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Inverse temporal changes of lipoxin A_4 and leukotrienes in children with Henoch–Schönlein purpura ☆

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ABSTRACT

The pathogenesis of Henoch–Schönlein purpura (HSP) is not clearly understood. It remains unclear how changes of lipoxin A_4 (LXA_4) that acts as a "braking signal" in inflammatory process occur in patients with HSP. In this study, we determined the temporal changes of blood and urinary LXA_4, Leukotriene (LT)B_4 and urinary LTE_4 in 49 children with HSP. Inverse temporal changes between gradually increased blood and urinary LXA_4 and gradually decreased blood and urinary LTB_4 and urinary LTE_4 were found in patients with HSP. Furthermore, both 15-S-hydroxyeicosatetraenoic acid and LXA_4 inhibited the LTB_4-induced chemotaxis of leukocytes and release of LTB_4 from leukocytes obtained from the patients in the active phase of HSP. In 22 children with HSP nephritis, concordant with the gradually increased severity of mesangial proliferation and proteinuria, the glomerular expressions of 15-lipoxygenase and the levels of urinary LXA_4 gradually decreased and the glomerular expressions of LTC_4 synthase and the urinary LTE_4 and LTB_4 gradually increased. The levels of blood and urinary LXA_4 in patients with HSP nephritis were lower than those in patients with purpura alone in early resolution of HSP. The levels of blood and urinary LTB_4 and urinary LTE_4 in the patients with HSP nephritis were higher than those in patients with purpura alone in early resolution of HSP. There was positive correlation between blood LTB_4 and serum C-reactive protein in 49 children with HSP. These data suggest that LTs may play a proinflammatory and profibrotic role in the pathogenesis of HSP, and insufficiency of LXA_4 may be responsible for the patients with HSP whose illness become more serious.

1. Introduction

Henoch–Schönlein purpura (HSP) is a form of vasculitis affecting skin and other organs, especially joints, the gut and kidneys. It is the most common vasculitis of childhood [1]. Until now, the pathogenesis of HSP, which often leads to sclerosis of the glomeruli, is not clearly understood [2]. Previous studies suggested that proinflammatory cytokines such as interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8 and tumor necrosis factor-α (TNF-α) may play an aggravating role in the development of inflammation and glomerular damage in HSP [3–6]. Leukotrienes (LTs) were also involved in the pathogenesis of HSP [7]. Lipoxin A_4 (LXA_4) is an endogenously produced eicosanoid, inhibits recruitment, activation of neutrophils and eosinophils, and promotes resolution of inflammation, and inhibits the production and/or action of many proinflammatory cytokines such as IL-1β, IL-6, IL-8, TNF-α, thereby acting as an endogenous "braking signal" in the inflammatory process [8–11]. However, it remains unclear that how changes of LXA_4 in patients with HSP occur. The present studies were therefore undertaken to find the dynamic changes of blood and urinary LXA_4, LTB_4 and urinary LTE_4 in children with HSP, and to explore the relationship between these eicosanoids and the degree of severity of the disease.

2. Patients and methods

2.1. Patients

Forty-nine patients with HSP from Department of Pediatrics, First Affiliated Hospital of Nanjing Medical University, Nanjing First Hospital Affiliated to Nanjing Medical University and Central Hospital of Tengzhou in Shandong Province were enrolled in this study. The diagnosis of HSP was based on the five clinical features described by Szer [2].These patients were divided into three groups. As shown in Table 1, the patients in group 1 only

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Table 1
Clinical data of the patients with Henoch–Schönlein purpura and controls.

Groups	*N*	Mean age	Symptom and sign				Proteinuria (g/24 h)	GFR (ml/min/1.73 m^2)	Serum CRP (mg/L)
			Skin	Nephritis	Joints	Gut			
Group 1	16	7.4	+	–	–	–	<0.15	107.4 ± 20.1	18.6 ± 12.1
Group 2	11	8.0	+	–	+	±	<0.15	113.6 ± 18.8	24.8 ± 13.8
Group 3	22	9.2	+	+	±	±	0.15–2.2	102.3 ± 18.4	41.8 ± 17.6
Controls	27	9.0	–	–	–	–	<0.15	116.8 ± 17.6	4.8 ± 2.6

Note: GFR: glomerular filtration rate; CRP: C-reactive protein.

manifested purpura. The patients in group 2 exhibited purpura and arthritis with or without abdominalgia and hematochezia. The patients in group 3 displayed nephritis with abnormal data of urinary examination, purpura with or without arthritis, abdominalgia and hematochezia. Eight out of the 22 patients in group 3 exhibited proteinuria less than 0.5 g/24 h on the day before treatment, and 11 out of 22 patients exhibited proteinuria 0.5–1.0 g/24 h, and 3 out of 22 patients exhibited proteinuria more than 1.0 g/24 h. Venous blood specimens were obtained from the patients on the day before treatment, named active phase, and in 3–8 days after disappearance of purpura, arthritis, abdominalgia, and hematochezia, and of gross hematuria and nephrotic range protenuria, named early resolution. Renal biopsies in the children of group 3 were taken on the day prior to the treatment. The patients of groups 2 and 3 were treated orally with prednisone. Twenty-seven normal healthy children were served as controls. The parents or guardians of all enrolled children had signed their name on the informed consent to a protocol approved by the Internal Review Board of our three Hospitals.

2.2. Assessment of blood LXA_4 and LTB_4

Whole blood samples were drawn into 5-ml tubes containing heparin and immediately exposed to the calcium ionophore A23187 (50 μM, Sigma, St. Louis, MO) 37 °C for 30 min [12]. After stimulation, reactions were stopped with 5 volumes of ice-cold methanol. The samples were centrifuged and the supernatants were diluted and acidified to pH 3.5 with HCl. Subsequently, eicosanoids were extracted with C_{18} Sep-Pak light column (Waters, Milford, MA) preactivated with methanol and deionized water. The column was washed with deionized water followed by petroleum ether. Materials in the methyl formate eluate were dried under a stream of N_2 and kept at −80 °C until LXA_4 and LTB_4 were measured by enzyme-linked immunosorbent assay (ELISA, Neogen, Lexington, KY) following the manufacturer's instructions.

2.3. Measurement of urinary LXA_4, LTB_4 and LTE_4

The extraction methods were carried out as described earlier [13]. Briefly, urinary samples were centrifuged, acidified and applied to a C_{18} Sep-Pak light column (Waters, Milford, MA). Materials were eluted with hexane/ethyl acetate and then dried. Residues were resuspended and applied to silica cartridges. Columns were washed with benzene/ethyl acetate and materials eluted with benzene/ethyl acetate/methanol. Eluates were dried and kept at −80 °C until LXA_4, LTB_4 and LTE_4 were measured by ELISA (Neogen, Lexington, KY) following the manufacturer's instructions. Urinary Cr was measured with enzymatic reaction. The results were expressed as LXA_4/mg Cr, LTB_4/mg Cr and LTE_4/mg Cr.

2.4. Glomerular pathologic studies

Cortical sections of formalin-fixed renal tissues were stained with hematoxylin and eosin, periodic acid-Schiff. Methanol-Carnoy-fixed cortical sections were immunohistochemically stained for IgG, IgA, IgM and C_3, and observed with light microscopy. The severity of the renal lesions in 22 patients with HSP nephritis were classified according to criteria agreed upon by pathologists of the International Study of Kidney Disease in Childhood (ISKDC) [1]. Expressions of LTC_4 synthase in the glomeruli were detected by indirect immune fluorescence after staining by using a rabbit anti-human LTC_4 synthase (LTC_4S) polyclonal IgG (Santa Cruz, Santa Cruz, CA) and subsequent fluorecein isothiocyanate-conjugated rat anti-rabbit IgG (DAKO, Copenhagen, Denmark). Expressions of 15-lipoxygenase (15-LO) in the glomeruli were determined by immunohistochemistry after staining by using goat anti-human 15-LO polyclonal IgG (V-17, Santa Cruz, Santa Cruz, CA) and subsequent biotinylated rabbit anti-goat IgG (Vector Lab, Burlingame, CA). Control sections were treated identically, but without the primary antibody, and did not show any positive staining. The mean ratio of LTC_4S positive or 15-LO positive cellular area to single glomerulus in 3–6 glomeruli of each patient was assessed by JD-801 computer-aided image analyzer under high power magnification (× 1000).

2.5. Chemotactic assays

Chemotactic assays were performed as described previously [14,15]. Phosphate buffered saline (PBS) containing LTB_4 (10 nM, Sigma, St. Louis, MO) was placed in the lower compartment of Transwell cluster plates (Costa Corning, Cambridge, MA) with two compartment chambers separated by a polycarbonate membrane filter. The leukocytes (2×10^6 cells/ml) obtained from 4 patients with HSP in the active phase were pretreated with the vehicle (1% ethanol in PBS), 15-S-hydroxyeicosatetraenoic acid (15-HETE, 30 nM, Biomol Res Lab, Plymouth Meeting, PA) or LXA_4 (30 nM, Sigma, St. Louis, MO) or 12-HETE (30 nM, Biomol Res Lab) for 20 min in a humidified incubator at 37 °C, and then added to the upper chamber and incubated at 37 °C for 30 min. Subsequently, the membrane filters were fixed and stained with hematoxylin and eosin. The polymorphonuclear leukocytes (PMNs) migrated from upper chamber were counted in duplicate per × 400 high power field (HPF) with light microscope. Pretreatment with 15-HETE, 12-HETE, LXA_4 and 1% ethanol did not alter PMN viability, which was measured as 95% by trypan blue vital dye exclusion.

2.6. Determination of LTB_4 released from leukocytes

The leukocytes (2×10^6 cells/ml) obtained from 4 patients with HSP in active phase were exposed to the A23187 (2 μM) plus vehicle (1% ethanol in PBS), or A23187 plus 15-HETE (30 nM), or A23187 plus LXA_4 (30 nM), or A23187 plus 12-HETE (30 nM) at