ORIGINAL ARTICLE

Differences in the Behavior of Advanced Glycation End Products and Advanced Oxidation Protein Products in Patients With Allergic Rhinitis

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Abstract

Background: The presence of oxidative stress in patients with asthma is well documented; however, the role of oxidative stress in allergic rhinitis has received less attention, although it is likely to be similar to that observed in patients with asthma.
Advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs) are compounds formed by the transformation of macromolecules, including proteins, which can serve as densitometric markers of oxidative stress and inflammation in several diseases.
Objective: The aim of this study was to investigate the role of AGEs and AOPPs as new markers of oxidative stress and inflammation in patients affected by allergic rhinitis.
Methods: AGE and AOPP levels were determined in the sera of 25 patients with allergic rhinitis and 64 healthy controls. AGEs and AOPPs were detected using spectrofluorimetry and spectrophotometry, respectively.
Results: AGE levels in patients were significantly higher than those in controls ($P<.0001$). These levels were not affected by the presence of asthma. No statistically significant differences were found between AOPP levels in patients or controls ($P=.38$).
Conclusions: Formation of AGEs and AOPPs may be accelerated in immunological and respiratory disorders such as asthma. Depending on the marker evaluated, the presence or absence of oxidative stress in allergic rhinitis is controversial. To our knowledge, this is the first study showing the possible involvement of AGEs in allergic rhinitis. The different behavior observed for these 2 biomarkers is very likely due to the activation of specific related biochemical pathways (eg, the myeloperoxidase pathway) associated with the condition under study.

Key words: Advanced glycation end products. Advanced oxidation protein products. Allergic rhinitis. Oxidative stress. Allergy.

Resumen

Antecedentes: La presencia de estrés oxidativo en pacientes con asma bronquial ha sido bien documentada, sin embargo el papel del estrés oxidativo en las rinitis alérgicas no ha sido estudiado.
Los productos finales de la glicación avanzada (AGEs), y los productos de la oxidación avanzada de proteínas (AOPPs) son compuestos formados por la transformación de macromoléculas, incluyendo proteínas, que pueden servir como marcadores densitométricos del estrés oxidativo y de la inflación en diferentes enfermedades.
Objetivos: El motivo de este estudio fue investigar el papel de AGEs y AOPPs como nuevos marcadores del estrés oxidativo en la rinitis alérgica.
Métodos: Estos marcadores fueron analizados en 25 pacientes con rinitis alérgica y en 64 sujetos sanos, mediante métodos de espectrofluorometría y espectrofotometría respectivamente.

Resultados: Los resultados confirman la existencia de niveles elevados de AGEs en pacientes respecto a los controles sanos (p<0.0001). Estos niveles no se vieron influenciados por la presencia de asma. No encontramos diferencias significativas entre los niveles de AOPPs en pacientes y controles (p=0.38).
Conclusiones: La formación de AGEs y AOPPs podría dispararse en alteraciones inmunológicas y patologías respiratorias tales como el asma bronquial. La presencia o no de estrés oxidativo en la rinitis alérgica es tema de controversia y depende del marcador evaluado. Este es el primer estudio que demuestra la posible implicación de AGEs en la rinitis alérgica.
El diferente comportamiento observado para estos dos biomarcadores podría ser debido a las vías bioquímicas específicas (por ejemplo la vía de la mieloperoxidasa) relacionadas con la condición patológica bajo estudio.

Palabras clave: Productos finales de la de la glicación avanzada. Productos de la oxidación avanzada de proteínas. Rinitis alérgica. Estrés oxidativo. Alergia.

Introduction

Oxidative stress is the result of an imbalance between endogenous production of free reactive oxygen species (ROS) and reduced effectiveness of antioxidant defense mechanisms. This imbalance can worsen inflammation and injury by enhancing the release of proinflammatory cytokines and altering enzymatic function [1].

Oxidative stress occurs in many allergic and immunologic disorders and has been well documented in patients with asthma [1]. However, the role of oxidative stress in allergic rhinitis has received little attention, although it is likely to be similar to the role it plays in patients with asthma [2]. Exposure to ozone has been shown to exacerbate allergic rhinitis in experimental animals [3] and in humans [4], and the molecular targets of oxidative stress in allergic rhinitis have been found in blood [5-10], nasal mucosa [6], nasal secretions [7], the nasal cavity [8], erythrocytes [11], and in exhaled air and exhaled breath condensate [12,13]. Reduced serum antioxidant levels have also been recorded in patients with allergic rhinitis and asthma [10,13,14]. However, some authors were unable to demonstrate the presence of markers of oxidative stress in patients with allergic rhinitis [15] or demonstrated different degrees of involvement depending on the marker of oxidative stress detected [16].

Advanced glycation end products (AGEs) and advanced oxidation protein products (AOPPs) are compounds formed by transformation of macromolecules, including proteins, which can serve as densitometric markers of oxidative stress and inflammation in several diseases and their complications [17,18]. Modified proteins can be used more efficiently than other biomarkers to monitor disease progression and outcome, since proteins generally play a key role in various structural and functional aspects of living organisms and their activity and function are strictly dependent on structure, conformation, and folding pattern. Thus, modification of the conformation/structure of the polypeptide chain in conditions of oxidative stress/inflammation can lead to dysfunction/function, loss of proteins, and inhibition of protein degradation (and, consequently, accumulation) and can also have a wide range of downstream functional consequences, such as cellular dysfunction, tissue damage, and disease onset and progression. Furthermore, as the techniques used to measure AGEs and AOPPs are simple, fast, and inexpensive, they can be applied in routine laboratory practice to assess and monitor oxidative stress in critically ill and several other types of patients. These biomarkers also have the advantages of other modified proteins (eg, carbonylated and nitrosylated proteins), namely, relative stability and consequent higher blood concentrations [19].

The aim of this study was to investigate the role of AGEs and AOPPs as new markers of oxidative stress and inflammation in patients affected by allergic rhinitis.

Materials and Methods

Patients

The study population comprised 25 patients aged ≥18 years with a diagnosis of rhinitis who were consecutively seen and evaluated in the outpatient allergy office of the Dipartimento BioMedico di Medicina Interna e Specialistica of the University of Palermo, Palermo, Italy. The evaluation consisted of a skin prick test (SPT), assay of serum total and specific immunoglobulin (Ig) E, and nasal lavage with eosinophil count. Symptoms (sneezing, rhinorrhea, nasal pruritus, and nasal obstruction) were graded according to the following scale: 0, absent; 1, mild (symptoms were present but not troublesome); 2, moderate (symptoms were present but did not interfere with normal activity); and 3, severe (symptoms were troublesome and interfered with normal activity) [20]. The severity of all nasal symptoms was evaluated by patients using a visual analog scale (VAS) and was classified, in accordance with the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline [21], as severe if patients were affected by 1 or more of the following items: sleep disturbance; impairment of daily activities, leisure, or sport; impairment of school or work; and troublesome symptoms. Rhinitis in the absence of these qualifiers was classified as mild. Nasal symptoms were also classified as intermittent or persistent, with intermittent defined as the presence of symptoms for >4 days a week or <4 weeks per year; persistent symptoms were defined as symptoms occurring with greater frequency.

None of the patients was treated with medications or immunotherapy when the biomarkers were assessed. During the previous pollen season, all patients had taken topical corticosteroids to control their symptoms. At the time of the study, none of the patients was receiving nasal corticosteroids

or antihistamines, although they used nasal saline irrigations when needed.

The control group comprised 64 healthy donors matched for sex and age. Participants gave their written informed consent, and the local ethics committee approved the study.

Skin Prick Test

Patients underwent SPT on the volar aspect of the forearm, using a standard panel of aeroallergens present in our geographic area (Alk Abelló). The panel consisted of the following extracts: grass (*Phleum pratense*, *Dactylis glomerata*, *Festuca arundinacea Schreb*, *Lolium perenne*, and *Poa pratensis*); weeds (mugwort [*Artemisia vulgaris*]), wall pellitory, or sticky weed [*Parietaria judaica*]); olive tree (*Olea europaea*) and cypress (*Cupressus arizonica*); house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*); molds (*Alternaria alternata*, *Cladosporium herbarum*, and *Aspergillus fumigatus*); animal dander (cat and dog); negative control (glycerinated saline); and positive control (histamine, 10 mg/mL). Positive responses were defined as any wheal with a diameter 3 mm greater than that of the negative control 15 minutes after application of the allergen. The diameter was assessed by adding the minimum and maximum diameter and dividing by 2. Wheal diameters were reported for each patient.

The SPT showed positivity for *Parietaria* (13 patients), house dust mites (12 patients), grass (11 patients), olive tree (8 patients), cypress (7 patients), cat (6 patients), mugwort (3 patients), and molds (1 patient). Five patients were monosensitive: 3 for *Parietaria* and 2 for house dust mites. The clinically relevant allergens were *Parietaria*, house dust mites, and grass.

Serum Total and Specific IgE

A blood sample was processed at the time of diagnosis. Serum total IgE and specific IgE were determined using a fluoroimmunoassay (UniCAP 100, Phadia) according to the manufacturer's instructions. Results for serum total IgE and specific IgE were expressed as kU/L and kU_A/L, respectively, and calibrated against the World Health Organization standard for IgE: 1 kU for total IgE and 1 kU_A for specific IgE were both equal to 2.4 ng/mL. Serum total IgE was determined with a detection limit of 2 kU/L and an upper limit of 5000 kU/L. Serum specific IgE was determined with a detection limit of 0.35 kU_A/L and an upper limit of 100 kU_A/L at the time of diagnosis and with a detection limit of 0.1 kU_A/L and an upper limit of 100 kU_A/L at the follow-up visit. Specific IgE was measured in all patients for the same allergens used in the SPT.

Eosinophil Counts in Nasal Fluid

Nasal lavage was performed using a disposable metered-dose nasal inhaler (Markos) filled with normal sterile saline solution at room temperature. This device consists of a plastic cup with 2 compartments. The central compartment was filled with sterile saline solution, while the external compartment collected the liquid after washing. The total input of saline solution was approximately 8 mL (4 mL per nostril for 5 minutes). To collect the nasal washings, participants were instructed to take an active breath during a Valsalva maneuver in order to harvest nasal fluid in the cup. The samples obtained were stored on ice and centrifuged at 400*g* for 10 minutes at 4°C. The individual variation in the volume recovered compared with the volume introduced was 86 ± 8%. Nasal eosinophil counts were performed on the nasal lavage fluid. One cytospin slide for each sample (1×10^{-4} cells in 170 μL per slide) was centrifuged at 10*g* for 10 minutes in a cytocentrifuge device (Shandon Southern Ltd.). The slides were immediately fixed in 95% ethyl alcohol, dipped in Wright-Giemsa stain, and examined using oil immersion light microscopy at a magnification of ×400. Eosinophils were expressed as a percentage of 300 cells [22,23]. All the specimens were examined by the same microscopist (SLP), who had no knowledge of the clinical histories, the results for SPT and specific serum IgE, or the severity of the rhinitis symptoms [23].

Sera Collection

A sample of blood was withdrawn from the antecubital vein. Sera were allowed to clot at room temperature for 2 hours, then separated by centrifugation at 1200*g* for 15 minutes and stored at –80°C until used.

Determination of AGEs and AOPP

AGEs and AOPPs were detected using spectrofluorimetry and spectrophotometry, respectively, as described by Spatari et al [24]. AGE was determined by diluting serum 1:50 with phosphate-buffered saline (PBS; pH, 7.4), and fluorescence intensity was recorded at maximum emission (~440 nm) upon excitation at 350 nm and expressed in arbitrary units (AU). The serum concentration of AGEs was normalized to the total protein amount determined by the Bradford assay and expressed in AU per gram of protein. AOPPs were determined by diluting 200 μL of blood serum 1:5 with PBS; 200 μL of chloramine-T (0-100 mol/L) was added for calibration, and 200 μL of PBS was applied as a blank on a microtiter plate. Ten microliters of 1.16 M KI and 20 μL of acetic acid were added, and absorbance was measured immediately at 340 nm. The serum concentration of AOPPs was normalized to the total protein amount determined by the Bradford assay and expressed as nanomoles of chloramine per milligram of protein. Each sample was analyzed in triplicate for determination of AGE and AOPP.

Statistical Analysis

The statistical analysis was performed with SPSS for Windows (version 13.0, SPSS Inc). Data were presented as median and 95% confidence interval (CI). Differences between data series were analyzed using the Mann-Whitney test. A multivariate regression model was constructed to assess the possible dependence of AGE and AOPP on nasal cytology (lymphocytes, neutrophils, and eosinophils).

Statistical significance was set at $P<.05$.

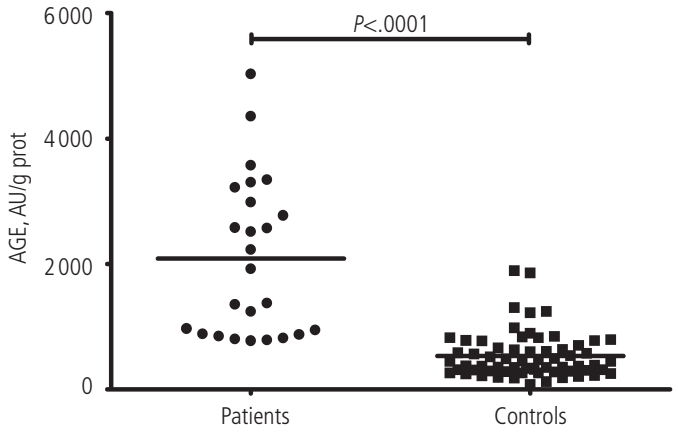

Figure 1. AGE concentrations in patients and controls. The lines represent means. AGE indicates advanced glycation end product.

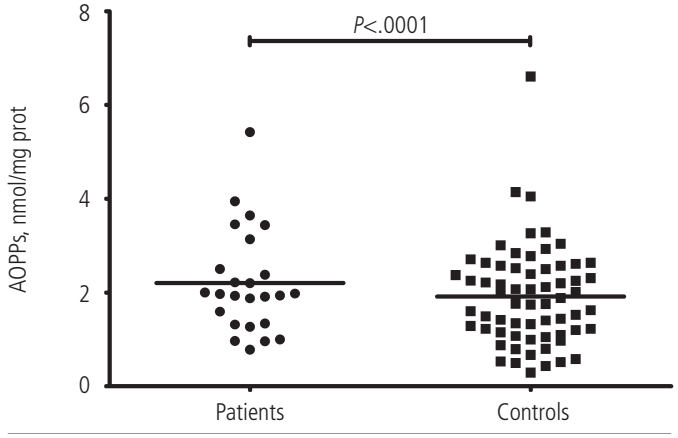

Figure 2. AOPP concentrations in patients and controls. The lines represent means. AOPP indicates advanced oxidation protein product.

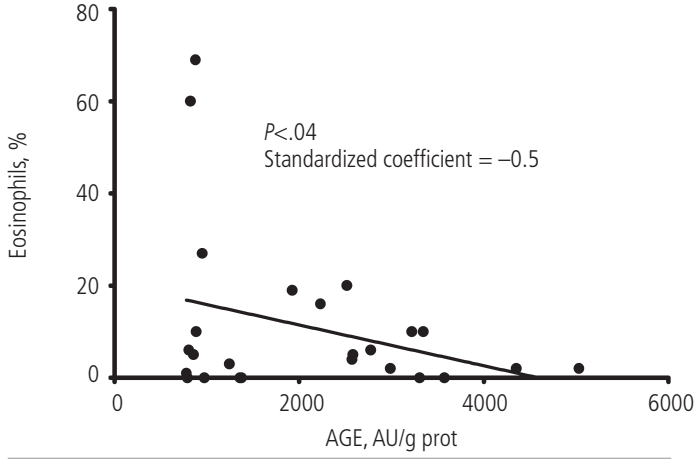

Figure 3. Relationship between AGE levels and eosinophils. AGE indicates advanced glycation end product.

Results

AGE levels were significantly higher in patients (1926.26 AU/g prot [95%CI, 910.65-2904.9]) than in controls (416.98 AU/g prot [95%CI, 339.13-567.22]) (*P*<.0001) (Figure 1). AGE levels were not affected by the presence of asthma (patients with rhinitis, 2373.97 AU/g prot [95%CI, 883.92-2997.89]; patients with rhinitis and asthma, 1376.7 AU/g prot [95%CI, 793.34-4057.11]) (*P*=.777).

No statistically significant differences were found between AOPP levels in patients (1.96 nmol/mg prot [95%CI, 1.44-2.45]) and controls (1.82 nmol/mg prot [95%CI, 1.42-2.22]) (*P*=.38) (Figure 2).

AOPP levels were not affected by the presence of asthma (patients with rhinitis, 1.95 nmol/mg prot [95%CI, 1.31-3.15]; patients with rhinitis and asthma, 1.98 nmol/mg prot [95%CI, 0.89-3.52]) (*P*=.91).

Neither serum AGEs nor AOPPs correlated with total and specific IgE and VAS values.

A statistically significant association was found between AGE levels and nasal cytology (*P*=.04), in particular with the proportion of neutrophils and eosinophils (standardized coefficient, –0.53 [*P*=.02] and –0.50 [*P*=.04], respectively) (Figure 3).

Discussion

Determination of circulating levels of AGEs and AOPPs in oxidative stress is an area of increasing interest, as is detection of inflammatory markers in several diseases.

AGEs are composed of a heterogenous group of bioactive compounds (eg, pentosidine, carboxymethyl lysine, and imidazolone) that are formed by nonenzymatic glycation of macromolecules [17]. Formation of AGEs is markedly increased in hyperglycemia, and AGEs are involved in the pathogenesis of diabetic complications. However, it is increasingly clear that AGEs also play a role in the pathogenesis of other diseases involving oxidative stress and inflammation, including atherosclerosis, chronic obstructive pulmonary disease, rheumatoid arthritis, and multiple sclerosis [25-29]. In fact, the formation and action of AGEs is linked both to oxidative stress and to inflammation. AGE levels can rise via auto-oxidation of sugars and other glycation intermediates and via lipid peroxidation of polyunsaturated fatty acids, both of which produce reactive carbonyl compounds. Additionally, some AGEs can be formed at inflamed foci via nicotinamide adenine dinucleotide phosphate oxidase and via myeloperoxidase action in glucose-independent pathways [30,31].

AOPPs are proteins (predominantly albumin and its aggregates) that are damaged by oxidative stress. They contain abundant dityrosines, which enable cross-linking, disulfide bridges, and carbonyl groups and are mainly formed by chlorinated oxidants (hypochlorous acid and chloramines) resulting from myeloperoxidase activity [18]. In addition to a common formation mechanism (oxidative stress) leading to macromolecule damage, the biological effects of AOPPs

are similar to those of AGEs and are considered to have a role in inflammatory processes and immune dysregulation [32].

Several studies have demonstrated that formation of AGEs and AOPPs may be accelerated in immunological and respiratory disorders such as asthma, which is often associated with allergy [33-36]. The patients enrolled in our study were affected by allergic rhinitis, a disease in which findings on the presence or absence of oxidative stress are controversial [15,16], depending on the marker evaluated.

We found higher levels of serum AGEs in patients than in healthy controls, although the serum levels of AOPPs in patients were similar to those of controls.

To our knowledge, this is the first study showing the possible involvement of AGEs in allergic rhinitis.

The different behavior observed for these 2 biomarkers is very likely due to the activation of specific biochemical pathways (eg, the myeloperoxidase pathway) associated with the disorder under study.

Some years ago, Aksoy et al [35] demonstrated the presence of significantly higher serum AOPP levels in patients with allergic rhinitis than in controls. Other than the different experimental conditions, the discrepancy between these findings and ours might be justified by variations in data calculations. In fact, while Aksoy et al expressed their results as micromoles per liter, we normalized the serum AOPP content to serum total protein content, taking into account that elevated circulating levels of allergen-specific and nonspecific proteins may be present in allergic patients [37].

In conclusion, this preliminary study evaluates the role of new biomarkers of oxidative stress in allergic rhinitis. However, other studies are needed to monitor these molecules during treatment and to evaluate the possible therapeutic use of antioxidants, whose role in allergic patients remains open to debate.

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