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Clinical trial

Serum level of advanced oxidation protein products (AOPPs) in patients with Henoch–Schonlein purpura and its relationship with aberrant glycosylation of IgA1 and Cosmc mRNA expression

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Abstract

Background Henoch–Schonlein purpura (HSP) is a systemic small vessel vasculitis that is mainly caused by IgA1-type immune complex deposition. Advanced oxidation protein products (AOPPs) are specific markers of protein oxidation.

Objective To explore the role of AOPPs in the pathogenesis of HSP.

Methods There are 51 HSP patients who were divided into four subgroups: (i) skin type – 20 cases; (ii) joint type – 8 cases; (iii) abdominal type – 12 cases; (iv) renal type – 11 cases; and 18 healthy volunteers were enrolled as controls. The serum levels of AOPPs and Gd-IgA1 were quantified by an HAA-lectin-based ELISA. The Cosmc mRNA expression in peripheral B lymphocytes was measured by RT-PCR.

Results 1. Advanced oxidation protein products in different subgroups of HSP patients are all higher than the controls, while the renal-type subgroup is the highest and the skin-type subgroup is the lowest. 2. Spearman correlation analysis shows that: (i) AOPPs and Gd-IgA1 in HSP patients are positively correlated; both of them are positively correlated with the disease severity scores; (ii) AOPPs are negatively correlated with the relative expression value (RQ) of Cosmc mRNA.

Conclusion Advanced oxidation protein products play an important role in the pathogenesis of HSP, especially in renal-type patients.

Introduction

Henoch–Schonlein purpura (HSP) is a systemic small vessel vasculitis that is mainly caused by IgA1-type immune complex deposition.[1] It can affect many body organs such as the skin, joints, gastrointestinal tract, and kidneys. At present, it is believed that galactose-deficient IgA1 (Gd-IgA1) and the molecular chaperone of T-synthase (core1β3-galactosyltransferase, C1GALT1), Cosmc (core1β3Gal-T specific molecular chaperone), play an important role in the pathogenesis of HSP. However, it is unclear how both cause vascular wall damage. Advanced oxidation protein products (AOPPs) are specific markers of protein oxidation. They are not only the products of oxidative stress but also exacerbate and perpetuate oxidative damage. Camilla *et al.*[2] discovered that AOPP levels and the aberrant glycosylation of IgA1 in the serum of children with IgA nephropathy were significantly higher than those of the control group and were found to positively correlate with proteinuria

levels in the course of the disease and negatively correlate with the rate of decline in renal function. It is speculated that the oxidative stress in children with IgA nephropathy may enhance the renal toxicity of Gd-IgA1.

In this study, serum AOPP and Gd-IgA1 levels and Cosmc mRNA expression in peripheral blood B lymphocytes in HSP patients with different clinical manifestations were studied, their correlations were analyzed, and their roles in the pathogenesis of HSP were explored.

Materials and methods

Subjects

Fifty-one patients, aged 6–42, were selected from the outpatient and inpatient populations diagnosed with HSP in the Second Hospital of Hebei Medical University, Shijiazhuang, China, from January to October 2016. There were 27 males and 24 females. The duration of disease ranged 2–60 days. The patients had normal platelet counts and coagulation functions and were not diagnosed with other allergic diseases, infections, autoimmune disorders, or other serious systemic conditions. The patients did not take glucocorticoids, antioxidants, immunosuppressive agents, or other drugs and were all selected based on the HSP diagnostic criteria[3] revised by the 2008 European Rheumatism Association. According to the clinical manifestations and laboratory tests, the patients were divided into four subgroups: (i) Skin subgroup: 20 patients, typical skin purpura without abdominal pain, joint pain, and kidney involvement, no abnormalities found with routine urinalysis and 24-hour urine protein quantification; (ii) Joint subgroup: eight patients, typical skin purpura accompanied by joint swelling and pain; (iii) Abdominal subgroup: 12 patients, typical skin purpura accompanied by abdominal pain and positive fecal occult blood test; (iv) Renal subgroup: 11 patients, typical skin purpura accompanied by varying degrees of renal damage, urinalysis positive for proteinuria and/or hematuria. The 51 HSP patients were scored according to the criteria for HSP disease severity[4,5] (Table 1). This study has been approved by the Ethics Committee of the Second Hospital of Hebei Medical University, and informed consents have been obtained from the patients and their families.

Table 1 Henoch–Schonlein purpura (HSP): Disease severity score sheet[4,5]

Subgroup	Symptom	Score
Skin type	No rash	0
	Affecting only one part[a]	1
	Affecting two parts	2
	Affecting more than two parts	3
Joint type	No joint symptoms	0
	Joint pain and/or mild swelling (walk normally)	1
	Joint pain and/or medium swelling (walk with difficulty)	2
	Joint pain and/or serious swelling (refuse to walk)	3
Abdominal type	No abdominal pain	0
	Mild abdominal pain and/or occult blood in the stool (+)[b]	1
	Medium abdominal pain and/or occult blood in the stool (++)	2
	Serious abdominal pain and/or occult blood in the stool (+++)	3
Kidney type	No proteinuria and/or < 5 RBC/HPF	0
	Proteinuria < 30 mg/dl and/or 6–10 RBC/HPF	1
	Proteinuria 30–150 mg/dl and/or 11–15 RBC/HPF	2
	Proteinuria > 150 mg/dl and/or > 15 RBC/HPF	3

[a] According to the HSP distribution area, the whole body surface is divided into five parts: I – feet and calves, II – upper limbs, III – thighs, IV – buttocks, V – other area.
[b] Fecal occult blood is used to detect blood in the stool. The results are determined by the positive color change caused by chemical reaction. The symbols in the table (+, + +, + + +) are determined by the color changes that are closely related to the number of red blood cells.

Reagents

The human peripheral blood lymphocyte separation fluid was obtained from Tianjin Haoyang Biological Manufacture Co., Ltd (Tianjin, China). The mouse anti-human CD19-PE antibody was obtained from Becton Dickinson (NJ, USA). Trizol Reagent was obtained from Invitrogen (CA, USA). First Strand cDNA Synthesis Kit was obtained from Thermo Scientific (MA, USA). TransStart Green qPCR SuperMix was obtained from Transgen Biotech (Beijing, China). Human serum Gd-IgA1 ELISA test kit and human AOPP ELISA test kit were obtained from Beijing Dongge Biotechnology Co., Ltd. (Beijing, China).

Detection of Cosmc mRNA expression in peripheral blood B lymphocytes

The peripheral blood mononuclear cells were separated according to the instructions for human peripheral blood lymphocyte separation. Two hundred microliters of PBS was added to prepare a cell suspension by pipetting repeatedly. Twenty microliters of mouse anti-human CD19-PE antibody was added to the cell suspension, mixed well, and incubated at room temperature for 15 minutes in the dark. After the unbound antibody was eluted, 1 ml PBS was added and mixed well by pipetting to prepare a cell suspension. The cells were sorted by flow cytometry and collected in tubes with RPMI1640 culture medium, which contained 10% fetal bovine serum. The suspension was centrifuged, washed, and the remaining precipitate of B lymphocytes was collected. The total RNA was extracted according to the Trizol instruction manual. The RNA purity and concentration were measured according to the

instructions for Nano Drop 2000c Spectrophotometer. Following the instructions for the First Strand cDNA Synthesis Kit, 1 μg of total RNA was reverse transcribed. All steps were carried out on ice, the total reaction volume was 12 μl, and the resulting cDNA was stored at − 70 °C. The fluorescence quantitative RT-PCR reaction volume was 20 μl; reaction cycles were 95 °C 15s, 60 °C 30s, 72 °C 15s for a total of 45 cycles. Melting curve analysis: 95 °C 1 min, 60 °C 30s, 95 °C 30s for 1 cycle. The relative expression of Cosmc mRNA was calculated by RQ = 2 − ΔΔCt relative quantification. Primer sequence: GAPDH-F: 5'-CAGTCAGCCGCATCTTCTTTT-3', GAPDH-R: 5'-GTGACCAGGCGCCCAATAC-3', Cosmc-F: 5'-TTTGAAGGGTGTGATGCTTG-3', Cosmc-R: 5'-ATGCGCTCATCCTCTGAAAT-3'.

Determination of the serum levels of AOPPs and Gd-IgA1

The measurements were obtained according to the instructions for the ELISA test kits.

Statistical analysis

All data were analyzed using SPSS 21.0 statistical analysis software with $P < 0.05$ as statistically significant. The data were tested for normality using the Shapiro–Wilk test, and the results were expressed as X ± S or MD (IQR), respectively, based on whether the assumption of normality was satisfied. For data that met the assumption of normality and variance homogeneity without modifying or after data conversion, one-way ANOVA single-factor variance analysis was used to compare in multiple groups, and LSD test was used for comparison between any two groups. Otherwise, Kruskal–Wallis *H* test for nonparametric tests was used. Spearman's rank correlation was used for correlation analysis, and the results were expressed as correlation coefficient *r*.

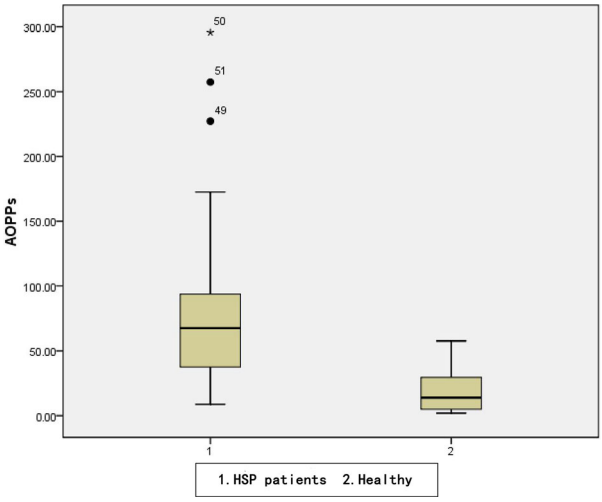

Figure 1 Henoch–Schonlein purpura (HSP):Serum levels of AOPPs in HSP patient group and control group

Results

The serum levels of AOPPs in HSP patients and controls did not satisfy the assumption of normality, and the results were expressed in MD (IQR). After square root conversion, the data met the assumption of normality ($P > 0.1$) with unequal variance ($P < 0.1$). The unequal variance *t*-test was used for comparison between two groups. The results show that the serum levels of

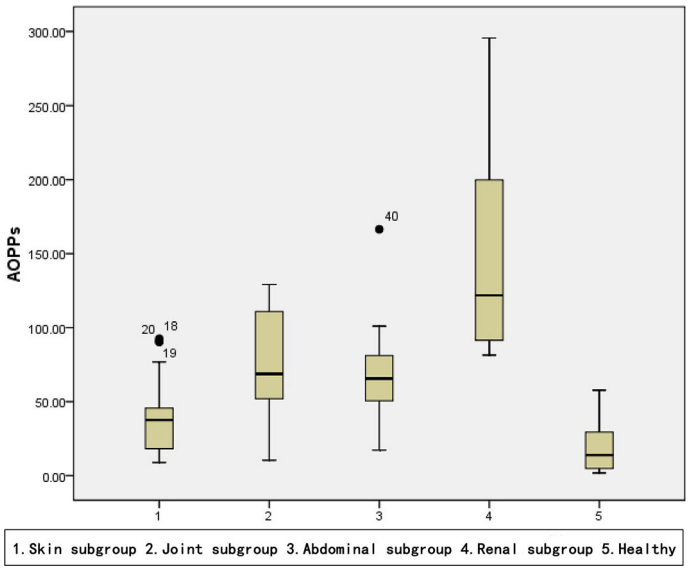

Figure 2 Serum levels of AOPPs in different subgroups of Henoch–Schonlein purpura (HSP) patients and control group

Table 2 Henoch–Schonlein purpura (HSP): Serum levels of advanced oxidation protein products (AOPPs) in HSP patients and control group

Group	Sample size	AOPPs (μmol/l) MD (IQR)
HSP patients	51	67.570 (60.80)
Controls	18	13.890 (25.09)
After square root conversion		$t' = 6.277$
Modified *t*-test		$P = 0.000$

Table 3 Henoch–Schonlein purpura (HSP): Serum levels of advanced oxidation protein products (AOPPs) in different subgroups of HSP patients and controls

Subgroup	Sample size	AOPPs (μmol/L) (MD(IQR))
Skin subgroup	20	37.585 (28.99)[ab]
Joint subgroup	8	68.610 (67.70)[abc]
Abdominal subgroup	12	65.710 (34.19)[abc]
Renal subgroup	114	121.750 (139.49)[ac]
Controls	18	13.890 (25.09)
After square root conversion		$F = 23.068$
Single-factor ANOVA		$P = 0.000$

[a]Significantly higher compared with control group $P < 0.01$.
[b]Significantly lower compared with renal-type subgroup $P < 0.01$.
[c]Significantly higher compared with skin-type subgroup $P < 0.01$.

AOPPs in HSP patients are significantly different from those in controls ($t = 6.277$, $P = 0.000$) (Fig. 1 and 2).

The serum levels of AOPPs in subgroups of HSP patients did not satisfy the assumption of normality, and the results were expressed in MD (IQR). After square root conversion, the data satisfied the assumption of normality and variance homogeneity ($P > 0.1$). One-way ANOVA single-factor variance analysis was used for comparison between multiple groups, and the LSD test was used for comparison between two groups. The results show that there is a significant difference between the serum levels of AOPPs in subgroups of HSP patients and controls ($F = 23.068$, $P = 0.000 < 0.05$), with the HSP patients having significantly higher AOPP levels than healthy controls. The renal subgroup has higher AOPP levels than the skin, joint, and abdominal subgroups ($P < 0.05$). The skin subgroup has lower AOPP levels than the other three subgroups ($P < 0.05$). There is no significant difference between the joint and the abdominal subgroups ($P > 0.05$) (Fig. 2 and Table 3).

Spearman's correlation analysis shows: (i) The serum levels of AOPPs and Gd-IgA1 in HSP patients are positively correlated ($r = 0.876$, $P < 0.01$); (ii) There is a significant negative correlation between serum levels of AOPPs and the expression of Cosmc mRNA in HSP patients ($r = -0.678$, $P < 0.01$); (iii) There is a positive correlation between the serum levels of AOPPs in HSP patients and HSP severity scores ($r = 0.796$, $P < 0.01$) (Figs. 3–5).

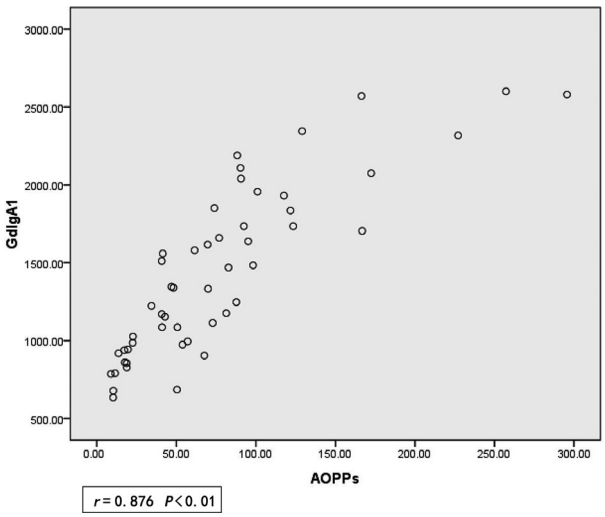

Figure 3 Correlation between the serum levels of AOPPs and Gd-IgA1 in Henoch–Schonlein purpura (HSP) patients

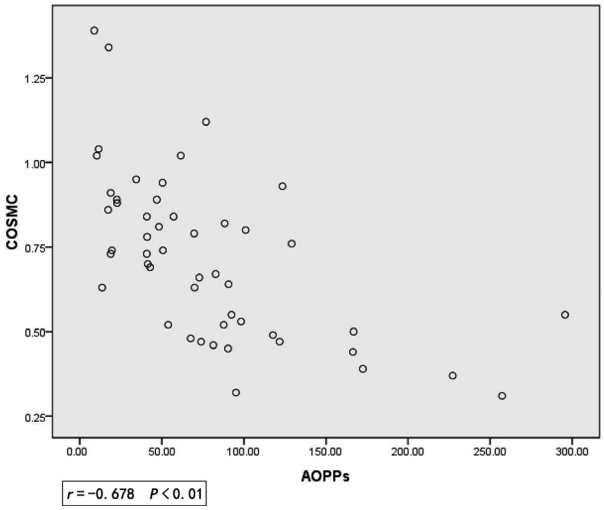

Figure 4 Correlation between the expression of Cosmc mRNA in peripheral B lymphocytes and the serum levels of AOPPs in Henoch–Schonlein purpura (HSP) patients

Discussions

Oxidative stress refers to the imbalance between oxidation and antioxidation actions. Oxidized intermediates and ROS will be produced if oxidation action is stronger than antioxidation action.

During oxidative stress, protein is more prone to oxidative modification than carbohydrates and fats and accumulates in the body. Advanced oxidation protein products are the final oxidation

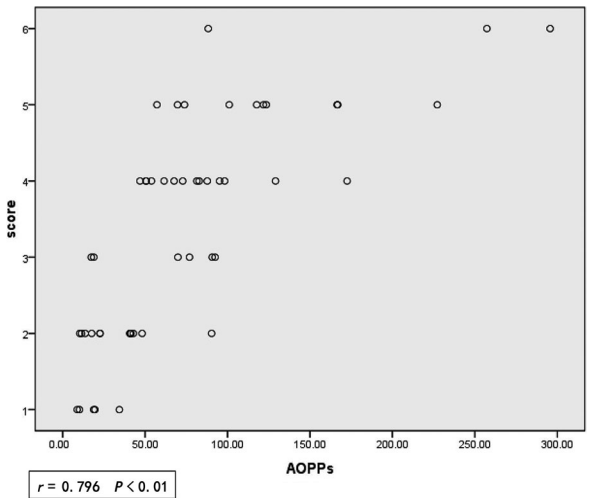

Figure 5 Correlation between the serum levels of AOPPs in Henoch–Schonlein purpura (HSP) patients and the disease severity scores

products of various proteins.[6] They are cross-linked products of serum albumin-based proteins oxidized by hypochlorous acid or chloramine generated by activated phagocytic cells in the process of chlorination and oxidation. AOPPs are considered to be a specific marker for protein oxidization and have a double tyrosine structure characteristic of oxidized proteins.[7]

The studies in vitro by Grzebyk *et al.*[8] show that AOPPs can stimulate neutrophils and monocytes to synthesize and release IL-8, TNF-α, and other inflammatory cytokines, triggering the respiratory burst and inducing vascular endothelial cells to produce ROS, which may lead to inflammatory damage and apoptosis of tissue cells. ROS can also directly or indirectly act on p53, jun, Akt, and other transcription factors to involve in the regulation of MAPK, NF-κβ, and other signaling pathways to mediate T and B lymphocyte differentiation, proliferation, and signal transduction.[9]

Camilla *et al.*[2] found that elevated serum AOPPs and Gd-IgA1 are strong risk factors during the progression of IgA nephropathy and that the synergistic effect of these two factors contributes to kidney damage.

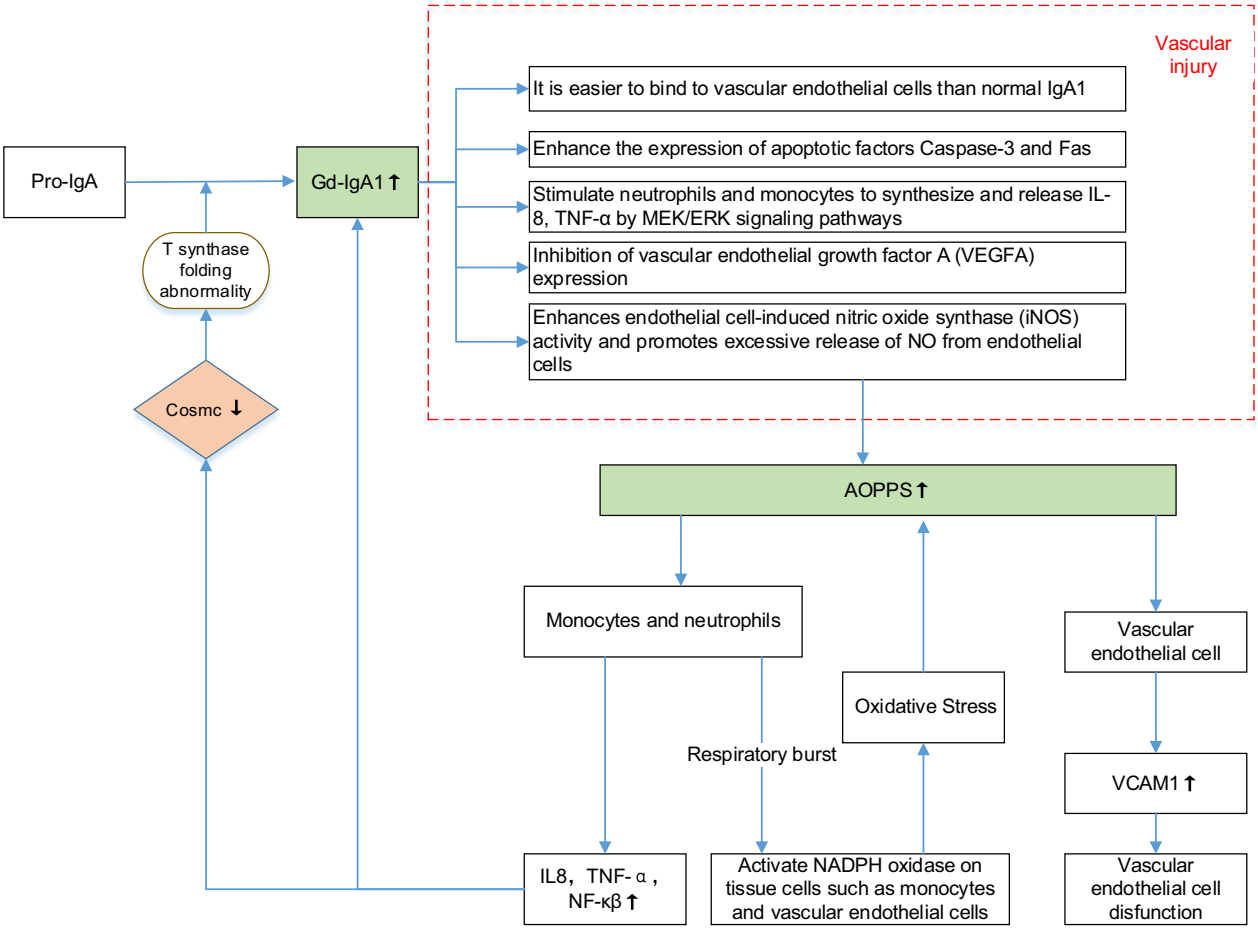

Figure 6 The possible relationship between AOPPS, Gd-IgA1, and Cosmc mRNA expression. Cosmc, core1β3Gal-T specific molecular chaperone; Gd-IgA1, galactose-deficient IgA1; AOPPs, Advanced oxidation protein products; NADPH, Nicotinamide adenine dinucleotide phosphate; VCAM, Vascular cell adhesion protein 1

Henoch–Schonlein purpura is a type of leukocytoclastic vasculitis. A large number of studies have shown that oxidative stress plays a key role in the pathogenesis. Nurcan Keskin *et al*. found that patients at the active stage had significantly higher AOPP levels than those at the remission stage of HSP and the controls. The mean AOPP levels of the patients with arthritis and/or arthralgia were significantly higher than those without joint involvement.[10] Our study also found that serum levels of AOPPs in HSP patients are significantly higher than those in healthy controls, but AOPP levels are more significantly elevated in the renal subgroup than the other three subgroups. Serum AOPP levels are positively correlated with serum Gd-IgA1 levels, and negatively correlated with the Cosmc mRNA expression in peripheral blood. These results suggest that AOPPs may play an important role in the development of HSP and that AOPPs and Gd-IgA1 act synergistically.

Advanced oxidation protein products (AOPPs) are not only the products of oxidative stress, they also induce monocytes and neutrophils to produce ROS and aggravate oxidative damage and chronic inflammation. AOPPs can also activate NF-κβ transcription factor and induce nitric oxide synthase.[11,12] The studies by Mahajan et al.[13,14] have shown that the serum levels of NO and nitric oxide synthase activity in patients with HSP were significantly higher in the acute phase than those in remission or the control group. AOPPs can also activate monocyte NADPH oxidase, release ROS, promote P38 phosphorylation, and result in increased secretion of TNF-α. Meanwhile, the resulting ROS can cause cell damage.[15] Gd-IgA1 can activate the NF-κβ transcription factor in monocytes.[16] These results suggest that NF-κβ may be the common target of AOPPs and Gd-IgA1 in the pathogenesis of HSP.

In our previous study, we found that the Cosmc expression in HSP patients is abnormal and the level of Gd-IgA1 is increased. AOPPs may induce and exacerbate tissue damage by decreasing the expression of Cosmc mRNA, increasing the level of Gd-IgA1, and acting synergistically with them.

In this study, it was found that the serum levels of AOPPs in HSP patients and the HSP severity scores are positively correlated, suggesting that AOPPs can be used as one of the indicators for disease severity and prognosis during the course of disease. Additionally, it provides a theoretical basis for vitamin C, vitamin E, and other antioxidants to be used in the treatment of HSP (Fig. 6).

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