

SHORT COMMUNICATION

Anti-allergic Effect of Pleuran (β -glucan from *Pleurotus ostreatus*) in Children with Recurrent Respiratory Tract Infections

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Recurrent respiratory tract infections (RRTIs) present a very important problem in paediatric praxis. As true immunodeficiencies are rare, one of the most important factors assumed to contribute to increased respiratory morbidity is atopy. Several preparations of natural origin have been used for the prevention of RRTIs, and some of the most effective immunomodulators are biologically active polysaccharides – e.g. β -glucans. In our randomised, double-blind, placebo-controlled study, we investigated the prevalence of atopy in a group of children with RRTIs and the potential anti-allergic effect of pleuran (β -glucan isolated from *Pleurotus ostreatus*) on basic laboratory markers of allergic inflammation. We confirmed that atopy may be an important factor contributing to the increased respiratory morbidity in children with RRTIs. The active treatment with pleuran resulted in a significant reduction of peripheral blood eosinophilia and stabilised the levels of total IgE in serum. This was more evident in atopic subjects. Pleuran showed a potential anti-allergic effect. This previously non-described effect could expand the application of this natural immunomodulator also as a complementary adjuvant therapy in allergic patients. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: allergic diseases; β -glucans; biologically active polysaccharides; natural immunomodulator; Pleuran; Recurrent respiratory tract infections.

INTRODUCTION

Recurrent respiratory tract infections (RRTIs) present a very frequent and important problem in paediatric praxis. According to epidemiological studies, approximately 6% of children younger than 6 years of age suffer from RRTIs. Physicians have to determine whether the high morbidity caused by respiratory infections in normal children is related to the physiological immaturity of the immune system (De Martino and Balloti, 2007) or to some another condition. In these children, usually only mild deviations in immune system parameters could be detected, as true immunodeficiencies are rare reasons for common RRTIs. Respiratory allergy, gastroesophageal reflux or ENT focal infections (e.g. adenoid hypertrophy) are the most important causes of RRTIs. Several preparations of natural origin have been used for the prevention of RRTIs, but only a few have scientific evidence for real clinical efficacy. Some of the most important immunomodulators of natural origin which are successfully used in the prevention of RRTIs are biologically active polysaccharides – as e.g. β -glucans. β -glucans are a group of biologically active polysaccharides of natural origin with a proven pleiotropic

immunomodulation effect. There are several known natural sources of β -glucans, such as fungi (e.g. pleuran, lentinan, grifolan), bacteria (e.g. curdlan), moulds and yeasts (e.g. yeast glucan, zymosan), seaweed (e.g. laminaran) and certain cereals. The most effective one with the highest immunomodulation activity are β -glucans from fungi. Their activity on immune system is based on the branched molecular structure (linear $\beta(1 \rightarrow 3)$ and lateral $\beta(1 \rightarrow 6)$ glycosidic linkages). The mechanism of their action in the organism is mediated through several receptors, especially the Dectin-1 receptors, Toll-like receptors (TLR-2, 4, 6), complement receptor 3, scavenger receptor and lactosylceramid. The most important is the Dectin-1 receptor expressed on many immune cells. After the binding of β -glucan to this receptor, it stimulates production of many cytokines or other mechanisms of immune and non-immune reactions. β -glucans are typical modifiers of biological activity in both directions: positive (immunostimulation) and also negative (immunosuppression) (Jesenak *et al.*, 2012). Pleuran (β -glucan isolated from *Pleurotus ostreatus* (Jacq.) P. Kumm.) possesses confirmed efficacy in preventing RRTIs through open and also double-blind, placebo-controlled studies (Jesenak *et al.*, 2010; Jesenak *et al.*, 2013).

As atopy is an important contributing factor to RRTIs in children [5,6], we investigated the potential anti-allergic effect of pleuran on basic laboratory markers of allergic inflammation.

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MATERIALS AND METHODS

This randomised, double-blind, placebo-controlled study enrolled children ($n=175$) with a history of RRTIs. Children between the age of 2 and 5 years and between the age of 6 and 10 years were included who had been diagnosed with >5 and >3 respiratory infections each year, respectively. The study was approved by the local Ethical Committee. The parents or legal guardians of the children signed consent forms. The study was performed according to the Declaration of Helsinki.

Children were randomised into two different treatment groups, which received either Imunoglukan P4H[®] syrup ($n=81$) or a placebo (vitamin C; $n=94$). Children that met the inclusion criteria were required to take 1 ml per 5 kg of Imunoglukan P4H[®] syrup (10 mg of pleuran and 10 mg of vitamin C in 1 mL of syrup) or placebo (10 mg of vitamin C in 1 mL of syrup) every morning on an empty stomach for 6 months. The active substance within this natural product was previously isolated, identified and chemically characterised by Karacsonyi and Kuniak (1994). Subjects were examined before the administration of Imunoglukan P4H[®] or placebo (V1), 6 months after the administration (V2), and after further 6 months without treatment (V3). At the end of each period, venous blood samples were collected. Patients were monitored using basic laboratory parameters for allergy, which included total IgE, specific IgE antibodies against a standardised panel of inhalant and food allergens and blood eosinophil cell counts (BEC). White and red components of the blood were examined by sampling with an 18-parameter haematological analyser Bayer Advia 60 (Siemens AG, Munich, Germany) using the company's reagents. IgE antibodies specific to common aeroallergens were measured by performing a solid-phase immunoassay (Frima, Stat). The levels ≥ 0.35 IU/ml were considered positive and indicated the atopic status of the examined subjects.

The results are presented as mean values $\pm$ standard deviation. Data were analysed with the software package SPSS version 9.0 (SPSS Inc. Chicago, IL, USA). Student's two-tailed *t*-test, Student's paired test and the chi-squared test were used for statistical comparisons of the parametric data. Non-parametric data were analysed using Mann-Whitney test and Fisher's exact test. *P* values lower than 0.05 were considered statistically significant.

RESULTS

The demographic characteristics of the studied population are presented in Table 1. The two groups of children did not differ according to gender, age and number of upper respiratory tract infections in the previous 12 months, proportion of atopic children, total IgE and blood eosinophil counts in peripheral blood. Atopy was detected in 43.4% of the studied subjects. The treatment was well tolerated, and we did not find any significant adverse effects related to the treatment.

Active treatment with Imunoglukan P4H[®] resulted in significant changes in general markers of allergy in subjects from the active group compared to those treated with placebo. Whereas the level of total IgE in serum remained stable across the whole study in the active group, in the placebo group, the levels of total IgE raised gradually from V1 to V3 (Table 2). When we divided the children into subgroups according to their atopic status, total IgE was stabilised in atopic children treated with the active preparation, whereas in atopic subjects from the placebo group, increase was evident (Table 2). The blood eosinophil count decreased significantly as a result of active treatment (V3 vs. V2), particularly among atopic subjects, but did not change in the placebo group (Table 2).

DISCUSSION

In our study, we confirmed that atopy may be important factor contributing to increased respiratory morbidity in children with RRTIs. Active treatment with pleuran resulted in a significant reduction of peripheral blood eosinophilia and stabilised the levels of total IgE in serum.

It was shown that RRTIs in childhood did not reduce the risk of atopic disease in young adulthood (Balemans *et al.*, 2006), but atopy may be important predisposing factor contributing to increased respiratory infections in children (Dellepiane *et al.*, 2009). Indeed, allergic children have more numerous and severe respiratory tract infections than non-allergic children (Ciprandi *et al.*, 2006). The prevalence of atopy in the studied group was more than 40%. According to our previous study of the general population of unselected children from the same geographical settings, the prevalence of atopy was only 21% (Ronchetti *et al.*, 2007). In other

Table 1. Demographic characteristics of the studied populations.

Parameter	Active group	Placebo group	<i>P</i>
Number	94	81	-
Males	54 (57.4%)	43 (53.1%)	n.s.
Age [years]	5.78 $\pm$ 2.53	5.51 $\pm$ 2.22	n.s.
Atopy	44 (46.8%)	32 (39.5%)	n.s.
Number of RTIs/12 months before the study	6.27 $\pm$ 2.10	6.86 $\pm$ 2.58	n.s.
Total IgE [IU/L]	84.05 $\pm$ 109.74	62.99 $\pm$ 101.47	n.s.
BEC [10^9 /L]	0.30 $\pm$ 0.24	0.24 $\pm$ 0.15	n.s.

BEC – blood eosinophil count, n.s. – non-significant, RTIs – respiratory tract infections

Table 2. The changes of basic markers of allergy during the study according to the atopic status of the subjects.

Parameter	Visit 1	Visit 2	Visit 3
Active pleuran group			
Total IgE [IU/L]	84.05 $\pm$ 109.74	93.27 $\pm$ 118.52	88.04 $\pm$ 116.34
BEC [10^9 /L]	0.30 $\pm$ 0.24	0.36 $\pm$ 0.78	0.25 $\pm$ 0.18 [†]
Atopic subjects			
Total IgE [IU/L]	100.89 $\pm$ 133.59	104.57 $\pm$ 117.39	108.05 $\pm$ 120.50
BEC [10^9 /L]	0.29 $\pm$ 0.24	0.41 $\pm$ 1.13	0.18 $\pm$ 0.11 ^{†††}
Non-atopic subjects			
Total IgE [IU/L]	71.57 $\pm$ 91.26	74.94 $\pm$ 88.44	60.82 $\pm$ 79.23
BEC [10^9 /L]	0.31 $\pm$ 0.26	0.31 $\pm$ 0.16	0.31 $\pm$ 0.21
Placebo group			
Total IgE [IU/L]	62.99 $\pm$ 101.47	86.87 $\pm$ 151.98*	100.66 $\pm$ 163.08*
BEC [10^9 /L]	0.24 $\pm$ 0.15	0.26 $\pm$ 1.18	0.26 $\pm$ 0.33
Atopic subjects			
Total IgE [IU/L]	50.12 $\pm$ 82.90	103.08 $\pm$ 188.61*	117.16 $\pm$ 215.58*
BEC [10^9 /L]	0.24 $\pm$ 0.15	0.26 $\pm$ 1.18	0.26 $\pm$ 0.33
Non-atopic subjects			
Total IgE [IU/L]	61.60 $\pm$ 80.72	66.63 $\pm$ 79.87	86.26 $\pm$ 105.98
BEC [10^9 /L]	0.24 $\pm$ 0.15	0.26 $\pm$ 0.18	0.26 $\pm$ 0.39

BEC – blood eosinophil count

Comparison among the three visits:

Visit 2 vs. Visit 1: * $P < 0.05$; Visit 3 vs. Visit 1: † $P < 0.05$; Visit 3 vs. Visit 2: †† $P < 0.05$, ††† $P < 0.001$.

studies, atopy was detected in almost half of all children enrolled in the study (Dellepiane *et al.*, 2009), while the presence of underlying immunological diseases was relatively uncommon and immune defects were mostly mild (Litzman *et al.*, 1999). Therefore, it can be suggested that these atopic children with RRTIs could benefit from anti-allergic treatment.

β -glucans are a group of biologically active polysaccharides of natural origin with a proven pleiotropic immunomodulatory effect. Based on *in vitro* experiments, and also on animal and human clinical studies, the anti-allergic potential of β -glucans was suggested (Jesenak *et al.*, 2012). In our study, the application of pleuran stabilised the levels of total IgE in serum. It was shown that total IgE is associated with a risk of the development of bronchial asthma and other allergic diseases (Burrows *et al.*, 1989) and that the symptomatic allergic patients have higher levels of total and specific IgE in their serum (Pastorello *et al.*, 1995). The effect of β -glucans on total IgE levels has not been previously reported. Application of β -glucan (imunoglukan) in a group of asthmatic patients yielded an increased production of anti-allergic tolerogenic cytokine interleukin 10 (Sarinho *et al.*, 2009). We also observed the effect of pleuran on the peripheral blood eosinophilia. The effect was observed with some delay after the end of the active treatment what can be attributed to the continuing gradual effect of the pleuran. It was shown that the full immunomodulation effect of β -glucans can

be achieved after the treatment for several months (Jesenak *et al.*, 2012). The dose-dependent reduction of eosinophilia in peripheral blood and bronchoalveolar lavage fluid was observed after the application of β -glucan in an animal model of asthma, and this effect was comparable to dexamethasone (Ku *et al.*, 2012). Peripheral blood eosinophilia is strongly associated with airway inflammation in asthmatic children (Barreto *et al.*, 2005), and the changes in total cells and differential counts of peripheral blood, especially in eosinophils, have been regarded as a valuable index in the efficacy test of anti-asthmatic agents (Maeda *et al.*, 1995).

In conclusion, pleuran showed a potential suppressive effect on the markers of allergic inflammation in peripheral blood, especially in atopic subjects. This previously undescribed effect could expand the application of this natural immunomodulator also as a complementary adjuvant therapy in allergic patients.

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Conflict of Interest

There is no conflict of interest in any of the authors

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