

Monitoring antioxidant enzymes in red cells during allergen immunotherapy

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The aim of this report was to answer the question how specific immunotherapy influences the antioxidant enzyme system in patients with respiratory allergy and in longer perspective to find markers suitable to assess the efficacy of treatment. In open prospective randomised study 28 patients (18 females and 10 males, age 14 – 48 years) with seasonal respiratory allergy were treated with allergen immunotherapy. Subjects received subcutaneous therapy with allergens absorbed on calcium phosphate or aluminium hydroxide and were analyzed by the established protocol at the beginning, after three and 12 month of the treatment. In all treatment group red cell superoxide dismutase and glutathione peroxidase activities were in the normal range in allergic patients both before and during the treatment. Catalase activity in the allergic patients was lower as compared with controls and a significant increase of the enzyme activity occurred during and at the end of the treatment. In patients treated with calcium phosphate adsorbed allergen there was a continuous increase of catalase activity from beginning up to the end of observation. In the case of the aluminium hydroxide treatment there was an increase from the baseline values up in the third month of the treatment and a decrease on the 12th month. In summary, the present results open the question that allergen immunotherapy may cause imbalance of oxidants and antioxidants. To support our findings larger controlled field studies are needed.

Key words: Allergen immunotherapy, Erythrocytes, Superoxide dismutase, Glutathion peroxidase, Catalase.

Imbalance between reactive oxygen species production and the activity of antioxidant systems (termed as oxidative stress) plays an important role in the pathogenesis of allergic disorders, especially in respiratory allergy (1, 6, 7). Most observations suggest its unambiguous role in pathogenesis of asthma, but in allergic rhinitis there is a paucity of data.

Allergen immunotherapy (AIT) is the only treatment that may affect the natural course of allergic diseases, and it also can prevent the development of asthma in patients with allergic rhinitis. AIT is application of gradually increasing quantities of an allergen extract to allergic subjects to ameliorate the symptoms associated with the subsequent exposure to the causative allergen. Monitoring the effectiveness of immunotherapy with inhalant allergens is based on the clinical response and the reduction of pharmacological treatment. Exact markers (*in vitro* or *in vivo*) to assess the efficacy of treatment are not available.

The main purpose of this preliminary study was to answer the question how does specific immunotherapy influence antioxidant enzyme activity in patients with allergic rhinitis and in longer perspective to find markers suitable to assess the efficacy of treatment.

Patients and methods

Patients.—28 patients, 18 females and 10 males in age from 14 to 48 (mean 29.4 ± 10.1 years) with pollen caused allergy were enrolled in this study. Eighteen of them suffered from seasonal allergic rhinitis, 6 had seasonal allergic rhinoconjunctivitis and 4 of them suffered from seasonal allergic rhinitis with stable seasonal bronchial asthma. At the beginning of the study all patients were asymptomatic and

were not taking any drug to relieve their symptoms. During the whole pollen season only rescue medication was allowed. Patients with clinically manifest symptoms of bronchial asthma, atopic dermatitis or combination of these diagnoses were excluded. 8 healthy subjects (4 females, 4 men, age from 23 to 48 years, nonsmokers, without any allergic condition) served as controls.

All patients had pollen allergy diagnosed according to their history, clinical evaluation, positive skin prick tests and the diagnosis was confirmed by specific IgE assay positive for at least one principal allergen. Before specific allergen immunotherapy all of them were treated in outpatient unit at least one year by symptomatic treatment during the pollen season.

Treatment protocol.—Allergen immunotherapy started at the end of the pollen season (middle October) with standard initial treatment regimen lasting 15 – 17 weeks. Subjects received subcutaneous therapy with allergens processed by different methods – calcium phosphate adsorbed allergen (Group A) and aluminium hydroxide adsorbed allergen (Group B); 18 and 10 patients, respectively. The primary endpoint of the study was to find changes of antioxidant enzyme activities associated with allergen immunotherapy in general. Treatment regimen was arranged as a prospective open randomized study while we did not expected differences between the groups. After finding the differences analysis was performed retrospectively (unexpected second endpoint). After initial stage, therapy was continued with maintenance doses applied every 4 – 6 weeks for the whole period of study. Effectiveness of the therapy was evaluated by the visual analogue scale

according to Wewers and Lowe (11). Peripheral blood samples were taken outside the pollen season (start – middle October 2006, end of initial treatment – before the pollen season – January/February 2007, end of study – middle October 2007 – end of pollen season).

Biochemical assays.– Red cell antioxidant enzymes were measured from frozen hemolysates of washed red cells (Hb concentration 30 ± 3 g/l). The activity of glutathione peroxidase (GPX; EC 1.11.1.19) and superoxide dismutase (SOD; EC 1.15.1.1) was measured on Cobas Mira S analyzer (Roche, Switzerland) with Ransel and Ransod kits respectively (Randox, Great Britain) according to Paglia and Valentine (9) and Fitzgerald (3) in our modification (10). Results are expressed as units/g Hb.

The activity of catalase (CAT; EC 1.11.1.6) was measured on Specord M 40 spectrophotometer (Zeiss Jena, Germany) at 240 nm according to Lück (5). Results are expressed as units/g Hb.

Immunoglobulins A, G, M, total IgE were analyzed with Roche kits on Cobas Integra 800, IgG subclasses with The Binding Site kits (Great Britain) on Cobas Mira S analyzer and specific IgE with EAST Hycor kits on Hytec 288 (Hycor Biomedicals, Germany).

Statistical methods.– Statistical analysis was performed using STATSDIRECT (version 2.5.2) software package. In addition to basic descriptive statistic one way ANOVA, Tukey – Kramer multiple comparisons and Kruskal – Wallis tests were used.

Results

Total IgE levels were increased in the third month after beginning the therapy and had a tendency to return to the initial levels thereafter (Fig. 1). Concentrations of IgM, IgA, IgG and subclasses of IgG were in the normal range in all patients during the whole treatment period and did not show any significant changes (data not shown). According to the visual analogue scale (VAS) there was a significant attenuation of the allergic symptomatology (Fig. 1) as a result of treatment.

Red cell superoxide dismutase and glutathione peroxidase activities were in the normal range in allergic patients both before and during the treatment. Catalase activity in the allergic patients was lower as compared with controls and a significant increase of the enzyme activity occurred during and at the end of the treatment (Table I).

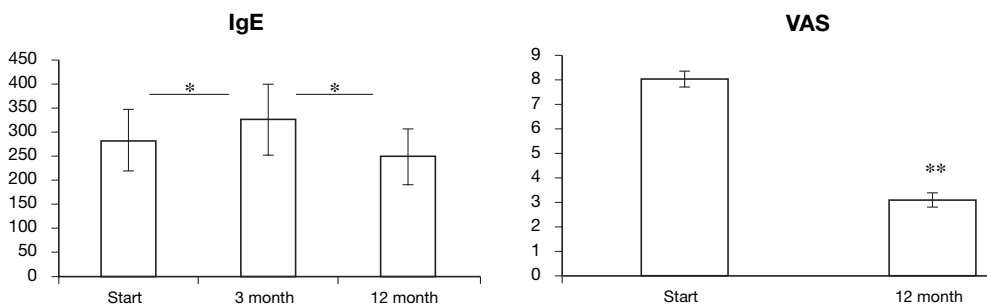


Fig. 1. IgE during therapy and visual analogue scale (VAS) after 12 months.

Values are expressed as mean $\pm$ SD; n=28; *p<0.05; **p < 0.01.

Specific immunotherapy was performed using allergens prepared by two different methods. From the results (Fig. 2), a different behaviour of catalase activity is evident. From this reason further statistical analysis was performed separately for treatment A and B respectively.

In patients treated with calcium phosphate adsorbed allergen there was a continuous increase of catalase activity from beginning up to the end of observation ($p = 0.0124$). In the case of the other treatment there was a significant increase from the baseline values up to the end of the initial stage of the treatment ($p = 0.007$) and a marginally significant decrease thereafter ($p = 0.0469$). On the other side there

were no differences between the two treatment groups throughout the study.

Discussion

In the present study we observed an increase in catalase activity after one year treatment with specific allergen immunotherapy in patients with seasonal allergic rhinitis.

In the allergic diseases, an imbalance between reactive oxygen species and antioxidant system activity has been postulated (7) and the authors analysed the possible associations between the changes of antioxidant system activity and the function of immune cells in allergic inflammatory diseases.

MATÉS *et al.* (6) found a nonsignificant increase of red cell catalase and significant increase of glutathione peroxidase and both forms of superoxide dismutase in symptomatic allergic patients (rhinitis, asthma). In our study we were not able to confirm these findings (normal superoxide dismutase and glutathione peroxidase activities, decreased catalase at baseline). The differences could be explained by different patient populations. In their study patients with pollen or house dust mite allergy were included. Another possibility of discordant results of both studies might be in different method used for red cell enzyme assays. The assays of both catalase and glutathione peroxidase used in two

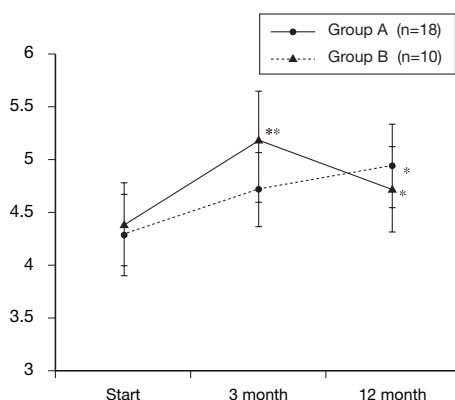


Fig. 2. Different profiles of CAT activity (units / g Hb) of red cells between two treatment groups. Group A = calcium phosphate adsorbed allergen, Group B = aluminium hydroxide adsorbed allergen (mean $\pm$ SD, * $p < 0.05$, ** $p < 0.01$).

Table I. GPX, CAT and SOD activity (units/g Hb) in red cells during allergen immunotherapy. (* $p < 0.01$ vs. control).

	GPX ($\pm$ SD)	CAT ($\pm$ SD)	SOD ($\pm$ SD)
Control (n=8)	37.78 $\pm$ 11.09	5.25 $\pm$ 0.64	1128.25 $\pm$ 143.26
Start (n=28)	40.72 $\pm$ 5.92	4.31 $\pm$ 0.63*	1133.45 $\pm$ 185.4
3 months (n=28)	41.79 $\pm$ 6.71	4.89 $\pm$ 0.62*	1181.38 $\pm$ 173.16
12 months (n=28)	37.81 $\pm$ 7.43	4.86 $\pm$ 0.53*	1194.91 $\pm$ 170.29

studies were essentially the same. However, the Ransod assay for superoxide dismutase in our study is based on another principle than that used in the study of MATÉS *et al.* (6).

The interpretation of increase and decrease of oxidative stress markers in different pathological conditions is not straightforward. In contrast to the results of the present study and MATÉS (6) other authors (2) observed a decrease of SOD activity in patients with bronchial asthma and they postulated that the activity of enzyme may serve as a marker of disease severity. Similar decrease in platelet glutathione peroxidase in patients with asthma was reported by MISSO *et al.* (8). Those authors postulated that reduced enzyme activity in blood may reflect mechanisms associated with the pathogenesis and the severity of disease. According to our view, decreased catalase activity in our patients might have been caused by imbalance of oxidants and antioxidants at the end of pollen season and before the allergen immunotherapy started. The decrease could reflect the severity of allergic inflammation in patients passing the pollen season.

Despite small treatment groups we found statistically significant differences, which may suggest different action of both agents on antioxidant status. In the treatment group B, the active substance is bound to aluminium vehicle. The explanation of this phenomenon could be derived from the study of GÓMEZ *et al.* (4) who postulated in an experimental model that aluminium exposure promotes oxidative stress in the rat hippocampus. On the other side aluminium as adjuvant down-regulates allergen driven Th 2 cytokine response while Th 1 cytokines are unaffected (12).

Further understanding of the mechanisms involved in oxidative stress, as well

as their relationship to the immunological response, need further work in this area and larger controlled studies to assess the impact of other similar interventions.

N. LUKAN, O. RACZ, I. MOCNEJOVA y I. TKAC. *Actividad de enzimas antioxidantes en los eritrocitos durante la inmunoterapia con alérgenos*. J Physiol Biochem, **64** (2), 143-148, 2008.

El objetivo del presente estudio es estudiar si la inmunoterapia con alérgenos puede influir en la actividad de las enzimas antioxidantes en pacientes con alergia respiratorias y, a largo plazo, encontrar los marcadores para seguir la efectividad de la medicación.

Participaron en el estudio 28 pacientes (18 mujeres y 10 hombres de 14 a 48 años) con alergia estacional tratados con inmunoterapia con alérgenos. Los pacientes recibían por vía subcutánea la vacuna con los alérgenos, absorbidos en fosfato cálcico o en hidróxido aluminico. Cada uno de los grupos fue evaluado al comienzo de la medicación, después de la medicación inicial (tres meses) y tras un año de terapia, según el protocolo propuesto. En ambos grupos, la actividad superóxido dismutasa y glutatión peroxidasa en los eritrocitos presentaban valores normales, antes y durante la terapia. La actividad catalasa en los pacientes alérgicos fue inferior a la del grupo control, pero aumentó significativamente después de tres y doce meses de medicación. En el grupo de los pacientes tratados con inmunoterapia, con el alérgeno absorbido en fosfato cálcico, la actividad catalasa aumentó de manera continua desde el inicio de la terapia hasta el fin. En el grupo del hidróxido aluminico, la actividad catalasa aumentó significativamente hasta los primeros tres meses de terapia y posteriormente disminuyó hasta los 12 meses. En suma, los resultados indican que la propia inmunoterapia con alérgenos puede provocar modificación en la actividad de los sistemas antioxidantes y ser causa del desequilibrio por estrés oxidativo.

Palabras clave: Inmunoterapia con alérgenos, Eritrocitos, Superóxido dismutasa, Glutatión peroxidasa, Catalasa.

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