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House dust-mites: Effect on antioxidant enzyme activities

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Summary

Background:

House dust-mites are potent allergens of the indoor environment and are common inhabitants of houses worldwide. Free radicals are constantly produced by cells, mostly as reactive oxygen species. Once produced, free radicals are removed by antioxidant defenses, including the enzymes SOD, GPx, and CAT.

Material/Methods:

The aim was to describe the importance of the antioxidant enzymes SOD, GPx, and CAT co-acting in human cells against toxic reactive oxygen species and their relationship with pathophysiological processes in subjects who have dust-mites in their homes.

Results:

The activities of erythrocyte GPx and SOD in skin-test-positive (dust-mite-positive/negative) patients were significantly lower than those in dust-mite- and skin-test-negative controls ($p < 0.05$). Among the skin-test-positive patients, SOD activity was found to be lower in dust-mite-positive than in dust-mite-negative patients ($p < 0.05$). There was not a statistically significant difference between the CAT levels of skin-test-positive (dust-mite-positive/negative) patients and dust-mite- and skin-test-negative controls ($p > 0.05$).

Conclusions:

This study clearly shows that dust-mite depresses the activities of SOD, GPx, and, to a small extent, CAT, which influence cellular reducing capacity and consequently may increase asthma risk more than other allergens.

key words:

superoxide dismutase • glutathione peroxidase • catalase • dust-mite • allergy

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BACKGROUND

Dust-mites are tiny bugs which inhabit the indoors. Dust-mites feed on pet and human dander and their waste is a major cause of allergies as well as asthma. Dust-mites like warm, humid, and dusty areas, and bed pillows, mattresses, carpets, and furniture are excellent habitats. Cleaning these objects can make a real difference in the number of dust-mites inhabiting them. House-mite allergy is a hypersensitivity reaction to proteins excreted by dust-mites. These proteins, when inhaled, can cause fever and asthma as well as aggravating atopic dermatitis in susceptible individuals [1–5].

Cells continuously produce free radicals and reactive oxygen species (ROS) as part of their metabolic processes. These free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) as well as numerous non-enzymatic antioxidants [6–9]. The maintenance of energy homeostasis, as well as the detoxification process, requires high-intensity oxygen metabolism. The human being has a wide range of systems capable of generating ROS [1]. An effective antioxidant defense system is necessary to control ROS metabolism in the organism. This system includes the antioxidant enzymes, such as CAT, GPx, and CuZn-SOD. The activity of these enzymes is higher in the liver than in other tissues [10,11].

GPx's principal function is to protect against damage from endogenously formed hydroxyperoxides and to catalyze the reduction of hydroxyperoxides by glutathione. CAT is an enzyme present in the cells of plants, animals, and aerobic (oxygen-requiring) bacteria. It promotes the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, to water and molecular oxygen ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). CAT also uses hydrogen peroxide to oxidize toxins such as phenols, formic acid, formaldehyde, and alcohols ($\text{H}_2\text{O}_2 + \text{RH}_2 \rightarrow 2\text{H}_2\text{O} + \text{R}$).

SOD, another important metabolic enzyme, protects and revitalizes cells and reduces the rate of cell destruction. It neutralizes some of the most dangerous free radicals, the superoxide radicals, before they can wreak havoc on the body. In addition to protecting cells from destruction, SOD also helps our bodies utilize zinc, copper, and manganese. Studies are being conducted on the potential use of SOD in anti-aging treatment [6,12–17].

CAT, along with SOD and GPx, controls the levels of oxygen-derived free radicals in mammalian cells. Together, these molecules may function as a somatic oxidant defense and they are known to play an important role in the inactivation of many environmental mutagens.

In the present study we aimed to investigate the role of the antioxidant enzymes SOD, GPx, and CAT against toxic reactive oxygen species in human cells and their relationship with pathophysiological processes in subjects who have dust-mites in their houses. For this purpose we assessed antioxidant enzyme levels in the erythrocytes of subjects who have dust-mites in their houses.

MATERIAL AND METHODS

Patients

Patients with positive skin test results (with unknown reason) to at least one allergen were selected as our study group. The patients' houses were thoroughly searched for dust-mites and the patients were subsequently divided into two sub-groups:

DPAP: Thirty patients whose houses were positive for dust-mites (DP, i.e. dust-mites were detected in their houses) and the skin test for an allergic symptom was positive (AP, i.e. suffering from rhinitis and/or asthma, pollen or fungus sensitivity, with skin test positive to at least one of the allergens).

DNAP: Thirty patients whose houses were negative for dust-mites (DN), but the skin test for allergic symptom was positive (AP).

The subjects ranged in age from 6 to 75 years (DPAP mean: 30.13 ± 15.39 , DNAP mean: 32.36 ± 13.21). Healthy individuals with a mean age of 27.2 ± 6.82 years (non-smoking, without any known pathological conditions) and no dust-mites in their houses served as controls (group DNAN). The solutions used in enzymatic assays were prepared from deionized water. The reagents were of analytical grade.

Mite detection

Dust was taken from mattresses, carpets, sofas, and chairs using a vacuum cleaner. Samples were collected from the vacuum cleaner filters and were sent to the laboratory in small sealed plastic bags. Large particles in each sample were removed. Ten milliliters of 90% lactic acid were added to 200 mg of the dust in a Petri dish. Using a stereo-microscope, mites from the surface of the mixture were collected and mounted in Hoyer's solution for future identification.

Blood samples and Erythrocyte extraction

Venous peripheral blood samples were collected in EDTA-coated tubes and centrifuged at $800 \times g$ for 30 min through a density gradient. Erythrocytes were washed three times with isotonic saline. Centrifugation for each washing was at $1400 \times g$ at 4°C . Erythrocytes were then lysed with hypotonic shocking and different dilutions were used as hemolysate [18].

Determination of SOD, CAT, and GPx activity

SOD enzymatic activity was measured using the method described by Sun et al. [19]. The method is based on nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase, which is used as a superoxide generator. One unit of SOD activity is defined as the amount of protein that inhibits the rate of NBT reduction by 50% and the enzyme activity is expressed as U/mg protein.

CAT activity was measured using the method described by Luck [20]. One unit of CAT activity is defined as the amount of enzyme which liberates half of the peroxide oxygen to H_2O_2 solution in 100 s at 25°C . The enzyme activity is expressed as U/mg protein.

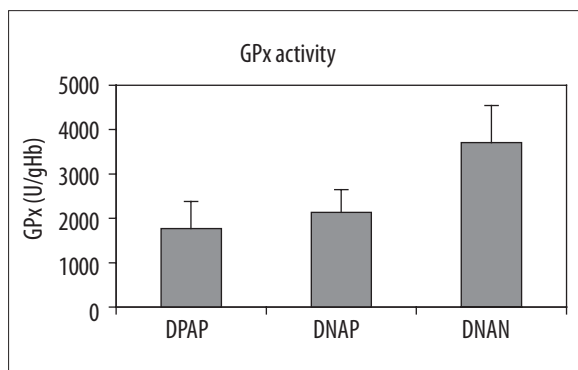


Figure 1. Variation in GPx activity in erythrocytes of patients suffering from allergic reaction to dust-mite or other allergen and controls. DPAP: dust-mite- & allergy-positive, DNAP: dust-mite-negative allergy-positive, DNAN: dust-mite- & allergy-negative.

GPx activity was determined according to Paglia and Valentina [21] using hydrogen peroxide as substrate. The reaction mixture contains 2.48 ml of a 50 mM phosphate buffer, pH 7 (Sigma), 0.01 ml of 112.5 mM sodium azide, and 4.6 U glutathione reductase (Type II, Sigma). The reaction was initiated by adding 0.1 ml of 2.2 mM H_2O_2 to the reaction mixture containing 500–1000 μ g protein. The change in the optical density was read at 340 nm for four min. The data were expressed as nmol NADPH oxidized to NADP/mg protein/min using the molar extinction coefficient of 6.200 at 340 nm. All data was corrected for the linear NADPH oxidation by H_2O_2 alone, in the absence of the enzyme protein, which was always <5% of the total reaction.

Protein assay

Protein concentrations were determined by the method described by Lowry [22]. Bovine serum albumin was used as the standard protein.

Hemoglobin concentration determination

Hemoglobin (Hb) concentrations were determined the method of Drabkin.

Statistical analysis

Statistical analysis was performed using the SPSS software package (Version 11.0 for Windows). Data were expressed as mean $\pm$ standard deviation (SD). For the comparison of two groups of continuous variables, independent samples *t*-test was used. A probability value of $p < 0.05$ indicated a statistically significant difference.

RESULTS

We used disease-free donors (both skin-test and dust-mite-count negative) as controls. Patients suffering from allergy were diagnosed with the disease and had not undergone any treatment prior to the time the blood samples were drawn. The erythrocytic GPx, CAT, and SOD activities, respectively, are presented in Figures 1, 2, and 3 for dust-mite-positive skin-test-positive (DPAP) and dust-mite-negative skin-test-positive (DNAP) patients and dust-mite-negative skin-test-negative (DNAN) controls.

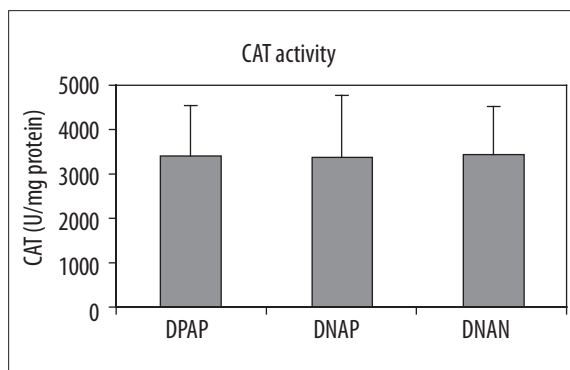


Figure 2. Variation in CAT activity in erythrocytes of patients suffering from allergic reaction to dust-mite or other allergen and controls. DPAP: dust-mite- & allergy-positive, DNAP: dust-mite-negative allergy-positive, DNAN: dust-mite- & allergy-negative.

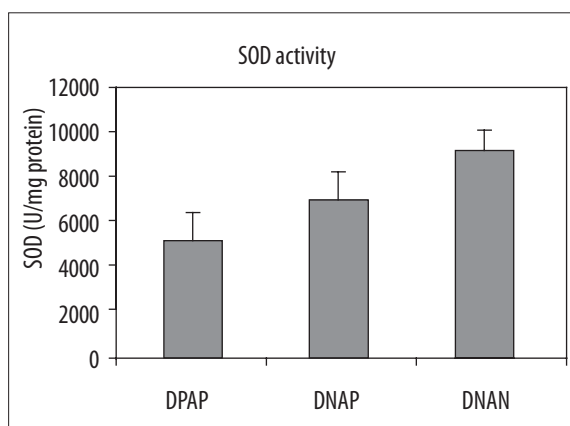


Figure 3. Variation in SOD activity in erythrocytes of patients suffering from allergic reaction to dust-mite or other allergen and controls. DPAP: dust-mite- & allergy-positive, DNAP: dust-mite-negative allergy-positive, DNAN: dust-mite- & allergy-negative.

The activities of erythrocyte GPx and SOD in skin-test-positive (dust-mite positive/negative) patients were significantly lower than in the dust-mite- and skin-test-negative controls ($p < 0.05$). Among the skin-test-positive patients, SOD activity was found to be lower in dust-mite-positive than in dust-mite-negative patients ($p < 0.05$). There was not a statistically significant difference between CAT levels of skin-test-positive (dust-mite positive/negative) patients and dust-mite- and skin-test-negative controls ($p > 0.05$).

DISCUSSION

House dust-mites are tiny (up to 0.3 mm) animals related to ticks and spiders and live in house dust. Although they are natural inhabitants of houses, some houses contain huge numbers of mites, whereas some contain almost none. This does not depend solely on cleaning habits, but also on the moisture of the environment; houses in very cold climates or on high mountains are less humid and have few mites, whereas houses in temperate climates and normal altitudes are more humid and apt to have more mites. House dust-mites feed on dander, which we continuously shed as our

skin replenishes. Dust-mites' droppings contain unused enzymes left over after digestion of the skin dander. These enzymes are, in fact, the most important cause of dust-mite-related asthma and other allergic diseases. House dust-mites and their droppings are reported to be the most important cause of asthma worldwide [1–5,23].

SOD, CAT, and GPx are important parts of the antioxidant defense system and they may be considered an enzyme family. Although these enzymes are present in almost every animal cell, their distribution among different cells is highly variable. Several factors abrogate the activity of the enzymes, some of which are internal individual factors resulting in significant variation in the enzyme activities of the different organs, age groups, and sexes. Endocrine regulation can also control enzyme activity, and environmental factors may also be important as they have a definite effect on enzyme action. CAT, which contains iron, SOD, which uses copper, zinc, and manganese, and GPx, some of which use selenium, all serve the purpose of terminating free-radical reactions in various components of the bodies' cellular structures. SOD, GPx, and CAT are important enzymes in the inactivation of many environmental toxic materials. They also act to protect cells from toxic oxygen [12–15].

ROS commonly exert a series of useful physiological effects. However, imbalance between pro-oxidant and antioxidant defenses in favor of pro-oxidants results in oxidative stress, associated with the oxidative modification of biomolecules such as lipids, proteins, and nucleic acids. Alone or in combination with primary etiological factors, free radicals are involved in the pathogenesis of more than a hundred diseases [6–9].

This study paid special attention to the relationship between allergic reaction due to the pathogenesis of dust-mite infection and immune-mediated inflammatory reaction (although the mechanism is unclear) and changes in the levels of antioxidant enzymes. The presented data show that GPx and SOD activities in erythrocytes were clearly lower in the patient groups than in the control group, indicating that GPx and SOD may be the principal antioxidant enzymes responsible for detoxification in dust-mite infection. In addition, interestingly, it seems that somehow dust-mite infection, rather than pollen, fungus, etc., was more decreased the activity of antioxidant enzymes of GPx and SOD. It is surprising that CAT activity did not vary among the two groups, and a possible explanation may be that CAT has much lower affinity and effectivity as an antioxidant enzyme in the detoxification mechanism.

As it is known, there are first-line defense antioxidants such as SOD, GPx, and CAT. SOD converts $O_2^{\bullet -}$ to H_2O_2 , which is further converted to H_2O with the help of GPx and CAT. SOD inhibits $\bullet OH$ production. SOD also acts as an antiproliferative agent and anticarcinogen (inhibitor at the initiation and promotion/transformation stage in carcinogenesis). GPx is another antioxidant enzyme which catalyses to convert H_2O_2 to H_2O .

Research applying molecular biology and biochemistry on human asthma and model systems of asthma over the last decade has revealed that numerous biologically active proinflammatory mediators lead to increased production of ROS [24]. The present study showed that concomitant

with increased generation of oxidative molecules in allergy was loss of protective antioxidant defense, specifically GPx and SOD, and partly CAT.

CONCLUSIONS

This study clearly showed that dust-mite contact depresses the activities of SOD, GPx, and, to a small extent, CAT, which influence cellular capacity and consequently may increase asthma risk. Therefore it may be concluded that SOD and GPx, and in part CAT, are linked to the anti-inflammatory effect and may have immunosuppressive properties. Additionally, it would be worth considering that the specific localization of SOD, CAT, and GPx and their rapid reaction suggest that antioxidant enzymes might also function as cell-signaling agents or regulators of cell signaling.

REFERENCES:

- Arlian LG, Morgan MS: Biology, ecology, and prevalence of dust-mites. *Immunol Allergy Clin North Am*, 2003; 23: 443–68
- Sheikh A, Hurwitz B: House dust-mite avoidance measures for perennial allergic rhinitis: a systematic review of efficacy. *Br J Gen Pract*, 2003; 53: 318–22
- Korsgaard J: House-dust-mites and asthma. A review on house-dust-mites as a domestic risk factor for mite asthma. *Allergy*, 1998; 53: 77–83
- Heinig JH, Mosbech H, Haugaard L: Diagnosis of house dust-mite allergy. *Allergy*, 1991; 46: 19–22
- Korsgaard J, Iversen M: Epidemiology of house dust-mite allergy. *Allergy*, 1991; 46: 14–18
- Djordjevic VB: Free radicals in cell biology. *Int Rev Cytol*, 2004; 237: 57–89
- Bergamini CM, Gambetti S, Dondi A, Cervellati C: Oxygen, reactive oxygen species and tissue damage. *Curr Pharm Des*, 2004; 10(14): 1611–26
- Bowler RP, Crapo JD: Oxidative stress in allergic respiratory diseases. *J Allergy Clin Immunol*, 2002; 110: 349–56
- Dworski R: Oxidant stress in asthma. *Thorax*, 2000; 55: 51–53
- Raychaudhury B, Banerjee S, Datta SC: Peroxisomal function is altered during leishmania infection. *Med Sci Monit*, 2003; 9(4): BR165–69
- Marczuk-Krynicka D, Hryniewicz T, Piatek J, Paluszak J: The effect of brief food withdrawal on the level of free radicals and other parameters of oxidative status in the liver. *Med Sci Monit*, 2003; 9(3): BR131–35
- Junqueira VB, Barros SB, Chan SS et al: Aging and oxidative stress. *Mol Aspects Med*, 2004; 25: 5–16
- Mates JM, Perez-Gomez C, Nunez de Castro I: Antioxidant enzymes and human diseases. *Clin Biochem*, 1999; 32: 595–603
- Fernandez V, Videla LA: Biochemical aspects of cellular antioxidant systems. *Biol Res*, 1996; 29: 177–82
- Irshad M, Chaudhuri PS: Oxidant-antioxidant system: role and significance in human body. *Indian J Exp Biol*, 2002; 40: 1233–39
- Cadenas E: Basic mechanisms of antioxidant activity. *Biofactors*, 1997; 6: 391–97
- Maier CM, Chan PH: Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. *Neuroscientist*, 2002; 8: 323–34
- Waterman MR: Spectral characterization of human hemoglobin and its derivatives. *Methods Enzymol*, 1978; 52: 456–63
- Sun Y, Oberley LW, Li Y: A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 1988; 34: 497–500
- Luck H: Catalase. *Methods of enzymatic analysis*. In: Bergmeyer H, ed., New York, Academic Press, 1965; 885–94
- Paglia DE, Valentina WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*, 1967; 70: 158–69
- Lowry O, Rosebrough NJ, Farr AL, Randal RJ: Protein measurements with the folin phenol reagent. *J Biol Chem*, 1986; 193: 265–75
- Arlian LG: Dust-mites: update on their allergens and control. *Curr Allergy Asthma Rep*, 2001; 1: 581–86
- Andreadis AA, Hazen SL, Comhair SA, Erzurum SC: Oxidative and nitrosative events in asthma. *Free Radic Biol Med*, 2003; 35: 213–25

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