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Original Article

Beneficial effects of tonsillectomy plus steroid pulse therapy on inflammatory and tubular markers in patients with IgA nephropathy

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A B S T R A C T

Background: IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide. Tonsillectomy plus steroid pulse therapy has been able to induce clinical remission in early-stage IgAN. However, its possible effect on systemic and local cytokines and tubular markers has not been fully investigated.
Methods: We obtained serum and urine samples from 38 patients just before renal biopsy and third steroid pulse therapy. Markers of tubular damage such as N-acetyl-β-D-glucosaminidase, and kidney injury molecule-1 and inflammation such as interleukin (IL)-6, monocyte chemotactic protein (MCP)-1, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 were measured by immunoassay.
Results: Before renal biopsy, only urinary inflammatory markers, except MCP-1, were associated with glomerular (proteinuria) and/or tubular damage markers. Proteinuria, hematuria, and estimated glomerular filtration rate dramatically improved after therapy. In addition, levels of serum IL-6 and ICAM-1 and all urinary markers declined significantly; however, serum MCP-1 and VCAM-1 levels did not. None of the urinary markers correlated with the serum inflammatory markers.
Conclusion: Tonsillectomy plus steroid pulse therapy for patients with IgAN might be useful for improving not only glomerular damage marker but also tubular damage markers through the improvement of local renal inflammation.

Introduction

IgA nephropathy (IgAN) is the most prevalent form of glomerulonephritis worldwide, especially in Asia, and is a major cause of end-stage renal disease. In glomerulonephritis, many factors such as aberrant glycosylation, upregulation of the renin–angiotensin system, and oxidative stress are involved in disease development and/or progression. It is also considered that paracrine and/or autocrine stimulation of various cytokines might influence glomerular cell proliferation and the expansion of mesangial matrices. We have previously shown that renal expression of intercellular adhesion molecule-1 (ICAM-1) is closely related to glomerular infiltration by lymphocytes and monocytes [1]; in addition, urinary interleukin-6 (IL-6) and monocyte chemotactic protein-1 (MCP-1) levels are related to disease activity in patients with IgAN [2,3].

Tonsillectomy plus steroid pulse (TSP) therapy has become a relatively common treatment in patients with IgAN, especially

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in Japan [4]. However, little is known about the effect of this therapy on inflammatory and tubular damage biomarkers. Thus, in this study, we evaluated the effect of TSP therapy on serum and urinary markers of inflammation [IL-6, MCP-1, ICAM-1, and vascular cell adhesion molecule-1 (VCAM-1)] and on urinary markers of tubular damage [N-acetyl-β-D-glucosaminidase (NAG) and kidney injury molecule-1 (KIM-1)] and glomerular damage (proteinuria) in patients with IgAN.

Methods

Patients and sample collection

The present study was conducted in 38 Japanese patients [19 men and 19 women, with a mean (SD) age of 32 (11) years] with biopsy-proven primary IgAN. None of the enrolled patients received steroid or immunosuppressive drugs. IgAN patients underwent tonsillectomy before steroid pulse therapy. Then, they received intravenous methylprednisolone pulse of 0.5 g/d for 3 consecutive days followed by oral prednisolone (0.5 mg/ideal body weight/d) on alternate days. They received the second and third round of steroid pulse therapy almost every other month. After final (third) steroid pulse therapy, continuous administration of oral prednisolone at a dose of 0.5 mg/ideal body weight/d on alternate days was performed for 4 weeks. Then, the dose of prednisolone was decreased by 5 mg every 4 weeks. Serum and urine samples were collected immediately before renal biopsy and third steroid pulse therapy and were stored at −80°C until use. The study protocol and informed written consent procedures were approved by the Institutional Review Board of Juntendo University Faculty of Medicine, Tokyo, Japan (No.: 26-170). All participants provided written informed consent, and the study adhered to the Declaration of Helsinki.

Assessment of exposure variables

The parameters of interest were measured in the clinical laboratory at Juntendo University Hospital. The estimated glomerular filtration rate (eGFR) of each patient was estimated by the following equation for the Japanese population: eGFR (mL/min/1.73 m^2) = 194 × [age (years)]$^{-0.287}$ × [serum creatinine (Cr) (mg/dL)]$^{-1.094}$ × 0.739 (for females) [5]. The urinary protein concentration was assayed using a pyrogallol red–based reagent kit (Protein Assay Rapid Kit; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Urinary Cr levels were measured using an automated machine (Hitachi 7170S Chemistry Analyzer; Hitachi Chemical Co., Ltd., Tokyo, Japan) and a commercial kit (CRE-S; Denka Seiken Co., Ltd., Tokyo, Japan). Urinary protein excretion was expressed as mg/g·Cr.

Measurements of inflammatory and tubular damage markers

IL-6 levels were measured using enzyme-linked immunosorbent assay (Cat # HS600B; R&D Systems, Minneapolis, MN, USA). We used a multiplex assay run on the Luminex platform to measure urinary levels of ICAM-1, VCAM-1, MCP-1, and KIM-1 (ICAM-1 and VCAM-1 using Cat # HCVD1-67AK; MCP-1 using Cat # HADK2-61K-B; and KIM-1 using Cat # HKTX3MAG-38K, MILLIPLEX; Merck Millipore, Austin, TX, USA). This multiplex assay is a particle-enhanced, sandwich-type, liquid-phase immunoassay with a laser-based detection system based on flow cytometry. All measurements were performed according to the manufacturer's instructions. Urinary NAG (Nittobo Medical Co., Ltd., Tokyo, Japan) levels were measured in the clinical laboratory at Juntendo University Hospital. Briefly, we measured the enzymatic activity of NAG using a colorimetric assay with 6-methyl-2-pyridyl-N-acetyl-1-thio-6-D-glucosaminide as the substrate. Urinary levels of inflammatory and tubular damage markers were normalized individually to urinary Cr levels.

Histologic examination

The renal biopsy specimens of IgAN patients were evaluated by light, immunofluorescence, and electron microscopy and were stained using four stains: hematoxylin–eosin, periodic acid–Schiff, Elastica–Masson, and periodic acid methenamine silver. The histologic grade (H-grade) was determined using the clinical guides for IgAN in Japan, third version, according to percentage of glomeruli with lesions (I: < 25%, II: 25–49%, III: 50–74%, and IV: > 75%) [6].

Data analysis

Continuous variables with a normal distribution are expressed as means ± SD. Variables with a skewed distribution are presented as medians (25–75% interquartile range). Categorical variables are described as frequencies or percentages. For paired samples, the Wilcoxon signed rank test was used to assess differences between groups. Spearman's regression analysis was used to analyze the correlation between 2 variables. Statistical analyses were performed using SPSS software (version 23; SPSS Inc., Chicago, IL, USA). A 2-sided $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics and levels of inflammatory, glomerular damage, and tubular damage markers in the study population

As shown in Tables 1 and 2, the mean (SD) age of the study population was 32 (11) years, and 19 (50.0%) patients were men. Blood pressure was almost within the normal range, although 4 patients received drugs targeting the renin–angiotensin system. The median (25th percentile, 75th

Table 1. Clinical characteristics of the study population

N	38
Male	19 (50.0)
Age (y)	32 ± 11
BMI (kg/m^2)	22.1 ± 2.6
SBP (mmHg)	115 ± 11
DBP (mmHg)	66 ± 9
ACEI or ARB Rx	4 (10.5)
Histologic grade	
I	7 (18.4)
II	23 (60.5)
III	8 (21.1)

Data are presented as mean ± SD or *n* (%).
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; Rx, treatment; SBP, systolic blood pressure.

percentile) levels of eGFR and urinary protein-to-creatinine ratio (UPCR) were 81 (63, 102) mL/min/1.73 m^2 and 487 (263, 807) mg/g·Cr, respectively. Most patients (80%) had chronic kidney disease stage 1 or 2.

After TSP therapy, the IgA/C3 ratio and UPCR were significantly declined from the median of 3.3 to 2.2 and from 487 to 162 mg/g·Cr, respectively. The degree of hematuria was dramatically improved. Moreover, eGFR was significantly improved from the median of 81 to 86 mL/min/1.73 m^2. The urinary markers of inflammation (IL-6, MCP-1, ICAM-1, and VCAM-1) and tubular damage (NAG and KIM-1) were significantly declined after therapy. Although serum levels of MCP-1 and VCAM-1 were not changed after therapy, serum and urinary levels of IL-6 and ICAM-1 were significantly declined.

Association between inflammatory markers and glomerular or tubular damage markers

Baseline (before renal biopsy)

As shown in Table 3, there were no correlations between serum inflammatory markers and glomerular or tubular damage markers, whereas there were significant positive correlations between urinary IL-6 or VCAM-1 and glomerular or tubular damage markers. Urinary ICAM-1 was significantly associated with glomerular damage markers and was marginally associated with KIM-1 ($P = 0.055$) or NAG ($P = 0.056$). On the other hand, there were no correlations between urinary MCP-1 and glomerular or tubular damage markers.

Table 2. Clinical characteristics and concentrations of inflammatory and tubular damage markers before and after TSP therapy in patients with IgA nephropathy

	Before Rx	After Rx	*P*
Clinical characteristics			
Serum IgA (mg/dL)	304 (227, 444)	201 (147, 261)	< 0.001
IgA/C3 ratio	3.3 (2.6, 4.4)	2.2 (1.5, 3.2)	< 0.001
eGFR (mL/min/1.73 m^2)	81 (63, 102)	86 (73, 110)	0.004
eGFR categories			
> 90	16 (41.0)	17 (43.6)	
60–90	15 (38.5)	18 (46.2)	0.166
30–60	7 (20.5)	3 (10.3)	
UPCR (mg/g·Cr)	487 (263, 807)	162 (83, 289)	< 0.001
Hematuria categories			
1–10/HPF	2 (5.1)	29 (76.9)	
11–30/HPF	13 (35.9)	7 (17.9)	< 0.001
> 30/HPF	23 (59.0)	2 (5.1)	
Urinary inflammatory markers			
IL-6 (pg/mg·Cr)	3.3 (1.5, 7.4)	2.0 (1.2, 3.4)	0.017
MCP-1 (pg/mg·Cr)	474 (243, 745)	212 (131, 330)	0.002
ICAM-1 (ng/mg·Cr)	1.1 (0.6, 1.7)	0.7 (0.5, 1.1)	< 0.001
VCAM-1 (ng/mg·Cr)	2.4 (1.1, 3.8)	0.9 (0.5, 2.1)	< 0.001
Serum inflammatory markers			
IL-6 (pg/mL)	2.9 (0.9, 24.3)	0.4 (0.03, 1.2)	< 0.001
MCP-1 (pg/mL)	161 (119, 215)	168 (116, 242)	0.712
ICAM-1 (ng/mL)	141 (95, 271)	81 (54, 117)	< 0.001
VCAM-1 (ng/mL)	845 (676, 964)	769 (684, 894)	0.365
Tubular damage markers			
KIM-1 (μg/g·Cr)	1.3 (0.7, 2.3)	0.8 (0.3, 1.2)	0.007
NAG (IU/g·Cr)	5.7 (3.5, 9.8)	4.1 (3.1, 6.3)	0.003

Data are presented as mean ± SD, median (quartiles), or *n* (%).
Cr, creatinine; eGFR, estimated glomerular filtration ratio; HPF, high power field; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemotactic protein-1; NAG, N-acetyl-β-D-glucosaminidase; Rx, treatment; TSP, tonsillectomy plus steroid pulse; UPCR, urinary protein-to-creatinine ratio; VCAM-1, vascular cell adhesion molecule-1.

Interestingly, none of the urinary markers correlated with the serum inflammatory markers (IL-6, $r = -0.05$; MCP-1, $r = -0.03$; ICAM-1, $r = 0.13$; VCAM-1, $r = -0.03$).

Percent reduction from baseline after TSP therapy

As shown in Table 4, the percent reduction of UPCR was significantly correlated with that of ICAM-1 ($r = 0.42$, $P = 0.008$) or VCAM-1 ($r = 0.51$, $P < 0.001$). On the other hand, the percentage reduction of KIM-1 was significantly correlated with the percent reduction of IL-6 ($r = 0.43$, $P = 0.008$) or ICAM-1 ($r = 0.32$, $P < 0.05$). There was no correlation between the percent reduction of NAG and that of each urinary inflammatory marker.

Discussion

In this study, we demonstrated that eGFR, UPCR, and hematuria in patients with relatively early-stage IgAN were dramatically improved after TSP therapy. Moreover, levels of urinary inflammatory (IL-6, MCP-1, ICAM-1, and VCAM-1) and tubular damage (KIM-1 and NAG) markers declined after therapy. Serum IL-6 and ICAM-1 levels also declined significantly, although serum MCP-1 and VCAM-1 levels did not. Considering the lack of correlation between any of the serum and urinary inflammatory markers, TSP therapy might directly improve local renal inflammation.

ICAM-1 and VCAM-1 mediate cell adhesion and maintain endothelial permeability, and their expression is upregulated by inflammatory cytokines such as tumor necrosis factor-α and interleukin-1 (IL-1). We previously reported that ICAM-1 was expressed only in glomerular capillary walls and the mesangial area of patients with advanced-stage IgAN but not in those of patients with mild IgAN [7]. In addition, serum ICAM-1 levels did not differ between patients with mild and advanced-stage IgAN. On the other hand, Arrizabalaga et al [8] demonstrated that tubular and interstitial expression of ICAM-1 can be a marker of tubulointerstitial disturbance and is associated with the severity of proteinuria in IgAN. The differences in ICAM-1 expression in the kidney might be caused by different antibodies used in each study. In the present study, we demonstrated that ICAM-1 levels in the urine, but not the serum, were associated with glomerular damage marker or marginally associated with tubular damage markers, suggesting that urinary ICAM-1 is excreted from the kidney but not from the systemic circulation. Moreover, the absence of any correlation between serum and urinary ICAM-1 levels might support this suggestion. We also showed that the percent reduction of urinary ICAM-1 by TSP therapy was associated with that of UPCR or KIM-1, suggesting that ICAM-1 plays an important role in the pathogenesis of not only glomerular damage but also tubular damage in patients with IgAN. Zhu et al [9] reported that serum VCAM-1 levels in IgAN were associated with not only clinical markers, such as eGFR and UPCR, but also histologic findings, such as tubular atrophy/interstitial fibrotic lesions and active crescentic lesions. Moreover, they demonstrated that IgA1 stimulation induced soluble VCAM-1 expression in the supernatant of cultured human umbilical vein endothelial cells. In the present study, urinary, but not serum, VCAM-1 levels were associated with both glomerular and tubular damage marker levels. In addition, serum VCAM-1 levels were not associated with eGFR (data not shown) or UPCR. The discrepancy between the results of Zhu et al [9] and those of our study might explain

Table 3. Correlation coefficient between inflammatory markers and glomerular or tubular damage markers before TSP therapy

	Urine				Serum			
	IL-6	MCP-1	ICAM-1	VCAM-1	IL-6	MCP-1	ICAM-1	VCAM-1
UPCR	0.52‡	0.21	0.46†	0.70‡	−0.18	−0.11	−0.01	0.20
KIM-1	0.49†	0.27	0.31	0.45†	−0.11	−0.08	−0.05	0.05
NAG	0.47†	0.29	0.31	0.34*	−0.27	−0.02	0.02	0.27

* $P < 0.05$
† $P < 0.01$
‡ $P < 0.001$
ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemotactic protein-1; NAG, N-acetyl-β-D-glucosaminidase; TSP, tonsillectomy plus steroid pulse; UPCR, urinary protein-to-creatinine ratio; VCAM-1, vascular cell adhesion molecule-1.

Table 4. Correlation coefficient between urinary inflammatory markers and glomerular or tubular damage markers in percent reduction from baseline after TSP therapy

	ΔUPCR	ΔKIM-1	ΔNAG
ΔIL-6	0.26	0.43†	0.15
ΔICAM-1	0.42†	0.32*	0.18
ΔVCAM-1	0.51‡	0.15	0.22

* $P < 0.05$
† $P < 0.01$
‡ $P < 0.001$
ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; KIM-1, kidney injury molecule-1; NAG, N-acetyl-β-D-glucosaminidase; TSP; tonsillectomy plus steroid pulse; UPCR, urinary protein-to-creatinine ratio; VCAM-1, vascular cell adhesion molecule-1.

the difference in disease severity or the frequency of hypertension. Actually, both UPCR and the frequency of hypertension were much lower in the present study compared with their study.

Tubular KIM-1 expression is significantly associated with tubulointerstitial injury and inflammation, and elevated urinary KIM-1 levels are strongly associated with tubular KIM-1 expression in IgAN [10]. Recently, Lin et al [11] reported that tubular KIM-1 expression was also associated with the aforementioned tubulointerstitial findings, but not glomerular findings such as endothelial proliferation and mesangial proliferation. Moreover, IL-6RNA expression was strongly upregulated in KIM-1–overexpressing LLC-PK1 cells, a pig kidney proximal tubules cell line. In the present study, the percent reduction of urinary IL-6 was associated with that of urinary KIM-1 but not UPCR. Thus, in IgAN, KIM-1–expressing tubular cells may play a role in the pathogenesis of tubulointerstitial injury and inflammation through the secretion of IL-6.

In conclusion, TSP therapy might be useful for the improvement in markers of both glomerular damage (hematuria and proteinuria) and tubular damage (KIM-1, NAG) through the improvement of local renal inflammation in patients with IgAN.

Conflicts of interest

All authors have no conflicts of interest to declare.

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