreased PKCbta protein levels (40 percent, 50 percent and 60 percent, respectively, as determined by western blotting) and mRNA levels. The specific inhibition was confirmed in immunoblots for other PKC (alpha, delta, epsilon, gamma, theta, and lambda lambda/tau) isoforms and in immunoprecipitates from representative (c2/asb2) clones. Stimulation of PKCbta-depleted clones significantly increased CD25 expression but decreased IL-2 production (similarly to as-PKCbta-pREP3 transfectants) and IL-2 message levels. CD69 expression and IL-8 secretion were significantly decreased, but TNFbeta message levels were highly increased in asb2/asb3 clones, without affecting TNFalpha secretion. Analysis of the mitogen-activated protein kinase (MAP Kinase) signalling pathway
showed unaltered extracellular signal regulated kinase 1/2 (ERK1/2) and p38 phosphorylation but increased activation of c-Jun N-terminal kinase (JNK1) and its substrate, the transcription factor ATF-2 (activated transcription factor-2), which are involved in IL-2 gene expression. Our results revealed new PKCbeta functions, affecting CD69 expression and IL-8 production, and support the requirement for PKCbeta in IL-2 secretion/transcription and IL-2R regulation. 

6. **Leptin and its receptors in obese patients with colorectal cancer**

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The purpose of this study is to examine serum concentration of leptin and that of the soluble form, the Ob-Re receptor, in patients with colorectal cancer, as well as to examine the level of leptin mRNA and that of its receptors, Ob-Ra and Ob-Rb, in large intestine specimens collected from patients with colorectal cancer, depending on cancer clinical and pathological progression and BMI. A total of 146 patients with colorectal cancer in a I-IV stage scale according to the TNM Classification were enrolled. The patients were divided into two groups according to BMI calculations based on body weight and height: a Study group (BMI≥25 kg/m2) of 75 patients aged 57 ± 4.5 years and a Control group (20<BMI<25 kg/m2) of 71 patients aged 60 ± 5 years. The experimental part of the work was performed in two stages: Stage I regarding the assay of leptin concentration and that of its soluble receptor, Ob-Re, in the serum of patients with the use of the ELISA method; and Stage II to determine the number of leptin mRNA copies and two isoforms of leptin receptors, Ob-Ra and Ob-Rb, using the QRT-PCR method in tissue specimens collected from 146 patients. In our results the concentration of serum leptin and Ob-Re was not dependent on the stage of clinical and pathological progression of the cancer. There was a statistically significant higher serum leptin level in colon cancer patients who were overweight or obese compared to patients with normal weight. No presence of mRNA of the gene encoding leptin was found in tissues collected from colorectal cancer patients. The number of mRNA copies of Ob-Rb was statistically significantly higher in all the study groups compared to the reference tissues. 

7. **Guanosine protects human neuroblastoma cells from oxidative stress and toxicity induced by Amyloid-beta peptide oligomers**

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Amyloid-beta (Abeta) peptide aggregation forms such as soluble oligomers (O) have a causal role in neuronal dysfunction and death associated with Alzheimer’s Disease (AD). The main efforts for the development of neuroprotective drugs are therefore focused on preventing Abeta production, aggregation or downstream neurotoxic events. We therefore investigated...
the effect of guanosine (GUO), a guanine based purine, that exerts neurotrophic and neuroprotective effects. The GUO showed the ability to reduce neuronal death in terms of apoptosis, but not necrosis, elicited by Abeta1-42O in human neuroblastoma SH-SY5Y cells. The neuroprotective effect was recorded only when the GUO was added simultaneously to treatment of the SH-SY5Y cells with Abeta1-42O. By contrast, the GUO treatment of SH-SY5Y cells before and after the appearance of beta1-42O toxicity had no neuroprotective effects. The employment of specific inhibitors showed the involvement of neuronal survival pathways, such as PI3K–Akt and MAPK-ERK for the GUO anti-apoptotic effects observed. In parallel, the SH-SY5Y cells treated with GUO, in experimental conditions similar to those adopted to evaluate neuronal death, showed a marked decrease of the early reactive oxygen species formation induced by Abeta1-42O and pro-oxidant H2O2. In the same neuronal model, GUO was also shown to inhibit the extra- and intra-cellular Abeta1-42 release as well as the beta-secretase activity evoked by H2O2 pro-oxidant action. Based on these findings, GUO and other guanine based purines appear to be a promising class of compounds with neuroprotective properties that may play an important role in the therapy of AD. J Biol Reg Homeost Ag 2010; 24:297-306.

8. An experimental study on minimally occlusive laser-assisted vascular anastomosis in bypass surgery: the importance of temperature monitoring during laser welding procedures

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Laser welding has been proposed as an alternative technique to conventional stitching in microvascular anastomosis, with the advantages of improving the vascular healing process and reducing the risk of malfunction of a bypass. Our group recently proposed a laser-assisted end-to-side anastomotic technique, providing the advantages of laser welding and reducing the occlusion time of the recipient vessel, that is important in neurosurgical bypass procedures, in order to reduce the risk of cerebral ischemia. This in vivo study focuses on the control of the temperature dynamics developing in the welded tissue. A jugular vein graft was harvested and implanted on the rabbit carotid artery by means of two end-to-side anastomosis. Laser welding procedure was then carried out to implant the bypass. A real-time monitoring of the temperature during welding was performed with an infrared thermocamera, in order to control the laser-induced heating effect on the external surface of the vessel walls. The temperature analysis highlighted the dynamic of the heating effect in space and time and enabled us to define an optimal temperature range in operative conditions. The temperature control provided safe tissue heating confined within the directly irradiated area, with negligible damage to surrounding tissues, as well as effective sealing and welding of the vessel edges at the anastomotic sites. The average occlusion time of the carotid artery was about 11 minutes. After a follow-up of 30 days, all the bypasses were patent and no signs of thrombosis or leak point pressure were present, thus confirming the safety of this laser-assisted anastomotic procedure. J Biol Reg Homeost Ag 2010; 24:307-315.

9. Subclinical candiduria in patients with gastrointestinal malignancies: a preliminary study on the protective effect of a natural phytocompound

There is a great concern for the increasing incidence of candidiasis in cancer patients following immune-suppressive, cytostatic or antibiotic treatment. There are cancer patients with repeat asymptomatic recovery of candida in the urine in whom the choice of treatment, if any, is still a matter of debate. The aim of the study is to test the efficacy and tolerability of a natural anti-fungal phytocompound in patients with tumors of the gastrointestinal tract with prior or ongoing candiduria. Thirty-nine patients with operated gastrointestinal malignancies (18 still under current chemotherapy) with a history of repeated candiduria were enrolled. Eleven patients showed candiduria on enrolment and were treated with K-712, a natural antifungal phytocompound. Genomic analysis was carried out on blood samples of all patients on a monthly basis for 6 months. Within 3 weeks all 11 treated patients had negative cultures in the urine (10 patients after 2 weeks), 7 patients remained free of candiduria throughout the study period while 4 required a new treatment course. Three patients had positive genomic tests for systemic candidiasis and were treated with fluconazole. Eighteen (64%) out of the 28 patients who were free of candiduria on enrolment, developed a urinary candida infection during the 6-month follow-up and all cases were successfully treated with K-712. Seven (38%) of these cases presented a further recurrence at a later stage and all responded to a new course of K-172. No positive genomic tests were observed during the follow-up period. These data suggest that a consistent part of patients, mostly with gastrointestinal malignancies develop urinary candida infection when following chemotherapy treatment. A therapeutic approach with a natural antifungal phytocompound seems a safe and effective measure and a tentative prophylactic approach might also be envisaged. J Biol Reg Homeost Ag 2010; 24: 317-324.

10. Physical exercise activates the poly(ADP-ribosyl)ation system in rat testes

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Given the high sensitivity of the male reproductive system to oxidative stress and to temperature changes, the amount of germ cell apoptosis and the activation of the poly(ADP-ribosyl)ation system (a very sensitive index of genotoxic stress) were evaluated in the testicular tissue of adult rats which underwent a 10-wk treadmill training, according to either a mild or a strong protocol; rats were sacrificed 24 h after the last training session or after a single bout of an additional stressing exercise (30 min of swimming). Controls were untrained rats (one resting group and one group with acute exercise). Both training and acute exercise increased marginally germ cell apoptotic indexes (caspase-induced poly(ADP-ribose) polymerase fragmentation and TUNEL-positive cells), while the activity of poly (ADP-ribose) polymerase and poly(ADP-ribose) glycohydrolase enzymes was affected in a
way that suggests that acute exercise is associated with reversible genotoxic stress, and that training induces adaptive responses, as demonstrated by the activation of poly(ADP-ribose) polymerase system without subsequent increase in apoptosis. *J Biol Reg Homeost Ag* 2010; 24:325-334.

11. Allergen-specific Ig classes in non-allergic individuals

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Allergic rhinitis (AR) is characterized by Th2 polarized immune response, such as increased IL-4 and reduced IFN-γ production, and by a functional defect of T regulatory cells. This impaired immune response profile influences the pattern of immunoglobulin production in allergic patients. However, there is no study that has explored the pattern of allergen-specific Immunoglobulin (Ig) classes in normal subjects. Therefore, the aim of this study is to investigate the allergen-specific IgE, IgG, IgG4, and IgA serum level pattern in a group of non-allergic individuals. Forty healthy non-allergic subjects were enrolled. Serum allergen-specific IgE, IgG, IgG4, and IgA for mites, cat, Parietaria, grasses, and birch were quantitatively determined by ELISA method. Allergen-specific IgE, IgG, IgG4, and IgA serum levels were significantly different for each tested allergen (p=0.0001 for each class). In conclusion, the present study provides the first evidence that immunoglobulin production pattern depends on the specificity of the allergenic response in non-allergic subjects as well as in allergic patients. In addition, this study is the first that quantitatively evaluates the Ig classes. *J Biol Reg Homeost Ag* 2010; 24:335-340.

12. Circadian rhythmicity of lymphocyte subpopulations and relationship with neuro-endocrine system

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Lymphocyte subpopulations present circadian variation of some of their subsets, this variation may influence magnitude and expression of the immune responses and may be related to the variation of neuro-endocrine humoral factors. In our study cortisol, melatonin, TRH, TSH, FT4, GH, IGF1 and IL2 serum levels were measured and lymphocyte subpopulation analyses were performed on blood samples collected every four hours for 24 hours from 11 healthy male subjects aged 38-55 years. A clear circadian rhythm was validated for cortisol serum levels, CD8, CD16, TcRδ1 with acrophase in the morning and at noon, and for melatonin, TRH, TSH, GH, CD3, CD4, CD4/CD8 ratio, HLA-DR, CD20 and CD25 with acrophase at night. Changes of serum levels of FT4, IGF1 and IL2 did not show circadian rhythmicity. In the photoperiod (06.00-18.00h) and in the scotoperiod (18.00-06.00h) there were significant correlations among the lymphocyte subpopulations and humoral factors studied. The results show that specific lymphocyte subsets present
different profiles of nyctohemeral changes and different timed relationships with neuro-endocrine hormones. *J Biol Reg Homeost Ag* 2010; 24:341-350.

13. Relationship between asymmetric dimethylarginine and asymptomatic carotid atherosclerosis

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Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide (NO) associated with an increased risk of cardiovascular disease (CVD). In this study we assessed the relationship between ADMA and asymptomatic carotid intima-media thickness (CIMT). Eighty subjects underwent a complete history and physical examination, determination of serum chemistries and ADMA levels, and carotid ultrasound investigation (CUI). None of the subjects had symptoms of carotid atherosclerosis and nor were they taking any medication. Statistical analyses showed that high plasma levels of ADMA were positively correlated to CIMT ($p$ less than 0.001). Total cholesterol, low density lipoprotein cholesterol, triglycerides and C-reactive protein plasma concentrations were significantly associated with asymptomatic carotid atherosclerosis ($p$ less than 0.001). High serum concentrations of ADMA were associated with early carotid atherosclerotic lesions as measured by CIMT and represent a new marker of asymptomatic carotid atherosclerosis. *J Biol Reg Homeost Ag* 2010; 24:351-358.

14. Effects of cigarette smoke on salivary superoxide dismutase and glutathione peroxidase activity

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Cigarette smoke contains oxidants such as oxygen-free radicals and volatile aldehydes, which are probably the major causes of damage to biomolecules exposed to cigarette smoke. However, saliva has an antioxidant defense system able to counter toxic activities of radical species that is formed by antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The purpose of this study is to verify the possible effects of cigarette smoke on SOD and GSH-Px. Forty-four patients (25 males and 19 females) were enrolled in this study. The participants were 20 smokers (12 males and 8 females) and 24 non-smokers (13 males and 11 females). Furthermore, 10 subjects of the control group were ex-smokers (9 males and 1 female). Their mean age ± standard deviation (SD) was 58.8 ± 15.9 years for the case group and 73.8 ± 10.6 years for the control group. All patients were underwent a careful anamnestic investigation and examination of the oral cavity. After rinsing the mouth with water, each subject put 3 cc of non-stimulated saliva inside a test tube.
The saliva was centrifuged and oral peroxidase and superoxide dismutase activity was measured according to a specific assay. Statistical analysis was performed to evaluate differences between the groups and significant differences were observed for p < 0.05. A significant decrease of GSH-Px activity was detected in the smoking group (p< 0.05), while the SOD activity was similar in the control and case groups. According to the sex, a significant decrease of GSH-Px activity was noted in males of the smoker group (p<0.05), while in the sample of females no significant difference of the enzymatic activity was found. Moreover, among ex-smokers, there was a significant difference in the values of GSH-Px between those who had not smoked for less than ten years and those who had not smoked for more than ten years. Cigarette smoke may alter the detoxification of hydrogen peroxide through a decrease of GSH-Px activity. The overproduction of H2O2 may lead to an oxidative stress that is involved in a large number of diseases, including precancerous and neoplastic lesions of the oral cavity. The effects of cigarette smoke on salivary antioxidant enzymes decrease after withdrawal from smoking and the benefits become more evident with the passage of time. *J Biol Reg Homeost Ag* 2010; 24: 359-366.

15. *Chlamydia pneumoniae* infection as a risk factor for accelerated atherosclerosis in hemodialysis patients

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Atherosclerotic cardiovascular disease is the main cause of morbidity and mortality for end-stage renal disease patients undergoing chronic haemodialysis (HD). Several studies in recent years have identified *Chlamydia pneumoniae*, a respiratory pathogen, as risk factor for cardiovascular diseases in the general population. The aim of our study is to evaluate chlamydial load, in peripheral blood mononuclear cells (PBMC) of HD patients. Furthermore, the correlation between DNA chlamydial load and markers of inflammation was also examined. PBMC specimens isolated from 49 HD patients and 46 blood donors were analyzed for the presence of *C. pneumoniae* DNA by real-time PCR and *ompA* nested touchdown PCR. In HD patients, plasma levels of several inflammatory markers were also determined. A significantly higher rate of *C. pneumoniae* DNA was found in HD patients (44.9%) than in blood donors (19.6%) (p=0.016); HD patients were also more likely to have a significantly high chlamydial load (p=0.0004). HD patients with atherosclerotic cardiovascular diseases have a significantly greater chlamydial load than HD patients without cardiovascular diseases (p= 0.006). A significantly higher value of C-reactive protein, IL-6 and advanced oxidative protein products was found in HD patients with a greater chlamydial load (p<0.05). Likewise, a significantly lower monocyte HLA-DR percentage (p=0.011) as well as a lower monocyte HLA-DR expression were found in such patients (p= 0.007). In conclusion, our results show that HD patients are at high risk of *C. pneumoniae* infection correlated with chronic inflammatory response which in turn can lead to accelerated atherosclerosis and other long-term clinical complications such as myocardial infarction and stroke. *J Biol Reg Homeost Ag* 2010; 24:367-375.
16. Influence of magnesium on fatty acids and their esters in isolated rat hepatocytes

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The aim of the study is to analyse the changes in the profile of fatty acids and their esters in rat hepatocytes that were incubated for 5 hours with different concentrations of MgCl2 (2 and 4mM) in hepatocyte culture medium. The methyl esters of fatty acids were identified with a GC-MS system included in the Hewlett-Packard quadrupolar mass spectrometer, coupled with a Hewlett-Packard 5890 gas chromatograph with an ionisation potential of 70 eV and recorded on a Vectra 386 computer. We observed differences in the amount of saturated, monounsaturated and polyunsaturated fatty acids among the examined samples. In the control sample, the largest component consisted of the pool of saturated and monounsaturated fatty acids. Analysing the changes in the profile of ester-bound fatty acids, we found statistically significant differences when 4 mM MgCl2 was presented. The amount of C18:2, C18:1b and C20:4a decreased in comparison with the control sample. J Biol Reg Homeost Ag 2010; 24:377-380.

17. Sodium iodide associated to salicylic acid in the topical management of chronic oral candidiasis: a randomized trial

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Candidiasis is a relevant problem in oral medicine practice. We compared the antimycotic activity of nystatin with a solution of sodium iodide associated to salicylic acid (SISA) in the topical management of chronic candidiasis. Consecutive patients affected by chronic candidiasis were randomly allocated to SISA (group A) or nystatin (group B). VAS and swab scores were recorded at the beginning and at the end of the study while the healing index was evaluated at the end of the study only. Data were analyzed by STATA 10 MP. Forty patients (20 male, 20 female) were randomized. SIAS was as effective as nystatin in affecting VAS (p>0.05) and swab score (p>0.05). A statistically significant reduction (p<0.05) of healing index was observed in both groups. No side effects were reported. SISA topical application, shows a comparable efficacy to the nystatin in the management of chronic oral candidiasis. Its use could represent an adequate alternative to the nystatin above all in the cases of drug-resistance. Further large scale randomized trials are warranted to confirm these preliminary findings. J Biol Reg Homeost Ag 2010; 24:381-384.