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The Role of Leukotrienes in the Pathogenesis of Steroid-Sensitive Nephrotic Syndrome

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Abstract: Endogenous leukotrienes (LT) levels in plasma and urine samples, and LT levels released from stimulated leukocytes in vitro were examined in children with steroid sensitive nephrotic syndrome (SSNS). Plasma LT concentrations were found to be higher in the SSNS group than in healthy subjects. Subjects with SSNS were also shown to have high levels of urinary cysteinyl-LTs and LTB_4, while healthy children had only urinary LTE_4 levels. In the second part of the study, peripheral leukocytes of patients and heatlhy children were stimulated by Ca ionophore, A23187. We detected significantly higher levels of LTs in patients with SSNS suggest that leukotrienes, with their biological effects, might be involved in the pathogenesis of steroid sensitive nephrotic syndrome.

Key Words: Steroid sensitive nephrotic syndrome, inflammation, leukotrienes, calcium ionophore.

Introduction

The glomeruli are very sensitive to inflammatory injury. Many studies are currently being done on cytokines and inflammatory mediators in various types of renal diseases. The spectrum of known proinflammatory compounds originating from glomeruli has expanded recently to include arachidonic acid-derived lipoxygenase metabolites and platelet activating factor (PAF) (1, 2). In recent experimental studies, the leukotrienes from the arachidonic acid lipoxygenase pathway have been demonstrated to play an important role in inflammatory reactions in glomeruli after immune or nonimmune injuries (3-5). The biological effects of cysteinyl-leukotrienes (LTs) (LTC_4, LTD_4 and LTE_4) on the kidneys include vasoconstriction, increase in arterial pressure, decrease in renal blood flow (RBF) and glomerular filtration rate (GFR), decrease in capillary pressure, proteinuria, ultrafiltration, oliguria, and mesangeal cell proliferation (4-7). LTC_4 causes epithelial cell proliferation and alters podocyte formation (8, 9). Nonpeptide LT (LTB_4), which is mostly released by endothelial cells, is a chemotactic factor for leukocytes. It mediates cell adhesion to endothelium and aggregation (6). Leukocytes and glomerular cells release PAF, Thromboxan A2, major basic protein (MBP), prostoglandins, hydroxyeicosatetraenoic acids (HETEs) and free radicals after activation by LTB_4. LTB_4 increases the permeability of the glomerular basement membrane (GBM), causes proteinuria, and decreases RBF and GFR (7, 10, 11).

Changes in LT levels in immune glomerular diseases have been implicated in many experimental studies by different authors (4, 6, 12-14). In our previous clinical studies, we determined increased urinary excretion of LTs and increased amounts of various LTs released by the leukocytes in different histopathological variants of glomerulonephritis such as mesangeal proliferative or membranoproliferative glomerulonephritis (15). The aim of this study was to demonstrate the possible roles of LTs in the pathophysiology of steroid-sensitive nephrotic syndrome (SSNS) by detecting concentrations of them in biological meterials.

Material and Methods

Patients: Seven patients (five male and two female, mean age 5.4±4.8 years, range 2.5-16 years) with SSNS hospitalized in the Department of Pediatric Nephrology of Ege University between 1993 and 1994 were included in the study in the acute period before the implementation of steroid treatment. Diagnosis of SSNS was made

Patient No. Age (years) Sex	Proteinuria (mg/m^2/h)	Serum albumin (g/L)	Serum T.Choles. (mg/dl)	Serum creatinine (mg/dl)	GFR (ml/min)	Clinicla status
1 (2.5, M)	72.5	20	295	0.7	80	First attack
2 (6, M)	95	25	386	0.9	129	First attack
3 (16, M)	93	23	661	0.8	128	Relaps, biopsy proven SSNS
4 (3, F)	52.5	25	486	0.4	144	First attack
5 (3, F)	60	24	486	0.6	81	Relaps, biopsy proven SSNS
6 (5, M)	52	25	370	0.4	133	First attack
7 (2.5, M)	75	24	565	0.3	153	Relaps, biopsy proven SSNS
Mean±SD	71.4± 17.8	23±1	464± 124.8	0.6± 0.2	121± 29	
Control Mean±SD	ND	50±4	149.5 ±24.6	0.5± 0.1	124± 35	
P value	<0.05	<0.05	<0.05	NS		

Table 1.   Laboratory and clinical data of the children with SSNS

NS: nonsignificant, ND: non detectable (less than 4 mg/m2/h))

according to the following criteria: 1) edema, 2) absence of hematuria either macroscopic or microscopic, 3) massive proteinuria (>40mg/m^2/h), 4) hypoalbuminemia (<25g/L), 5) normo-complementemia, 6) selective proteinuria, and 7) response to steroid. The clinical and laboratory features of the patients are shown in Table 1. Eight healthy children aged 3-14 years (mean 7 years, two female and six male) with normal urine analysis results and BUN levels were used as the control group.

Renal biopsy confirming minimal changes in glomeruli had been performed on three of the patients. The mean urinary protein concentration was 71.4±17.8 mg/m^2/h (Table 1).

Methods

Leukotrienes were obtained from Plasma by solid phase extraction using Sep-PAK C18 (Waters, Millford, USA) cartridges. The urinary LTs were extracted by the same method using Maxi-Clean C18-900 mg cartridges (Altech, USA) (16). LTs were separet from the samples at a flow rate of 1 ml/min in acetonitrile: water: methanol: acetic acid gradient (300:420:100:0.8) at pH:5.1 in a Waters HPLC system by Ultraspher ODS C$_{18}$ (5 micron) analytic columns (250x4.6 nm ID) and precolumns (100x4.6 nm ID). The separation and retention time of the LTs were obtained with the synthetic standards of LTC$_4$, LTD$_4$, LTE$_4$ and LTB$_4$ (Sigma, St. Louis, USA). The collected samples were dried under liquid nitrogen in a vacuum speed concentrator.

Quantitative measurement of LTs was done using specific LT(H^3) RIA kits (TRK-910, TRK-940, Amersham, UK) and a b-liquid scintillation analyser system (TRI-CARB-1600 TR, LSA-Packard, Canberra Company). Results were expressed as pmol/ml for plasma and pmol/mg urine creatinine for urine.

Following preparation of the leukocyte suspension, it was preincubated in a water bath at 37°C and incubated with A23187 for 15 min (17). At the end of incubation the supernatant was separated by centrifugation and LTs were extracted by using Sep-Pak C18 concentration columns, as in the extraction of LTs from plasma. The separation of LTs was performed by HPLC using Ultrasphere ODS C18-5μ, 250x4.6mm analytic and 100x4.6mm guard columns in acetonitrile-water-TFA eluent. Spectral analysis of LTs was done using synthetic LTC$_4$, LTD$_4$, LTE$_4$ and LTB$_4$ standards (Sigma, St.Louis, MO, USA). Amounts of LTs were measured by the Baseline 815 HPLC Software System using PGB$_2$ as an internal standard for LTs. The results were expressed as ng LT/10^7 leukocytes. The minimum detectable amount was 1.0 ng/sample for all leukotrienes.

Statistics

Results are expressed as mean and standard deviation. The Mann-Whitney test was used to analyze the difference between patients with SSNS and controls. The relationships between the parameters were tested by regression analysis.

| Biological material | Groups | Leukotrienes | | | | Table 2. | LT levels of plasma und urine in SSNS patients and in controls (Mean±SD) |
		C_4	D_4	E_4	B_4		
Plasma	Normal	ND	ND	ND	ND		
(pMol/l)	SSNS	ND	ND	2.66±1.04	2.20±0.95		
	P value	NS	NS	<0.001	<0.001		
Urine	Normal	ND	ND	0.07±0.01	ND		
(pMol/mg creatinine)	SSNS	0.34±0.24	ND	1.10±0.37	0.58±0.23		
	P value	<0.001	NS	<0.01	<0.001		

(ND: Nondetectale) (NS: Nonsignificant)

| Biological material | Groups | Leukotrienes | | | | Table 3. | LT levels released from peripheral leukocytes stimulated in vitro by Ca^{+2} ionophore-A23187 (Mean±SD) |
		C_4	D_4	E_4	B_4		
Leukocytes stimulated with A23187 in vitro (5×10^7)	Normal	2.7±0.6	ND	ND	2.0±0.4		
	SSNS	15.5±5.4	0.8±0.5	7.1±3.8	18.1±6.5		
	p value	<0.01	<0.01	<0.001	<0.01		

ND: Nondetectable

Results

Leukotriene concentrations in the plasma and urine of both the SSNS and the control groups are given in Tables 2 and 3. The level of urinary LTE_4, a nonactive metabolite, was 0.07±0.012 pMol/mg ceratinine in healthy children. However, LTB_4 and biologically active cysteinyl LTs (LTC_4 and LTD_4) were not detected in this group. Urinary LTE_4 levels were found to be higher (mean 1.10±0.37, range 0.55-1.59 pMol/mg creatinine) in the SSNS group than in the control group (p<0.01). Children with SSNS also had significantly higher levels of urinary LTB_4 (0.58±0.23 pMol/mg creatinine-range 0.29-0.85) and LTC_4 (0.34±0.24 pMol/mg creatinine-range 0.10-0.52) than the controls did (p<0.001). The daily urinary protein excretion of the SSNS group had a significant positive correlation with concentrations of urinary LTC_4 (r=0.80, p<0.05), LTE_4 (r=0.77, p<0.05) and LTB_4 (r=0.78, p<0.05).

Patients in the SSNS group had significantly higher (2.20±0.95 pMol/l) plasma LTB_4 concentrations than the controls (p<0.001). Plasma LTE_4 levels were found to be higher 2.66±1.04 pMol/l than in the controls (p<0.01).

Plasma LTE_4 was alsofound to be correlated with proteinuria (r=0.76, p<0.05).

The peripheral leukocytes of the healthy children were demonstrated to release LTC_4 and LTB_4, but not LTD_4 and LTE_4, after stimulation by Ca^{+2} ionophore-A23187. Concentrations of LTC_4 and LTB_4 were found to be 2.7±0.6 ng/1×10^7 cells, and 2.0±0.4 ng/1×10^7 cells, respectively. The leukocytes of the children with SSNS were found to have markedly higher releasing activity of LTC_4, LTB_4, LTD_4 and LTE_4, than those of the healthy subjects (p<0.01, p<0.01, p<0.01, and p<0.001, respectively) (Table 3). The levels of LTC_4 and LTB_4 were 15.5±5.4 ng/1×10^7 cells and 18.1±6.5 ng/1×10^7 cells in the SSNS group. Healthy subjects had no detectable amounts of LTD_4 and LTE_4.

Discussion

The proteinuria mechanism in nephrotic syndrome is explained through a decrease in the anionic load of the glomerular endothelial basement membrane and of the epithelial foot processes. The factors determining proteinuria caused by glomerular polyanion loss are not

yet known. The aim of this clinical study was to examine the possible roles of LTs in the pathogenesis of SSNS.

Endogenous LT concentrations in plasma and urine samples were found to be higher in the SSNS group than in the control group (Table 2). The urinary concentrations of cysteinyl-LTs and LTB_4 in SSNS group, and of LTE_4 in the heatlhy children were thought to be due to the kidneys, since previous experimental studies demonstrated that normal kidney tissue had substrates and enzymes needed for LT spnthesis (3, 18).

Cysteinyl-LTs have contraction effects on smooth muscles and endothelial cells. LTB_4 has both direct and indirect effects on GBM. The effects of LTs include increased GBM permeability, decreased anionic load of GBM, and leakage to microvascular space resulting in local proteinuria and systemic edema (19-21). In this study it was demonstrated that there were higher levels of plasma and urinary LTB_4 and cysteinyl-LTs in the urine of the SSNS group, but not of LTs in the plasma of the control group. We also found positive correlations between proteinuria and urinary LTC_4, LTD_4, LTB_4, and plasma LTB_4 in the same individual with SSNS, suggesting that these biologically active substances might be related to the development of proteinuria and adema.

The circulating polymorphonuclear and mononuclear leukocytes that were activated in the glomeruli were determined to play a role in anion load alterations of GBM in the pathophysiology of SSNS (22). In both humans and animals, peripheral leukocytes and glomerular cells synthesize and release LTs when stimulated separately. In this study in was thought that increased amounts of LTs in plasma and urine might be released not only by glomerular cells, but also by circulating leukocytes which might be activated in glomeruli. For this purpose, peripheral blood leukocytes of the SSNS group were stimulated in vitro by Ca^{+2} ionophore A23187, and the LT release in response to this stimulation was examined (Table 3).

The substantial amounts of LTs released by the cirulating leukocytes of the SSNS group in response to the ionophore suggested that these cells were active and functional, or that structural alterations occurred in their cytoplasmic membranes. The determination of LT release by peripheral leukocytes in vitro with high activity suggests that the hypothesis of these leukocytes might be activated by the LTs released from the injured glomerular cells. Peripheral leukocytes have specific receptors of cysteinyl-LTs and LTB_4. LTs locally synthesized by glomerular cells may activate intact leukocytes as well as circulating leukocytes with their LT release in glomerulus may also activate intact glomerulus and its cells. Continuation of this activation cascade causes local glomerular lesions, impairing GBM functions in particular. Specific symptoms of nephrotic syndrome may occur by their local effects.

In this study, endogenous LT concentrations in the plasma and urine of SSNS patients were determined. It was also demonstrated that the patient's peripheral leukocytes have a very high releasing capacity of endogenous LTs when stimulated. On the basis of our results and those reported in the literature, we propose that LTs are importand mediators in the pathogenesis of SSNS.

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