

Urinary leukotriene E₄ in Henoch–Schonlein purpura

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Summary

Background Children with Henoch–Schonlein purpura (HSP) occasionally have allergic disease. We have previously shown that pranlukast hydrate was effective for purpura in HSP. Pranlukast hydrate is a leukotriene (LT) receptor antagonist; therefore, it is likely that LTs take part in the cause of HSP. Urinary leukotriene E₄ (LTE_{4u}) levels are a useful index of whole-body cysteinyl LT production *in vivo*. In this study, LTE_{4u} was examined in children with HSP.

Objective The purpose of this study was to examine the relation between the level of LTE_{4u} and the cause of HSP.

Methods Eighteen HSP children (six boys and 12 girls) and six healthy children were enrolled.

Results LTE_{4u} levels in patients with HSP were significantly higher ($P < 0.05$) at the onset than those in healthy children. Four weeks therapy with pranlukast hydrate lowers LTE_{4u} levels in patients with HSP ($P < 0.05$). There were no differences in LTE_{4u} between the group of HSP patients with purpura nephritis and the group of HSP patients without purpura nephritis.

Conclusion These results indicate that cysteinyl LTs may play a role in the pathophysiology of purpura in HSP.

Keywords cysteinyl leukotriene, Henoch–Schonlein purpura, leukotriene E₄, pranlukast hydrate

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Introduction

Henoch–Schonlein purpura (HSP) is a vasculitis of small vessels over the whole body. It is the most common cause of non-thrombocytopenic purpura in children. HSP is more frequent in children than adults, mostly at pre-school age. The classical presentation is with purpura, arthritis, hemorrhagic gastrointestinal involvement and glomerulonephritis. Most cases of this disease are benign and self-limiting, but recurrence is common.

The cause of HSP is unknown, but this illness follows bacterial infection (Streptococciosis, Mycoplasma and others), viral infection (Epstein-Barr, herpes, cytomegalovirus, adenovirus and others), drug allergy, food allergy, insect bites, immunization and other triggers [1–4]. These findings suggest that allergic reactions participate in the onset of this disease.

This disease tends to be regarded as a special form of allergic vasculitis. It is also known as ‘anaphylactoid purpura’. We have studied the serum IgE levels in HSP patients, and higher levels of serum IgE were observed in HSP patients. From the medical history of these patients, allergic diseases (atopic dermatitis, bronchial asthma and food allergy) are often existent. Davin et al described that IgE play a possible pathogenic role in HSP [5]. At present, there are no effective treatments directed at the basic cause of HSP. Therefore, we

started anti-allergic therapy for this disease after gaining informed consent. Anti-allergic drugs (sodium cromoglicate, tranilast, ketotifen fumarate, pranlukast hydrate and montelukast sodium) were prescribed to patients with allergic disease. Leukotriene (LT) receptor antagonists (pranlukast hydrate and montelukast sodium) had a fair efficacy against the purpura of this disease. We therefore started a study of urinary leukotriene E₄ (LTE_{4u}) in HSP patients, as LTE_{4u} serves as an indicator of whole-body cysteinyl LT production [6].

Materials and methods

Subjects

Eighteen patients (six boys, 12 girls, age range 4–15 years, average age 6.59 ± 2.57 (1SD (standard deviation))) were investigated. All subjects had normal renal function. Patients whose serum creatinine was over 1.0 mg/dL or creatinine clearance was under 80 mL/min/1.73 m² were excluded. None of the patients studied had taken any steroidal or non-steroidal anti-inflammatory drugs. As a control group, six healthy subjects (3 boys, 3 girls, age range 3–10 years, average age 6.5 ± 2.88 (1SD)) were studied. These children had no allergic history, no familial history on allergic diseases and their serum IgE levels were under 30 IU/mL. Informed consent was obtained from parents whose children participated in this study.

The HSP diagnosis was based on the association of non-thrombocytopenic and characteristic purpura, arthritis and gastrointestinal symptoms (colic pain, diarrhea with bleeding).

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Measurement of endogenous urinary leukotriene E₄

Urine samples were obtained from first, morning samples and those were stored at -80°C until analysis. Analysis of LTE_{4u} was carried out by enzyme immunoassay (EIA) and internal standard recovery, after reversed-phase high-performance liquid chromatography (HPLC) purification of urine samples. Urine samples (10 mL) were centrifuged, acidified to pH 4.5 by addition of 0.1 M HCl, homogenized and passed through activated Sep-Pak C18 cartridges (Waters Associates, Millipore, Milford, MA, USA). The cartridges were washed with distilled water and eluted with 5 mL aqueous methanol containing 1 mM HTMP and 0.5 mM EDTA. The eluates were evaporated to dryness under reduced pressure and resuspended in 30% ice-cold aqueous methanol. The samples were then injected into a C18 Hypersil column (4.6×250 mm, 5- μm particles; Shandon, Runcorn, UK) with a C18 precolumn and eluted isocratically through an HPLC system at a constant flow rate of 1 mL/min, using a mixture of methanol/water (60:40, v/v), with the aqueous part containing 0.1% acetic acid, 1 mM EDTA and adjusted to pH 5.6 with ammonium hydroxide. Fractions corresponding to the elution of radioactivity were pooled, dried *in vacuo*, resuspended in EIA buffer and assayed directly by EIA (Cayman Chemicals, Ann Arbor, MA, USA). For measurement of absorbance Spectra MAX 250 (Molecular Devices, Orleans, USA) was used.

Measurement of urinary creatinine

Urine samples were deproteinized with trichloroacetic acid. Creatinine was measured in the alkalinized supernatants with picric acid. The results of LTE_{4u} were expressed as pmol of excreted mediator per mmol of creatinine.

Pranlukast hydrate therapy

Patients with HSP were administered pranlukast hydrate at 5 mg/kg, orally, twice a day. Pranlukast hydrate was provided by ONO Pharmaceutical (Osaka, Japan).

Purpura nephritis

Purpura nephritis was defined as proteinuria (over 50 mg/dL) or hematuria (over 5 RBC/hyper powered field).

Statistical analysis

The Mann–Whitney *U*-test and two-way repeated-measures ANOVA were used for statistical comparison between urinary LT metabolite concentrations from patients and control subjects. Differences were considered significant at $P < 0.05$. Values are expressed as mean $\pm$ SD.

Results

Serum IgE level

Serum IgE levels of 18 patients with HSP were measured. The median was 127.5 IU/mL. The mean $\pm$ SD values were

119.4 ± 56.2 IU/mL. The standard serum IgE level of 6-year-old healthy Japanese children is 23.87 IU/mL. Serum IgE level with HSP was higher than that of healthy children.

Urinary leukotriene E₄ in HSP children at the onset

LTE_{4u} in HSP children was 132.67 ± 77.78 (pmol/mmol Cr). LTE_{4u} in healthy children was 61.17 ± 26.22 (pmol/mmol Cr).

Patients with HSP excreted significantly ($P = 0.0125$) higher amounts of LTE_{4u} than did healthy subjects.

LTE_{4u} was higher in children with HSP at onset than in healthy children.

Urinary leukotriene E₄ in HSP children after 4 weeks of pranlukast hydrate therapy

Table 1 shows the mean $\pm$ SD. LTE_{4u} values at onset and after 4 weeks pranlukast hydrate therapy in HSP patients studied, demonstrating a significant difference in LTE_{4u} excretion ($P = 0.037$).

Comparison of urinary leukotriene E₄ levels in Henoch–Schönlein purpura patients with or without nephritis

Table 2 shows the mean $\pm$ SD. LTE_{4u} values in HSP patients with or without nephritis. There was no significant difference in LTE_{4u} excretion at onset and after 4 weeks of pranlukast hydrate therapy.

Discussion

The results of the present study demonstrate that LTE_{4u}, at the onset of HSP, was higher than in healthy children. These experiments may suggest that LTs participate in the onset of HSP. Furthermore, they may suggest that the cause of vasculitis in HSP was an allergic reaction. Davin et al. [5] reported that the incidence of increased plasma IgE levels according to age-matched normal values was significantly higher in HSP patients. In our study, serum IgE levels in HSP

Table 1. LTE_{4u} after pranlukast hydrate therapy

At onset	132.67 ± 77.78 (pmol/mmol Cr)
Four weeks after	81.35 ± 55.78 (pmol/mmol Cr)
<i>P</i> -value	0.037

Values are the mean $\pm$ SD.

LTE_{4u}, urinary leukotriene E₄; SD, standard deviation.

Table 2. Comparison of LTE_{4u} (pmol/mmol Cr) levels in HSP patients

	Onset	Four weeks after
With nephritis ($n = 11$)	168.14 ± 78.7	100.53 ± 62.5
Without nephritis ($n = 7$)	106.94 ± 29.6	23.71 ± 23.7
<i>P</i> -value	0.11	0.065

Values are the mean $\pm$ SD.

HSP, Henoch–Schönlein purpura; LTE_{4u}, urinary leukotriene E₄; SD, standard deviation.

patients were also higher than healthy children. Moreover, IgE deposits were demonstrated in Langerhans and mast cells in four of six patients with HSP nephritis. They concluded that stimulation of IgE-sensitized mast cells by specific antigens in the presence of IgA circulating immune complexes (CIC), releases vasoactive substances, which were causing increased capillary permeability and perivascular deposition of IgA CIC. The specific pathogenesis of HSP is not known, but from histopathology results this illness is considered to be an IgA-mediated vasculitis of small vessels. Immunofluorescence techniques show deposition of IgA and C3 in the small vessels of the skin and renal glomeruli, but the role of complement activation is controversial. In our study, we could not investigate IgA CIC. In addition, we think that serum IgE levels are not necessarily reflected allergic state in patients, but are one of the important indicators on allergic background.

Kumlin [6] described that LTE_{4u} is a reliable means to measure *in vivo* production of cysteinyl LTs and that LTE₄ in human urine may be useful in further clinical trials with anti-LT drugs. Denzlinger et al. [7] provide the direct evidence that LTs may be involved in anaphylactic reactions in human. Measurement of urinary markers of inflammation (9 α ,11 β -PGF₂, N⁷-methylhistamine and LTE₄) could prove useful not only in elucidating basic mechanisms, but also in the diagnosis and monitoring of patients with inflammatory disorders [8]. Wennergren [9] described that urinary excretion of LTE₄ reflects the production of cysteinyl LTs. In the future, monitoring of cytokines may be used in clinical asthma to monitor, control and aid in the prognosis of the disease in young children. Increased LTE_{4u} is indicative of ongoing inflammatory activity in the airways or other organs [10]. On the contrary, Kopp et al. [11] mentioned that the LTE₄ levels in urine are not helpful in monitoring patients treated with anti-IgE and specific immunotherapy. For the present, we think that LTE_{4u} is a useful marker of the inflammatory activity in man. It remains to be determined whether LTE_{4u} might be useful for the monitoring of the disease activity. These complex issues await further studies.

Significant attenuation of LTE_{4u} was achieved by administration with a LT receptor antagonist, pranlukast hydrate. This suggests that the pranlukast hydrate affects the attenuation of LTs. Pranlukast hydrate is a highly potent, selective and competitive antagonist of peptide LTs [12, 13]. These findings were consistent with our hypothesis that LTs take part in the onset of HSP. Although the number of patients is small in this study, we already reported successful pranlukast hydrate treatment to the symptom of purpura in HSP [14]. Hence, the efficacy of the pranlukast hydrate treatment to the HSP is needed to examine the control study.

The level of LTE_{4u} in HSP patients without nephritis after four weeks pranlukast hydrate treatment was lower than those observed in controls. In contrast, the level of LTE_{4u} in HSP patients with nephritis after four weeks pranlukast hydrate treatment are still elevated than those observed in controls. These results suggest that nephritis was one of the factors, which influenced the level of LTE_{4u}.

In the present study, we could not demonstrate the sex- and age-matched control data. Furthermore, to our knowledge, there have been no reports of the normal range of LTE_{4u} on every age. The normal range of LTE_{4u} on every age remains to be determined. Therefore, we have to study on these points in future, and to test our hypothesis on the etiology on HSP, further studies are warranted.

In addition, HSP is a self-limiting disease. We think that the indication of pranlukast hydrate treatment for HSP is required to be prudent.

Conclusions

Data obtained suggest that pranlukast hydrate may be efficacious for the treatment of purpura in HSP, through reducing the effect of LTs. These results indicate that LTs may play a role in the development and persistence of purpura in HSP.

References

- 1 Ackroyd JF. Allergic purpura, including purpura due to foods, drugs and infections. *Am J Med* 1953; 14:605–31.
- 2 Burke DM, Jellinek HL. Nearly fatal case of Schonlein–Henoch syndrome following insect bite. *Am J Dis Child* 1954; 88:772–5.
- 3 Jensen B. Schonlein–Henoch's purpura. Three cases with fish or penicillin as antigen. *Acta Med Scand* 1955; 152:61–70.
- 4 Sharan G, Anand RK, Sinha KP. Shonlein–Henoch syndrome after insect bite. *Brit Med J* 1966; 1:656.
- 5 Davin JC, Pierard G, Dechenne C et al. Possible pathogenic role of IgE in Henoch–Schonlein purpura. *Pediatr Nephrol* 1994; 8:169–71.
- 6 Kumlin M. Measurements of leukotrienes in the urine: strategies and applications. *Allergy* 1997; 52:124–35.
- 7 Denzlinger C, Haberl C, Wilmanns W. Cysteinyl leukotriene production in anaphylactic reactions. *Int Arch Allergy Immunol* 1995; 108:158–64.
- 8 O'Sullivan S, Roquet A, Dahlen B, Dahlen SE, Kumlin M. Urinary excretion of inflammatory mediators during allergen-induced early and late phase asthmatic reactions. *Clin Exp Allergy* 1998; 28:1332–9.
- 9 Wennergren G. Inflammatory mediators in blood and urine. *Pediatr Respir Rev* 2000; 1:259–65.
- 10 Kumlin M. Measurement of leukotrienes in humans. *Am J Respir Crit Care Med* 2000; 161:S102–6.
- 11 Kopp MV, Mayatepek E, Engels E et al. Urinary leukotriene E₄ levels in children with allergic rhinitis treated with specific immunotherapy and anti-IgE (Omalizumab). *Pediatr Allergy Immunol* 2003; 14:401–4.
- 12 Obata T, Okada Y, Motoishi M, Nakagawa N, Terawaki T, Aishita H. *In vitro* antagonism of ONO-1078, a newly developed anti-asthma agent, against peptide leukotrienes in isolated guinea pig tissues. *Jpn J Pharmacol* 1992; 60:227–37.
- 13 Yonetomi Y, Fujita M, Nakagawa N, Obata T. Preclinical pharmacology of pranlukast. *Clin Exp Allergy Rev* 2001; 1:210–7.
- 14 Tsuji Y, Narui K, Takayanagi T et al. Effectiveness of pranlukast hydrate for Henoch–Schonlein purpura. (English abstract) *Jpn J Pediatr Nephrol* 1998; 11:185–90.