High Levels of Urinary Pentosidine, an Advanced Glycation End Product, in Children With Acute Exacerbation of Atopic Dermatitis: Relationship With Oxidative Stress

Hirokazu Tsukahara, Rumiko Shibata, Naoko Ohta, Shuko Sato, Masahiro Hiraoka, Setsuko Ito, Eisei Noiri, and Mitsufumi Mayumi

Pentosidine is an advanced glycation end product formed by sequential glycation and oxidation. The formation of pentosidine is increased in diseases associated with oxidative stress, such as inflammatory conditions. The aim of the present study was to determine the urinary concentration of pentosidine in atopic dermatitis (AD) and its relationship to the inflammatory status of AD. Urine samples of 32 children with AD and 30 age-matched healthy control subjects were assayed for pentosidine, pyrraline (another advanced glycation end product formed by nonoxidative glycation), and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (an established marker of oxidative stress). Of these 3 markers, urinary concentrations of pentosidine were significantly higher in patients with acute exacerbation of AD than in healthy controls and patients with stable AD. Urinary concentrations of 8-OHdG were significantly higher in AD patients with and without acute exacerbation than in healthy controls. Urinary pentosidine levels correlated significantly with those of 8-OHdG when all data of healthy controls and AD patients were plotted. In patients with acute exacerbation of AD, both urinary pentosidine and 8-OHdG significantly decreased after 7 to 9 days of treatment. Our findings in patients with acute exacerbation of AD suggest that pentosidine levels are partly determined by the prevailing oxidative stress in these patients.

PENTOSIDINE IS one of several chemically characterized advanced glycation end products (AGEs).[1,2] Pentosidine exists in human tissues, such as skin, kidney, cartilage, aorta, and lens protein and in blood and urine, and its concentrations in tissues increase with age and are increased in diabetes and, more overtly, in uremia. Blood pentosidine levels are also elevated in the latter 2 conditions.[3,4] Pentosidine is formed by sequential glycation and oxidation (thereby termed "glycoxidation") reactions between carbohydrate-derived carbonyl group and protein amino group, known as the Maillard reaction.[5] Its formation requires aerobic conditions, while an antioxidative condition inhibits such reaction. On the other hand, pyrraline, another AGE, is derived from nonoxidative chemistry.[6] Therefore, it is hypothesized that formation of pentosidine, but not of pyrraline, is accelerated in diseases accompanied by oxidative stress.

Atopic dermatitis (AD) is a chronic, relapsing inflammatory disease characterized by extreme pruritus, typically distributed eczematous skin lesions, and a personal or family history of atopic diseases.[7] Patients afflicted with AD suffer greatly, as it adversely affects their quality of life. Population studies suggest that the prevalence of AD has been increasing since World War II, and in most countries, it now affects more than 10% of children at some point during childhood. At present, there is no treatment directed at the basic cause of AD. Therefore, exploring the mechanisms involved in the complex inflammatory cascades could lead to the generation and acceptance of new improved therapies for this common and potentially debilitating disease.

AD may be associated with a state of increased oxidative stress due to systemic inflammation, as indicated by increased urinary concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG),[8,9] as well as enhanced release of reactive oxygen intermediates (ROI) from leukocytes[10,11] in these patients. These findings suggest a possible increase in glycoxidation reaction and augmented pentosidine formation in AD patients, especially those with acute exacerbation.

Based on this background, the present study was designed to measure pentosidine concentrations in urine of AD patients with and without acute exacerbation of AD. Our results demonstrated for the first time the presence of significantly higher pentosidine concentrations in urine of patients with acute exacerbation of AD compared with those of patients with stable AD and normal healthy subjects. We also found that urinary pentosidine concentrations of AD patients correlated significantly with those of 8-OHdG. Therefore, a plausible explanation for the increased formation of pentosidine in our patients with acute exacerbation of AD is enhanced oxidative stress.

MATERIALS AND METHODS

Patients and Controls

Thirty-two patients with AD who fulfilled the criteria of the 1999 Guideline for Atopic Dermatitis (of the Ministry of Health, Labor and Welfare, Japan[9]) participated in this study. Twenty patients had stable AD without recent flare-up. These patients were using topical corticosteroids and/or nonsteroidal anti-inflammatory agents at the time of the study. The patients were 7 boys and 13 girls (age, 5.8 ± 2.8 years, mean ± SD; range, 2.8 to 12.1 years). The remaining 12 had acute exacerbation of AD with purulent skin infection caused by *Staphylococcal aureus* and were thus admitted to the hospital. The patients were 3 boys and 9 girls (age, 5.4 ± 2.4 years; range, 1.9 to 10.9 years). They were treated with systemic antibiotics, topical antiseptics, and corticosteroids. All showed satisfactory clinical response to treatment during hospitalization. All participating patients had normoglycemia and nor-

From the Department of Pediatrics, Fukui Medical University, Fukui; Department of Pediatrics, National Minami-Fukuoka Hospital, Fukuoka; Department of Pediatrics, Takeda Hospital, Kyoto; and the Department of Nephrology and Endocrinology, The University of Tokyo, Tokyo, Japan.

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Address reprint requests to Hirokazu Tsukahara, MD, PhD, Department of Pediatrics, Fukui Medical University, Fukui 910-1193, Japan.

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mal renal function. We also recruited 30 age-matched healthy control children (12 boys and 18 girls; age, 5.8 ± 2.3 years; range, 1.4 to 10.1 years) who were free of allergic or inflammatory diseases at the time of recruitment. All subjects in the present study were nonsmokers. Informed consent was obtained from the parents of the subjects, and the study was approved by the hospital ethics committee.

Urine Samples

Urine samples were obtained from each subject. For patients with acute exacerbation of AD, urine samples were obtained on admission and the seventh to ninth hospital days. The samples were centrifuged and the supernatants were stored at −20°C until analysis. All analyses were performed in duplicate, and the examiner was blinded to the clinical and laboratory results.

Determination of Urinary Pentosidine

Urinary concentrations of pentosidine were determined using the method of Yoshihara et al.[12] The washing solution was a mixture of *n*-butanol: acetic acid: hydrochloric acid (8:1:1, vol/vol). CF-1 slurry was prepared by making a 5% (wt/vol) suspension of CF-1 cellulose powder in the washing solvent. The pretreatment column was prepared by adding 8 mL CF-1 slurry to a Poly-Prep chromatography column (0.8 mm × 40 mm internal diameter [ID], Bio-Rad, Hercules, CA). A total of 250 μL urine was hydrolyzed with an equal volume of concentrated hydrochloric acid at 108°C for 18 hours. The cooled hydrolysate (250 μL) was mixed with 250 μL CF-1 slurry, 250 μL acetic acid, and 2 mL *n*-butanol and then loaded to the pretreatment column. After washing the column with 35 mL of the washing solvent, pentosidine was eluted from the column with 9 mL of 50 mmol/L hydrochloric acid and dried under N_2 gas flow. The dry residue was then dissolved in 250 μL 1% *n*-heptafluorobutyric acid (vol/vol), and an aliquot (10 μL) of each sample was applied to analytical high-performance liquid chromatography (HPLC). The HPLC eluent was 7% acetonitrile (vol/vol) containing 0.1% *n*-heptafluorobutyric acid. The HPLC system was equipped with an L-6200 intelligent pump (Hitachi, Ibaragi, Japan), an F-1050 fluorescence detector set at excitation and emission wavelengths of 335 nm and 385 nm, respectively, (Hitachi) and Symmetry RP18 column (3.5 μm, 4.6 mm × 150 mm, ID, Waters, Milford, MA). The flow rate was maintained at 0.8 mL/min, and the column was kept at 30°C. Standard pentosidine was synthesized and purified as described in detail previously.[12] The mean concentration of urinary pentosidine in healthy adults (n = 64) determined using this method is 28.2 ± 12.0 pmol/mg creatinine (Cr).

Determination of Urinary Pyrraline

Urinary concentrations of pyrraline were determined using the method of Yoshihara et al.[13] Urine samples were treated in solid-phase extraction cartridges (Oasis HLB, 3 mL, Waters). The cartridge was preconditioned with 1 mL methanol and equilibrated with 1 mL distilled water before loading the urine sample. A 500-μL urine sample was applied to the cartridge, followed by washing the cartridge with 1 mL 0.1% acetic acid (vol/vol). Pyrraline was eluted from the cartridge with 3 mL of 60% acetonitrile, and the eluent was dried under N_2 gas flow. The dried residue was then dissolved in 500 μL 0.1% trifluoroacetic acid (vol/vol) and the aliquot (20 μL) of each sample was applied to the analytical HPLC system. The HPLC eluent was 7% acetonitrile (vol/vol) containing 0.1% trifluoroacetic acid (vol/vol). The HPLC system was equipped with an L-7100 intelligent pump, an L-7400 UV detector set at 298 nm and Capcellpak UG120 column (3 μm, 4.6 mm × 150 mm, ID, Shiseido, Tokyo, Japan). The flow rate was maintained at 0.8 mL/min and the column was kept at 35°C. Standard pyrraline was synthesized and purified.[13] The mean concentration of urinary pyrraline in healthy adults (n = 27) determined using this method is 25.3 ± 30.0 nmol/mg Cr.

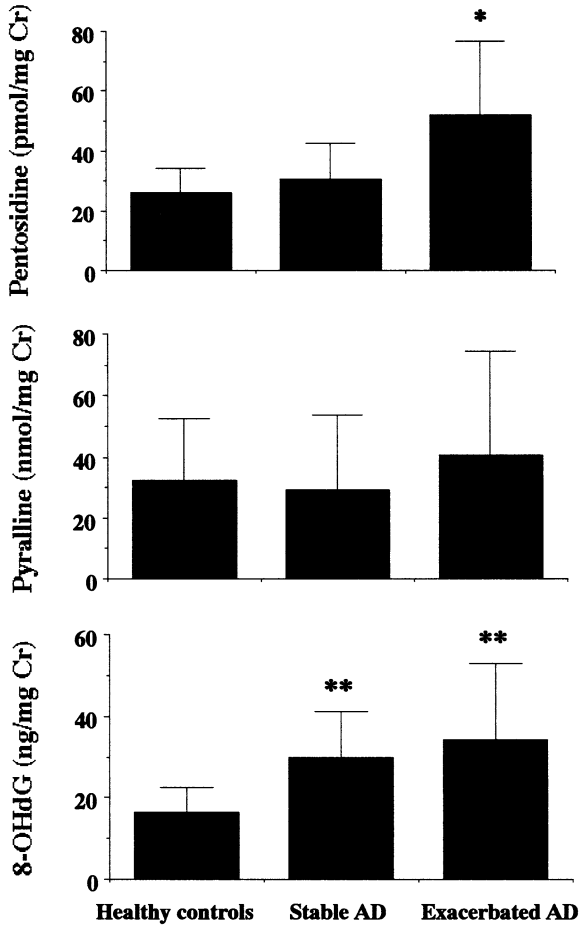

Fig 1. Comparisons of urinary concentrations of pentosidine, pyrraline, and 8-OHdG among healthy control subjects, patients with stable AD, and patients with acute exacerbation of AD. Data are shown as mean and SD. *$P < .001$ *v* healthy control subjects and patients with stable AD; **$P < .001$ *v* healthy control subjects.

Determination of 8-OHdG and Cr

The concentration of 8-OHdG was determined using a competitive enzyme-linked immunosorbent assay (8-OHdG Check, Institute for the Control of Aging, Shizuoka, Japan), as described previously.[9,14] All the above urinary markers were expressed relative to urinary Cr concentration, which was measured enzymatically using the Creatinine HR-II Test kit (Wako Pure Chemical, Osaka, Japan).

Statistical Analysis

Data are presented as mean ± SD and range. Differences between groups were examined for statistical significance using 1-way analysis of variance followed by the Scheffe test or the paired t test as appropriate. Correlations between variables were assessed by linear regression. A *P* value < .05 denoted the presence of a statistically significant difference.

RESULTS

As shown in Fig 1, the concentrations of pentosidine, pyrraline, and 8-OHdG in urinary samples of 30 healthy children

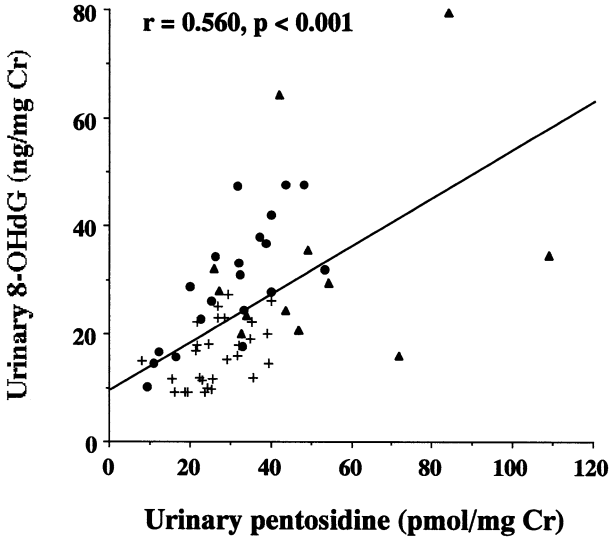

Fig 2. Relationship between urinary concentrations of pentosidine and 8-OHdG in all subjects studied. Healthy control subjects (+), patients with stable AD (●), and patients with acute exacerbation of AD (▲). The correlation between pentosidine and 8-OHdG was significant (n = 62, *r* = .560, *P* < .001).

were 25.9 ± 8.2 pmol/mg Cr (range, 8.0 to 40.0 pmol/mg Cr), 32.6 ± 19.7 nmol/mg Cr (range, 2.3 to 80.8 nmol/mg Cr), and 16.5 ± 5.9 ng/mg Cr (range, 9.1 to 27.2 ng/mg Cr), respectively. The concentrations of pentosidine, pyrraline, and 8-OHdG in urinary samples of 20 children with stable AD were 30.4 ± 12.3 pmol/mg Cr (range, 9.5 to 53.3 pmol/mg Cr), 29.1 ± 24.5 nmol/mg Cr (range, 5.4 to 93.3 nmol/mg Cr), and 29.7 ± 11.4 ng/mg Cr (range, 10.1 to 47.7 ng/mg Cr), respectively. Of these 3 markers, only urinary levels of 8-OHdG were significantly higher in these patients than in healthy controls ($P < .001$). The concentrations of pentosidine, pyrraline, and 8-OHdG in urinary samples of 12 children with acute exacerbation of AD on admission were 51.8 ± 25.0 pmol/mg Cr (range, 26.0 to 109.0 pmol/mg Cr), 40.5 ± 33.8 nmol/mg Cr (range, 3.6 to 97.0 nmol/mg Cr), and 34.0 ± 18.9 ng/mg Cr (range, 16.0 to 79.4 ng/mg Cr), respectively. Of these 3 markers, urinary levels of pentosidine and 8-OHdG, but not pyrraline, were significantly higher in these patients than in healthy controls ($P < .001$, each). Urinary pentosidine was also significantly higher in children with acute exacerbation of AD compared with those of patients with stable AD ($P < .001$). There was a significant positive correlation between pentosidine and 8-OHdG when all data of healthy controls and AD patients were entered into the analysis (n = 62, $r = .560$, $P < .001$) (Fig 2).

In children with acute exacerbation of AD, the concentrations of pentosidine and 8-OHdG significantly decreased from the day of admission to the seventh to ninth hospital day ($P < .05$, each) (Fig 3). The concentrations of pentosidine, pyrraline, and 8-OHdG on the seventh to ninth hospital day were 35.1 ± 15.0 pmol/mg Cr (range, 20.1 to 74.1 pmol/mg Cr), 28.2 ± 26.2 nmol/mg Cr (range, 3.7 to 96.0 nmol/mg Cr), and 20.9 ± 8.6 ng/mg Cr (range, 9.7 to 35.6 ng/mg Cr), respectively, in these patients.

DISCUSSION

Urinary 8-OHdG, derived from DNA or the nucleotide pool via an endonuclease or nucleotide excision repair, has been used as a marker of oxidative stress.[14] Our results showed that the urinary concentrations of 8-OHdG of AD patients with or without acute exacerbation were significantly higher than those of healthy children. This result supports previous findings of the involvement of oxidative stress in the pathophysiology of AD.[8,9] Of greater significance is the fact that the urinary concentrations of pentosidine of patients with acute exacerbation of AD were significantly higher than those of patients with stable AD and healthy control subjects.

Although we do not know the exact reason for the increased pentosidine in patients with acute exacerbation of AD, the involvement of redox imbalance and oxidative stress may be relevant. Yoshihara et al[12] (unpublished results), using the same methodologies applied in our study, examined the relationship between blood and urinary concentrations of pentosidine in 26 healthy subjects and in 44 patients with impaired renal function. Urinary pentosidine levels closely correlated with blood pentosidine concentrations, indicating that pentosi-

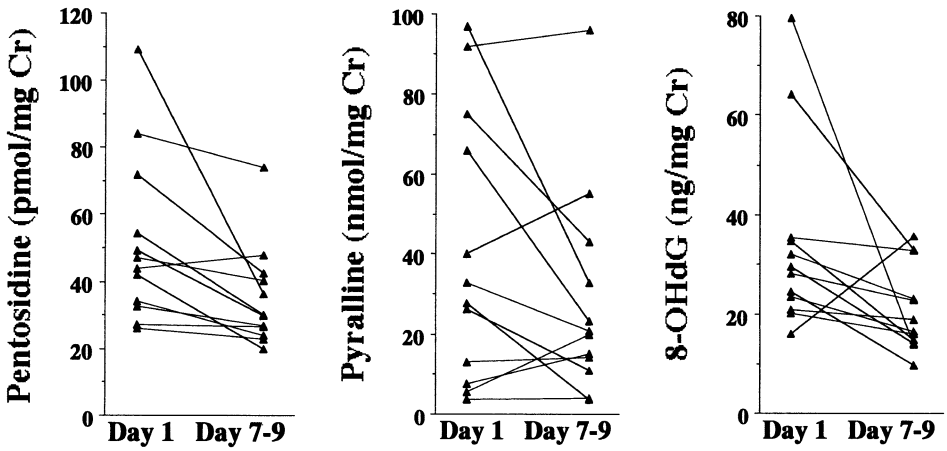

Fig 3. Effect of treatment on urinary concentrations of pentosidine, pyrraline, and 8-OHdG in patients with acute exacerbation of AD. The concentrations of pentosidine and 8-OHdG decreased significantly after 7 to 9 days of treatment (*P* < .05, each).

dine in the circulation is excreted into the urine according to its blood concentration. Therefore, we assume that the increased excretion of pentosidine observed in our patients reflects a greater synthesis and accumulation in the blood, tissues, and organs.

AGE accumulation, including pentosidine, is enhanced in diabetes as a result of sustained hyperglycemia.[1,2] However, hyperglycemia per se is not the direct reason of increased pentosidine levels in peripheral blood, because these levels are markedly increased in uremia, despite normal levels of blood glucose in patients with renal failure.[3,4] Furthermore, pentosidine levels in blood, synovial fluid, and urine are elevated in patients with rheumatoid arthritis in the absence of hyperglycemia or impaired renal function.[15-17] It is more plausible that the increased pentosidine levels in blood and synovial fluid are associated with the systemic inflammatory activity of rheumatoid arthritis. Pentosidine formation is closely linked not only to glycation, but also to oxidative processes, hence its qualification as a "glycoxidation" product. Wolff and Dean[18] demonstrated that reducing sugars could be autoxidized by metal-catalyzed oxidative processes and generate ROI and ketoaldehydes, which contribute to chromo- and fluorophoric alterations of proteins now taken as characteristic of AGEs. That pentosidine is the product of "glycoxidation" is further supported by in vitro evidence that the absence of oxygen in the incubation medium prevents pentosidine formation.[5] Because rheumatoid arthritis is associated with increased oxidative stress due to systemic inflammation, it appears likely that oxidative stress contributes to the generation of AGEs, including pentosidine.[15-17] The oxidative stress theory in increased pentosidine production is supported in uremia by the recent demonstration in uremic plasma of a close correlation between the levels of pentosidine and oxidative stress markers (ie, oxidized ascorbic acid, highly oxidized proteins).[19,20] In addition, the colocalization of AGEs with markers of lipid peroxidation in vascular and renal tissues indicates that both glycation and oxidation reactions contribute to pathologic lesions in diabetic atherosclerosis and nephropathy.[2,21] In the present study, we demonstrated that urinary pentosidine levels correlated significantly with those of 8-OHdG when all data of healthy controls and AD patients were plotted.

Acute skin inflammation of AD is characterized histopathologically by intense infiltration of T lymphocytes, monocyte-macrophages, and eosinophils.[7] These cells release bioactive substances, such as cytokines, chemokines, and tissue degradative enzymes and ROI, such as O_2^-, H_2O_2, $ONOO^-$, upon immunologic and nonimmunological stimulation.[7,11,22,23] Moreover, keratinocytes and endothelial cells can influence the inflammatory responses by elaborating cytokines, chemokines, adhesion molecules, and ROI. Purulent skin infection caused by *Staphylococcal aureus* infection may precipitate the exacerbation of AD.[24] Numerous infiltrating neutrophils are activated and show enhanced ROI release.[10] Activated neutrophils produce hypochlorite (HOCl) through the action of myeloperoxidase-H_2O_2-chloride system. HOCl can oxidize free amino acids to the corresponding aldehydes.[25] Anderson et al[25] showed that HOCl converts serine to glycolaldehyde, which in the presence of protein, generates carboxymethyllysine, another glycoxidation product. Similar reactions, initiated by HOCl or ROI, may lead to the generation of reactive carbohydrate intermediates, such as dicarbonyls or pentoses, which would readily react with proteins to generate pentosidine. In patients with acute exacerbation of AD, the high urinary concentrations of pentosidine, as well as 8-OHdG, decreased as the patients started to recover from the disease. The changing pattern of these markers allows us to suggest that some adaptation process occurred in these patients. Acute exacerbation of AD may be characterized by a state of enhanced oxidative stress, and this could lead to the upregulation of the antioxidant systems and the resistance to oxidative stress.[14] We observed no significant increase in the generation of pyrraline, which does not require oxidative conditions, in AD patients with or without acute exacerbation. Taken together, the enhanced prooxidant milieu may offer a reasonable explanation for the increased pentosidine formation in patients with acute exacerbation of AD.

Formation of AGEs, including pentosidine, is accompanied by a cross-linking of proteins causing alterations of structural and functional properties of macromolecules. Furthermore, AGEs are endowed with diverse biologic activities, such as increase in endothelial permeability, activation of macrophages and endothelial cells with secretion of cytokines and growth factors, which in turn, accelerate inflammation and enhancing oxidative stress.[1,2] Therefore, some of the possible mechanisms leading to acute exacerbation of AD may be related to an increase in the formation of AGEs, including pentosidine. Further studies are necessary to investigate whether the increased AGEs actively participate in the acute worsening of AD.

The observations described in the present study may not be specific to acute exacerbation of AD, but represent changes common to inflammatory or infectious conditions. However, in a series of preliminary studies, we found that the urinary concentrations of pentosidine, pyrraline, and 8-OHdG of children with acute bronchitis were not significantly different from those of aged-matched healthy controls (unpublished results). Further investigation is necessary to determine concentrations of AGEs in other conditions with activated immune and inflammatory responses.

In conclusion, our findings of increased urinary concentrations of pentosidine and 8-OHdG and their close relationship, together with normal pyrraline concentrations, in patients with acute exacerbation of AD are interpreted as evidence that pentosidine levels are partly determined by the prevailing oxidative stress in these patients. Pentosidine could thus be used as a marker of the disease status in patients with AD.

REFERENCES

1. Bucala R, Vlassara H: Advanced glycosylation end products in diabetic renal and vascular disease. Am J Kidney Dis 26:875-888, 1995

2. Suzuki D, Miyata T: Carbonyl stress in the pathogenesis of diabetic nephropathy. Intern Med 38:309-314, 1999

3. Makita Z, Bucala R, Rayfield EJ, et al: Reactive glycosylation

endproducts in diabetic uraemia and treatment of renal failure. Lancet 343:1519-1522, 1994

4. Miyata T, Kurokawa K, van Ypersele de Strihou C: Advanced glycation and lipoxidation end products: Role of reactive carbonyl compounds generated during carbohydrate and lipid metabolism. J Am Soc Nephrol 11:1744-1752, 2000

5. Dyer DG, Blackledge JA, Thorpe SR, et al: Formation of pentosidine during nonenzymatic browning of proteins by glucose: Identification of glucose and other carbohydrates as possible precursors of pentosidine in vivo. J Biol Chem 266:11654-11660, 1991

6. Hayase F, Nagaraj RH, Miyata S, et al: Aging of proteins: Immunological detection of a glucose-derived pyrrole formed during Maillard reaction in vivo. J Biol Chem 263:3758-3764, 1989

7. Leung DY: Atopic dermatitis: New insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 105:860-876, 2000

8. Tsuboi H, Kouda K, Takeuchi H, et al: 8-hydroxydeoxyguanosine in urine as an index of oxidative damage to DNA in the evaluation of atopic dermatitis. Br J Dermatol 138:1033-1035, 1998

9. Omata N, Tsukahara H, Ito S, et al: Increased oxidative stress in childhood atopic dermatitis. Life Sci 69:223-228, 2001

10. Hoeger PH, Niggemann B, Schroeder C: Enhanced basal and stimulated PMN chemiluminescence activity in children with atopic dermatitis: Stimulatory role of colonizing staphylococci ? Acta Paediatr 81:542-546, 1992

11. Kapp A, Zeck-Kapp G, Czech W, et al: The chemokine RANTES is more than a chemoattractant: Characterization of its effect on human eosinophil oxidative metabolism and morphology in comparison with IL-5 and GM-CSF. J Invest Dermatol 102:906-914, 1994

12. Yoshihara K, Nakamura K, Kanai M, et al: Determination of urinary and serum pentosidine and its application to elder patients. Biol Pharm Bull 21:1005-1008, 1998

13. Yoshihara K, Kiyonami R, Shimizu Y, et al: Determination of urinary pyrraline by solid-phase extraction and high performance liquid chromatography. Biol Pharm Bull 24:863-866, 2001

14. Toyokuni S: Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 49:91-102, 1999

15. Takahashi M, Suzuki M, Kushida K, et al: Relationship between pentosidine levels in serum and urine and activity in rheumatoid arthritis. Br J Rheumatol 36:637-642, 1997

16. Rodriguez-Garcia J, Requena JR, Rodriguez-Segade S: Increased concentrations of serum pentosidine in rheumatoid arthritis. Clin Chem 44:250-255, 1998

17. Miyata T, Ishiguro N, Yasuda Y, et al: Increased pentosidine, an advanced glycation end product, in plasma and synovial fluid from patients with rheumatoid arthritis and its relation with inflammatory markers. Biochem Biophys Res Commun 244:45-49, 1998

18. Wolff SP, Dean RT: Glucose autoxidation and protein modification. The potential role of 'autoxidative glycosylation' in diabetes. Biochem J 245:243-250, 1987

19. Witko-Sarsat V, Friedlander M, Capeillere-Blandin C, et al: Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 49:1304-1313, 1996

20. Miyata T, Wada Y, Cai Z, et al: Implication of an increased oxidative stress in the formation of advanced glycation end products in patients with end-stage renal failure. Kidney Int 51:1170-1181, 1997

21. Miyata T, Inagi R, Asahi K, et al: Generation of protein carbonyls by glycoxidation and lipoxidation reactions with autoxidation products of ascorbic acid and polyunsaturated fatty acids. FEBS Lett 437:24-28, 1998

22. Taha RA, Minshall EM, Leung DY, et al: Evidence for increased expression of eotaxin and monocyte chemotactic protein-4 in atopic dermatitis. J Allergy Clin Immunol 105:1002-1007, 2000

23. Wakita H, Sakamoto T, Tokura Y, et al: E-selectin and vascular cell adhesion molecule-1 as critical adhesion molecules for infiltration of T lymphocytes and eosinophils in atopic dermatitis. J Cutan Pathol 21:33-39, 1994

24. Nomura I, Tanaka K, Tomita H, et al: Evaluation of the staphylococcal exotoxins and their specific IgE in childhood atopic dermatitis. J Allergy Clin Immunol 104:441-446, 1999

25. Anderson MM, Hazen SL, Hsu FF, et al: Human neutrophils employ the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxy-amino acids into glycolaldehyde, 2-hydroxypropanal, and acrolein. A mechanism for the generation of highly reactive α-hydroxy and α, β-unsaturated aldehydes by phagocytes at sites of inflammation. J Clin Invest 99:424-432, 1997