

The Effect of Dust-Mites on Nitric Oxide Production

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Aim: We aimed to investigate the effect of house dust mite on the level of nitric oxide in patients who had dust mite in their houses and suffering from rhinitis and/or asthma and/or eczema-like allergic symptoms and were positive for skin tests (Prick).

Materials and Methods: The effect of dust-mites on nitric oxide concentration was investigated using Griess method in patients who had allergic symptoms and were positive for skin tests.

Results: The plasma nitric oxide level was found to be statistically higher in patients who had dust mite in their houses and suffering from allergic symptoms and were positive for skin tests than in patients who did not have dust mite in their houses but were positive for skin tests or negative for skin tests. In addition, there was no statistical difference in the level of nitric oxide between the patients who did not have dust mite in their houses but were positive for skin tests and the patients who did not have dust mite in their houses and were negative for skin tests.

Conclusions: There seems to be an effect of house dust mite on the level of nitric oxide. However, the molecular mechanism underneath of this effect is not yet clear. A plausible explanation for this might be the intrinsic radical properties of nitric oxide and its protective effect against the parasitic agents.

Key Words: Dust-mite, nitric oxide

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Ev Tozu Akarlarının Nitrik Oksit Üzerine Etkisi

Amaç: Bu çalışmada üst ve/veya alt solunum yolu semptomları astım ve/veya rinit ve/veya egzema gibi alerjik semptomları olan ve (Prick) alerjik deri testi pozitif bulunan hastaların evlerindeki ev tozu akarlarının bu hastalardaki nitrik oksit seviyelerine etkisi araştırıldı.

Yöntem ve Gereç: Alerjik semptomu ve alerjik deri testi pozitif olan 30 hastada ev tozu akarlarının bu hastalarda nitrik oksit seviyelerine etkisi Griess metodu ile araştırıldı.

Bulgular: Plazma nitrik oksit seviyeleri; alerjik semptomu ve alerjik deri testi pozitif olan evlerinde ev tozu akarları bulunan hastalarda, evlerinde ev tozu akarları bulunmayan alerjik deri testi pozitif ve alerjik deri testi negatif olan hastalardan istatistiksel olarak anlamlı derecede yüksek bulunmuştur. Evlerinde ev tozu akarları bulunmayan alerjik deri testi pozitif ve negatif olan iki ayrı hasta grubunun da ise nitrik oksit seviyeleri açısından istatistiksel olarak anlamlı bir fark bulunmamıştır.

Sonuç: Sonuçlar ev tozu akarlarının nitrik oksit üzerine alerjiden bağımsız olarak etkili olduğunu göstermiştir. Ancak parazitik enfeksiyonlarda nitrik oksitin rolünün ve bunun insan sağlığı ile olan klinik bağlantısının daha ileri çalışmalarla aydınlatılması gerekmektedir.

Anahtar Sözcükler: Ev tozu akarları, nitrik oksit

Introduction

Dust-mites are not insects but are more closely related to spiders and ticks. Due to their very small size, dust-mites are not visible to the naked eye. Though they live in many homes, only people who are positive for allergy skin tests know they are there. When dust-mites grow, they shed their skin. The shed skin and feces are what cause allergic symptoms in people. Allergic symptoms range from itchy noses and eyes to severe asthma attacks. In fact, house dust-mites and their droppings are the most important cause of asthma worldwide (1-3).

Nitric oxide (NO) is an essential mediator for a variety of biological functions, including defense against a range of pathogens. However, excessive production of NO, as a result of immunological stimulation, will lead to important immunopathologies. Therefore, the production of NO is necessarily under tight regulation. The regulatory

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mechanisms so far known to control NO synthesis include cytokines, feedback inhibition by NO itself, inhibition by pretreatment with glycoinositol-phospholipids, and up-regulation by lipophosphoglycan from the parasite (4).

NO is known to possess anti-parasitic effects on both protozoa and metazoa in vertebrate definitive and intermediate hosts (5). It is also implicated as an integral component of the host armament against invading parasites. In addition, several appreciated forms of interaction between NO and parasites are also important; including the role of NO as an immunosuppressive or tissue-destructive molecule during the course of infections, the regulation of antioxidant systems by host cell-derived NO, the contribution of NO to parasite stage conversion, the induction or suppression of macrophage iNOS by microbial products, and the existence of endogenous NO synthase pathways in certain parasites (6).

The past decade has witnessed a veritable explosion of interest in the simple molecule NO as a vasodilator, neurotransmitter, and antimicrobial agent. NO and other reactive nitrogen intermediates exhibit cytostatic or cytotoxic activity against a remarkable breadth of pathogenic microorganisms. It is known that mammalian cells, including human cells, produce nitric oxide both constitutively and inducibly in response to inflammatory stimuli (7).

In the present study, we aimed to investigate the effect of house dust mite on the level of nitric oxide in plasma. Subjects of this study were the patients who had dust mite in their houses and suffering from rhinitis and/or asthma and/or eczema-like allergic symptoms and were positive for skin tests (Prick). Comparisons were made with controls that were the patients who did not have dust mite in their houses but were positive for skin tests and the patients who did not have dust mite in their houses and were negative for skin tests.

Materials and Methods

Subjects

Non-smoking subjects who had rhinitis and/or asthma and/or eczema-like allergic symptoms were included the study. Patients who had any known systemic diseases, such as diabetes mellitus and chronic heart diseases, were excluded. Subjects' houses were thoroughly searched for dust-mites and the subjects were divided into 3 groups and each group consisted of 30 subjects:

Group 1. The patients who had dust mite in their houses and were positive for skin tests.

Group 2. The patients who did not have dust mite in their houses but were positive for skin tests.

Group 3. The patients who did not have dust mite in their houses and were negative for skin tests.

Although house dust mite is not present in tree pollens, grass pollens, microorganisms, animal proteins, and food samples, they were included in skin tests.

Subjects ranged in age from 6 to 75 (mean ages were 30.13 ± 15.39 , 32.36 ± 13.21 , and 27.2 ± 6.82 respectively).

Nitrite assay

All plasma samples were deproteinized before assay. Briefly; for every 200 μ l sample, 400 μ l of 0.5 N sodium hydroxide and 400 μ l of 10% zinc sulfate was added. The samples were then vortexed and centrifuged at 25,000 \times g for 5 min at 4 °C. Nitric oxide metabolites (nitrate and nitrite) were assayed by first reducing nitrate to nitrite. Nitrate reductase (0.05 unites/ml) along with reduced nicotinamide adenine dinucleotide (90 μ mol/l) and flavin adenine dinucleotide (3.12 μ mol/l) were added to each sample to convert nitrates to nitrites. Nitrite production was then determined with the spectrophotometric Griess reaction. For this study, 100 μ l of plasma or water blank was mixed with 50 μ l of 0.32 mol/l potassium phosphate pH 7.5 and 10 μ l of nitrate reductase with cofactors. To the sample mixture, 40 μ l of Griess reagent (10% sulfanilamide and 1% naphthylethylenediamine dihydrochloride in 85% phosphoric acid) was added. The mixture was incubated for 10 min at room temperature and the absorbance (550 nm) was read (8).

Mite-detection

Dusts were taken up from mattresses, carpets, sofas, and chairs using a vacuum cleaner. Samples were collected from vacuum cleaner filters and were sent to the laboratory in small sealed plastic bags. Large particles in each sample were removed. The dust samples were examined by the lactic acid precipitation method, which was modified from the Spieksma-Boezeman's method, as described briefly below (9).

Ten milliliters of 90% lactic acid were added to 200 mg of 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.

Statistical Analysis

Differences among groups' means were determined by the Student's *t*-test. All the data were expressed as mean \pm SD. A *P* value of less than 0.05 was considered to be significant.

Results

As illustrated in the Figure, the level of nitric oxide in the plasma of group 1 was found significantly higher than the levels of nitric oxide in the plasmas of groups 2 and 3 ($P < 0.05$). However, there was no statistically significant difference observed in the nitric oxide levels of groups 2 and 3 ($P > 0.05$) indicating that the level of nitric oxide was directly related to the presence of house dust mite.

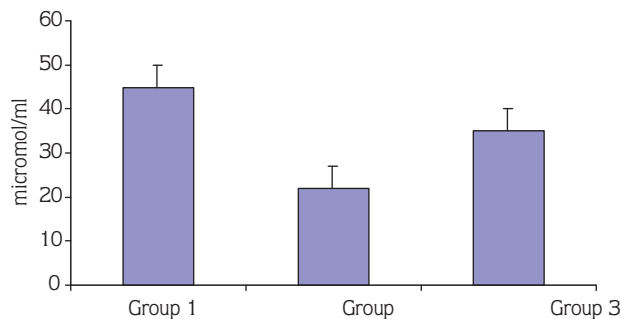


Figure. Distribution of plasma nitric oxide concentration in groups.

Discussion

Complete elimination of dust-mites is unlikely. Reducing their population is the only likely way to reduce allergens in the air. Reducing humidity in the home using a de-humidifier may help reduce populations, but reducing humidity levels in microclimates, such as in bed fibers or carpet fibers, is impossible. Chemical control is not necessary, nor will it have a lasting effect on dust-mite populations. Regular cleaning and vacuuming will have a greater impact (1,2,3).

NO has emerged as an important cytotoxic and cytostatic effector for a number of pathogens, including parasites. When the microbicidal effect of NO occurs, the NO-mediated S-nitrosylation of cysteine containing proteins (e.g., cysteine proteases) appears to be a common and widespread mechanism (10,11,12).

NO produced by an inducible NO-synthase also plays a major microbicidal role in murine macrophages and its

importance is now emerging also in animal and human models (13). It has been recently demonstrated that macrophages in vitro infected with *Leishmania infantum* produced NO (14).

One of the most prominent functions of NO is its participation in antimicrobial and antiviral defense. The evidence for this function compiles the infectious agents, which are currently thought to be controlled via high output generation of NO as it occurs in activated macrophages and other cells expressing the inducible isoform of NO-synthase (6).

NO is produced by a number of different cell types in response to cytokine stimulation and thus has been found to play a role in immunologically mediated protection against a growing list of protozoan and helminth parasites in vitro and also in animal models. The biochemical basis of its effects on the parasite targets appears to involve primarily inactivation of enzymes crucial to energy metabolism and growth, although it has other biologic activities, as well. NO is produced not only by macrophages and macrophage-like cells commonly associated with the effector arm of cell-mediated immune reactivity but also by cells commonly considered to lie outside the immunologic network, such as hepatocytes and endothelial cells, which are intimately involved in the life cycle of a number of parasites. NO production is stimulated by gamma interferon in combination with tumor necrosis factor alpha or other secondary activation signals and is regulated by a number of cytokines and other mediators, as well as through its own inherent inhibitory activity. The potential for the design of prevention and/or intervention approaches against parasitic infection on the basis of induction of cell-mediated immunity and NO production appears to be great, but the possible pathogenic consequences of overproduction of NO must be taken into account (15).

Although the results of the present study imply that house dust mite increases the level of NO, we cannot hide the fact that this relationship may not be a direct one. Because house dust mite is not a human parasite and is not able to dwell inside our body, the parasite should not be directly responsible for the increase in the level of NO. Indirect and unrevealed mechanisms might be responsible for the observed effect.

There is a definite knowledge about the beneficial and protective effects of NO against parasites. We believe that the indirect effect comes from the effect of house dust

mite on the increase of allergic symptoms. Such increases in allergic symptoms may trigger the production of NO and consequently causes an increase in the levels of NO in the plasma. This increase may not help and provide protection against the increase of allergic symptoms

caused by house dust mite. More research on the role and regulation of NO with respect to the presence of environmental parasites is needed before its possible clinical relevance can be determined.

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