THE EFFECTS OF 1.3-1.6 BETA-GLUCAN (IMUNEKS®) IN VITRO PROLIFERATION OF LYMPHOCYTES IN TREATMENT OF RECURRENT APHTHOUS STOMATITIS

1.3-1.6 BETA-GLUCAN (IMUNEKS®) IN TREATMENT OF RECURRENT APHTHOUS STOMATITIS

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ABSTRACT

Purpose : The aim of this study was to investigate the effects of 1.3-1.6 Beta-glucan (Imuneks®) on the response of lymphocytes with and without phytohemagglutinin A (PHA) , a T lymphotocyte mitogens in treatment of Recurrent Aphthous Stomatitis (RAS).

Material and method: The study consisted of 37 patients with RAS and 42 healthy control group without RAS. Of the 37 patients with RAS, 27 patients were given 10 mg Imuneks® twice per day and 10 patients given a placebo twice per day. RAS, estimated by the Ulcer Severity Score (USS), shows ulcer characteristics (number, size, duration, ulcer-free period, site and pain). For the proliferation of in vitro lymphocytes, peripheral blood leukocytes cells from control and patients (PBL) isolated by means of density gradient centrifugation were suspendend in culture medium (RPMI 1640 and 10% FCS). The cells($10x10^5$ / 200 μL) were cultured in 96 – well round bottom plates at 37^oC in a 5%CO_2 atmosphere with culture medium and with culture medium/20μl PHA (10 μl/ml). Lymphocytes were cultured for 96 hours under the same conditions as described above. Proliferative responses were determined by means of ^3H thymidine incorporation (0.5μCi /200μL) over the last 18 hours of culture. The response of lymphocytes to PHA and USS values were recorded before the treatment, at the end of the treatment and once again 1 month after that. During this month following the end of treatment, the patients received no treatment. Lymphocytes without PHA were also recorded before the treatment in the 37 patients with RAS and 42 healthy control group without RAS.

Results: Proliferation of lymphocytes without PHA was significantly lower in the 37 patients with RAS (mean=24.89) when compared to the 42 healthy control group without RAS (mean=53.31), ($z=-5.492$ $p< 0.001$). Response of lymphocytes to PHA was significantly higher in the 27 patient study group when compared to the 10 patients in the placebo group ($z=-1.966$, $p< 0.05$). At the same time USS values were significantly lower in the 27 patients ($p<0.05$).

Conclusions: According to our results, we found that Imuneks® had an immunostimulator effect. We therefore believe that Imuneks® can be used in the treatment of patients with RAS.

Introduction

Recurrent aphthous somatitis (RAS), is a disorder characterized by recurring ulcers confined to the oral mucosa in patients with no other signs of disease (Epstein 2003,). It is among the most common oral mucosal lesions, with a prevalence of 10% to 30 % in the general population. Many specialists and investigors in oral medicine no longer consider RAS to be a single disease but rather several pathologic states with similar clinical manifestations. Immunologic disorders, hematologic deficiences and allergic or psychological abnormalities have all been implicated in causes of RAS. Minor aphthae are the most common form, and they present clinically as small, panful, round ulcers 3-6 mm in diameter, covered by a whitish-yellow membrane and surrounded by a thin red halo. The lesions may be single or multiple and they heal without scarring in 7-12 days. The fist episodes of RAS most frequently begin during the second decade of life and in individual patients may be precipitated by minor trauma, menstruation, upper respiratory infections, or contact with certain foods. The cause remains unclear. Several predisposing factors have been reported, such as trauma, allergy genetic predisposition, endocrine disturbances, emotional stress, hematological deficiencies and AIDS. There are many agents available for treatment of patients with RAS. For most of them local therapy is sufficient to control disease. Topical steroids can be use for treatment. In more severe cases the use of topical corticosteroid preparations is helpful in decreasing the healing time of the lesions. Preparations such as triamsinolone or fluacinolone may be applied topically to the lesions theree or four times daily (Laskaris, 1998). The aim of this study was to investigate the effects of 1.3-1.6 Beta-glucan (Imuneks®) on the response of lymphocytes with and without phytohemagglutinin A (PHA) , a T lymphotocyte mitogens in treatment of RAS.

Material and method:

Patient selection

The study consisted of 37 patients with RAS and 42 healthy control group without RAS. Of the 37 patients with RAS, 27 patients were given 10 mg Imuneks® twice per day and 10 patients given a placebo twice per day. RAS, estimated by the Ulcer Severity Score (USS), shows ulcer characteristics (number, size, duration, ulcer-free period, site and pain).

Lymphocytes proliferation investigation:

For the proliferation of in vitro lymphocytes, peripheral blood leukocytes cells from control and patients (PBL) isolated by means of density gradient centrifugation were suspendend in culture medium (RPMI 1640 and 10% FCS). The cells($10x10^5$ / 200 μL) were cultured in 96

– well round bottom plates at 37^{o}C in a 5%CO_2 atmosphere with culture medium and with culture medium/20µl PHA (10 µl/ml). Lymphocytes were cultured for 96 hours under the same conditions as described above. Proliferative responses were determined by means of ^{3}H thymidine incorporation (0.5µCi /200µL) over the last 18 hours of culture. The response of lymphocytes to PHA and USS values were recorded before the treatment, at the end of the treatment and once again 1 month after that. During this month following the end of treatment, the patients received no treatment. Lymphocytes without PHA were also recorded before the treatment in the 37 patients with RAS and 42 healthy control group without RAS.

Statistical Analysis

Results:

Proliferation of lymphocytes without PHA was significantly lower in the 37 patients with RAS (mean=24.89) when compared to the 42 healthy control group without RAS (mean=53.31), ($z=-5.492$ $p< 0.001$). Response of lymphocytes to PHA was significantly higher in the 27 patient study group when compared to the 10 patients in the placebo group ($z=-1.966$, $p< 0.05$). At the same time USS values were significantly lower in the 27 patients ($p<0.05$).

Discussion:

RAS is one of the most painful oral mucosal inflammatory ulcerative conditions and can cause pain on eating, swallowing and speaking (Miller and Ship 1977). Since the etiology of RAS remains unknown, and the cyclic nature of the disease makes it difficult to treat certain. Therefore there is no definitive treatment. Treatment is symptomatic, the goal being to decerase symptoms, reduce ulcer number and size, increase disease-free periods (Scully et al 2003) . According to our results, USS values were significantly lower in study group when compared the placebo group. Our treatment approach is in concordance with resarcher's approach.

The pathogenesis of RAS involves a predominantly cell-mediated immune response in which tumor necrosis factor α, or TNF α, plays a major role. A mononuclear (lymphocytic) cell infiltrate in the epitelhelium in the preulcerative stage is followed by a localized popular swelling due to keratinocyte vacuolation surrounded by a reactive erythemathous halo

representing vasculitis. The painful papule then ulcerates and a fibrinous membrane overs the ulcer, which is infiltrated mainly by neutrophils, lymphocytes and plasma cells (Natah et al 2000, Scully et al 2003).

The best treatment is that which will control ulcers for the longest period. The treatment approach should be determined by disease severity (pain), the patient's medical history, the frequency of flare-ups and the patient's ability to tolerate the medication.

Efficacy of beta-glucan being immune modulator has been investigated by various researchers and successful results have been reported (Liu and Balasubramanian 2001, Kournikakis et al 2003, Yun et al 2003). The immune modulator effect of beta-glucan was shown in several studies. Our study group includes 37 patients with RAS and their proliferation of lymphocytes without PHA was significantly lower when compared to the 42 healthy control group without RAS. It means study group has immunologic weakness. According to our results, response of lymphocytes to PHA was significantly higher in the 27 patient study group when compared to the 10 patients in the placebo group. In this study we found that the response of lymphocyte was significantly higher in study group when compared the placebo group.

Investigations using more sophisticated immune assays have suggested a role of lympohocytotoxicity, antibody-dependent cell mediated cytotocity and defects in lymphocyte cell populations of RAS patogenesis.

RAS is a common oral disorder of uncertain etiopathogenesis for which symptomatic therapy only is available. According to our results, USS values were significantly lower in study group when compared the placebo group. Therefore we suggested that Immuneks® had immunosimulator effect on RAS lession. beta-glucan can be alternatively use in the treatment of patients with RAS.

Conclusions:

According to our results, we found that Imuneks® had an immunostimulator effect. We therefore believe that Imuneks® can be used in the treatment of patients with RAS.

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