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Oral adsorbent AST-120 ameliorates tubular injury in chronic renal failure patients by reducing proteinuria and oxidative stress generation

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Abstract

AST-120 is an oral adsorbent that attenuates the progression of chronic renal failure (CRF) and improves the prognosis of the patients under dialysis. Although tubulointerstitial injury is more important than glomerulopathy in terms of renal prognosis in patients with CRF, effect of AST-120 on tubular injury in CRF patients remains unknown. In this study, we examined whether and how AST-120 treatment could improve tubular damage in nondiabetic CRF patients. Fifty nondiabetic CRF patients were enrolled in the present study and divided into 2 groups: one was the AST-120–treated group (15 men and 10 women) and the other was the age-, sex-, and clinical variables–matched non–AST-120-treated control group. Patients were followed up for 12 months. We investigated the effects of AST-120 on serum levels of interleukin-6 (IL-6), proteinuria, and urinary excretion levels of 8-hydroxydeoxyguanosine (8-OHdG) and L-fatty acid binding protein (L-FABP), markers of oxidative stress and tubular injury, respectively. AST-120 treatment (6 g/d), but not control treatment, for 12 months significantly reduced IL-6, proteinuria, and urinary excretion levels of L-FABP and 8-OHdG, and inhibited the increase in serum creatinine in CRF patients. In univariate analyses, L-FABP levels were correlated with age, proteinuria, 8-OHdG, and IL-6. In multiple stepwise regression analysis, proteinuria and urinary 8-OHdG levels were independently related to L-FABP levels (R^2 = 0.605). Our present study demonstrated for the first time that AST-120 improved tubular injury in nondiabetic CRF patients. AST-120 may exert beneficial effects in CRF patients by protecting tubular damage partly via reduction of proteinuria and oxidative stress generation.

1. Introduction

AST-120 (Kremezin; Kureha Chemical, Tokyo, Japan) is an oral adsorbent that attenuates the progression of chronic renal failure (CRF) by removing uremic toxins such as indoxyl sulfate (IS) and advanced glycation end products (AGEs), thus resulting in the delay of dialysis in patients with chronic kidney disease (CKD) [1-4]. Furthermore, recently, AST-120 treatment in predialysis period was shown to improve the prognosis of CKD patients under dialysis as well [5].

It has recently been recognized that changes within tubulointerstitium are more important than glomerulopathy in terms of renal prognosis in patients with CKD [6,7]. Furthermore, there is accumulating evidence that proteinuria is not merely a biomarker for the progression of CKD, but also a mediator of CKD [8,9]. Indeed, clinical data show a positive correlation of the extent of proteinuria with the severity of tubulointerstitial damage in CKD patients [8,9]. In addition, oxidative stress and inflammatory reactions have been reported to play a role in tubulointerstitial injury in CKD as well [10-13]. These observations led us to speculate that AST-120 treatment could exert beneficial effects on CKD by protecting tubular injury in CRF patients partly via reduction of proteinuria as well as suppression of oxidative stress and inflammatory reactions. However, as far as we know, there is no report to show the effect of AST-120 on tubular injury in

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CRF patients. Therefore, in this study, we examined whether and how AST-120 treatment could improve tubular damage in CRF patients. For this, we first examined the effects of AST-120 treatment on serum levels of interleukin-6 (IL-6), proteinuria, and urinary excretion levels of 8-hydroxydeoxyguanosine (8-OHdG) and L-fatty acid binding protein (L-FABP), markers of oxidative stress and tubular injury, respectively [14,15], in nondiabetic CRF patients and then investigated their relationships in these subjects.

2. Subjects and methods

2.1. Subjects

Fifty nondiabetic CRF patients were enrolled in the present study and divided into 2 groups: one was the AST-120–treated group (15 men and 10 women; immunoglobulin [Ig] A nephropathy, n = 12; non–IgA-type proliferative glomerulonephritis, n = 4; nephrosclerosis, n = 3; polycystic kidney disease, n = 2; membranous nephropathy, n = 2; focal glomerular sclerosis, n = 2; mean age, 56.8 ± 7.8 years) and the other was the age- and sex-matched AST-120–nontreated control group (14 men and 11 women; IgA nephropathy, n = 11; non–IgA-type proliferative glomerulonephritis, n = 4; nephrosclerosis, n = 3; polycystic kidney disease, n = 3; membranous nephropathy, n = 2; focal glomerular sclerosis, n = 2; mean age, 59.5 ± 6.9 years). We excluded any patients with chronic pulmonary disease, collagen disease, liver disease, and neoplastic disorders and those who had recent (<6 months) acute coronary syndromes, stroke, and any acute infections. Patients who were assigned to AST-120 group were treated with 2 g AST-120 (Kureha Chemical) 3 times a day for 12 months. Each group patients received antihypertensive drugs, statins, or antiplatelet agents at baseline; and those therapies were not changed during the study period. The study protocol was approved by the local ethical committee of Shinmatsudo Central General Hospital, and informed consent was obtained from all study participants. The study complied with the principles of the Helsinki Declaration.

2.2. Data collection

Blood pressure was measured in the sitting position twice after 2 minutes of rest using an upright standard sphygmomanometer. Renal function was evaluated by serum creatinine (sCr) levels and estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease equation modified for the Japanese population [16]. Twenty-four–hour urine collections were made during the study period. Urinary excretion levels of 8-OHdG, a marker of oxidative stress, were measured by an enzyme-linked immunosorbent assay (ELISA) as reported previously [14]. Urinary L-FABP and serum IL-6 levels were measured with ELISA kits (Human L-FABP ELISA kit; CIMC, Tokyo, Japan, and Quantikine IL-6; R&D Systems, Minneapolis, MN) according to the manufacturers' protocols [15,17]. Total protein excretion levels were determined with a pyrogallol red method (Wako, Osaka, Japan).

2.3. Statistical methods

Data were expressed as mean ± standard deviation. One-way analysis of variance was used for the statistical comparisons among the parameters at baseline and at 6 or 12 months after the study. Statistical comparisons between control and AST-120–treated groups were performed with unpaired *t* test. Correlations between urinary L-FABP levels and clinical variables were determined by a linear regression analysis. To determine independent determinants of urinary excretion levels of L-FABP, multiple stepwise linear regression analysis was performed. Statistical significance was defined as $P < .05$. All statistical analyses were performed with the use of the SAS system (SAS Institute, Cary, NC).

3. Results

There were no gut-related adverse effects of AST-120, and no one discontinued the treatment. Furthermore, adherence to AST-120 therapy was monitored by each family. All patients were found to take 90% or more of the test drug. Background of the patients is shown in Table 1. Control and AST-120–treated group patients received antihypertensive drugs, statins, or antiplatelet agents at baseline. However, there were no significant differences of baseline therapies between the 2 groups (Table 1). Clinical variables at baseline also did not differ between the 2 groups. As shown in Table 1 and Fig. 1, AST-120 treatment (6 g/d) significantly reduced urinary excretion levels of protein, L-FABP, and 8-OHdG and decreased serum level of IL-6, although it did not affect blood pressure levels. Compared with the values of control group, urinary excretion levels of protein, L-FABP, and 8-OHdG and serum levels of IL-6 were significantly lower in AST-120–treated group. AST-120 treatment significantly inhibited the increase in sCr levels, but it did not affect the decrease in eGFR during the study period. In univariate analyses, L-FABP levels were correlated with age, urinary excretion levels of protein and 8-OHdG, and IL-6. Because the parameters could be closely correlated with each other, to determine independent determinants of urinary excretion levels of L-FABP, multiple stepwise regression analysis was performed. This analysis showed that proteinuria and urinary 8-OHdG levels ($P < .0001$) were independently related to L-FABP levels in our CRF patients ($R^2 = 0.605$).

4. Discussion

In the present study, we demonstrated for the first time that AST-120 treatment for 12 months significantly

Table 1
Clinical variables of control and AST-120-treated patients

Variables	Control			AST-120 treated		
	Basal	6 mo	12 mo	Basal	6 mo	12 mo
Age (y)	59.5 ± 6.9			56.8 ± 7.8		
n (male)	25 (14)			25 (15)		
SBP (mm Hg)	128.3 ± 2.8	127.7 ± 2.0	127.6 ± 1.3	129.0 ± 3.9	128.1 ± 2.7	127.4 ± 2.0
DBP (mm Hg)	75.8 ± 2.0	75.5 ± 1.6	76.3 ± 1.5	75.8 ± 2.9	75.8 ± 2.1	75.5 ± 1.9
sCr (mg/dL)	2.4 ± 0.4	2.7 ± 0.5†	3.0 ± 0.6†	2.4 ± 0.4	2.5 ± 0.4	2.6 ± 0.4§,¶
eGFR (mL/min)	21.1 ± 5.1	18.5 ± 4.2†	16.8 ± 3.8†	20.1 ± 5.2	19.7 ± 5.1	18.6 ± 4.5§
Proteinuria (g/d)	1.3 ± 0.3	1.5 ± 0.3†	1.6 ± 0.3†	1.4 ± 0.3	1.2 ± 0.3§,¶	1.0 ± 0.2§,¶
L-FABP (μg/g Cr)	47.9 ± 13.8	50.5 ± 12.7†	56.9 ± 14.0†	52.1 ± 14.9	42.9 ± 12.8§,¶	34.5 ± 11.7§,¶
8-OHdG (ng/mg Cr)	34.4 ± 4.9	36.8 ± 5.0†	40.2 ± 5.1†	34.4 ± 5.8	28.9 ± 5.0§,¶	25.3 ± 4.4§,¶
IL-6 (pg/mL)	28.1 ± 7.2	29.6 ± 7.5†	33.4 ± 7.9†	28.2 ± 7.3	24.2 ± 4.7§,¶	20.6 ± 3.6§,¶
Antihypertensive drugs	n			n		
ARB	16			17		
ACEI	9			8		
CCB	7			8		
α1-Blockers	6			6		
Others	6			6		
Antiplatelet agents	n = 13			n = 12		
Statins	n = 5			n = 4		

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; ARB, angiotensin II type 1 receptor blockers; ACEI, angiotensin-converting enzyme inhibitors; CC,: calcium channel blockers.

† $P < .01$ compared with the basal values of control patients.

§ $P < .01$ compared with the basal values of AST-treated patients.

¶ $P < .01$ compared with the values of control patients.

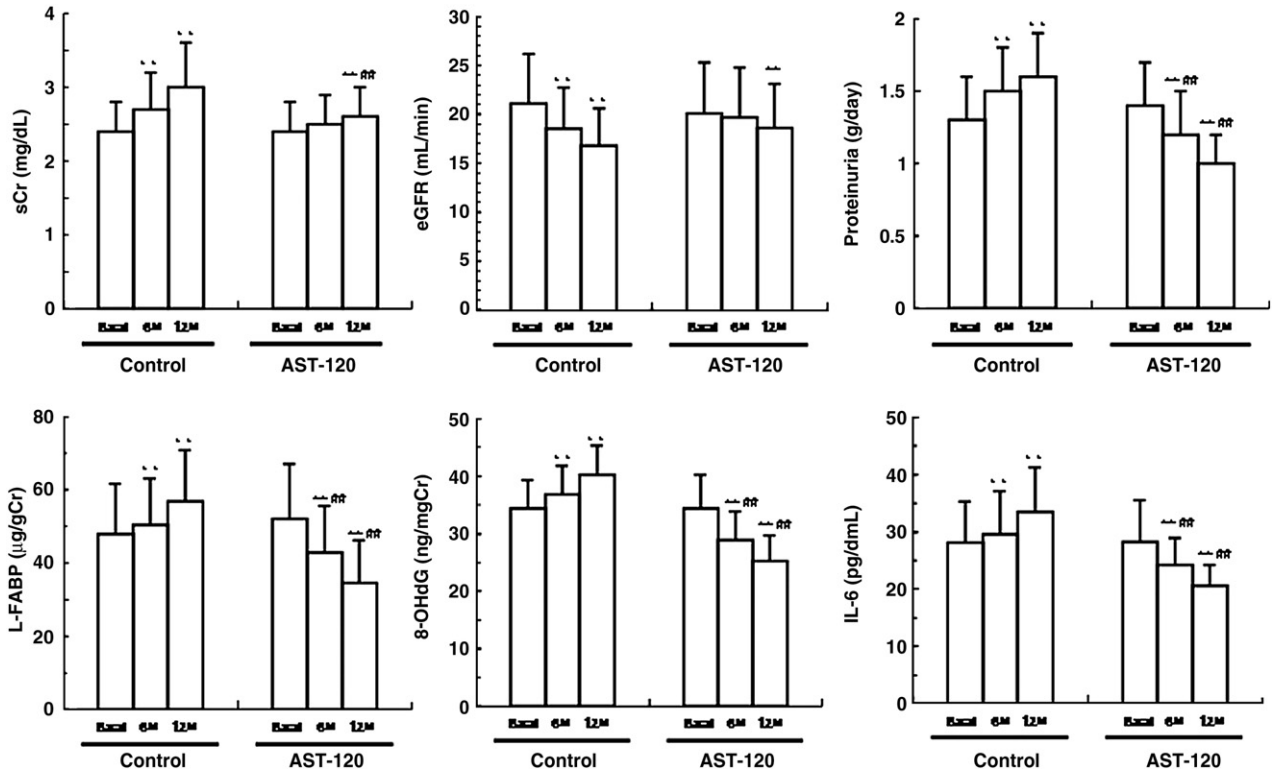

Fig. 1. Effects of AST-120 on various markers for renal injury. *$P < .05$ and **$P < .01$ compared with the basal values of control patients. $^{+}P < .05$ and $^{++}P < .01$ compared with the basal values of AST-treated patients. $^{\#}P < .05$ and $^{\#\#}P < .01$ compared with the values of control patients.

decreased urinary excretion levels of L-FABP in nondiabetic CRF patients. Urinary L-FABP, a marker of tubular damage, is more sensitive than urinary protein in predicting the progression of CKD [15]. Furthermore, changes within tubulointerstitium are shown to be more important than glomerulopathy in terms of renal prognosis in patients with CKD [6,7]. These observations suggest that salutary effects of AST-120 treatment on renal function and prognosis in CRF patients reported previously [1-4] may be ascribed, at least in part, to its protective properties against tubulointerstitial injury in these patients. In this study, we did not evaluate urinary excretion levels of β2-microglobulin and/or *N*-acetyl-glucosaminidase, classic markers of tubular damage [18]. However, because urinary excretion levels of L-FABP are reported to accurately reflect the severity of tubulointerstitial damage [19], our present findings suggest that AST-120 treatment may be effective against tubulointerstitial injury in nondiabetic CRF patients.

In this study, AST-120 treatment significantly decreased proteinuria and prevented the increase in sCr levels in CRF patients compared with control treatment. Furthermore, proteinuria was found to be one of the independent determinants of L-FABP levels in our subjects. There is accumulating evidence that proteinuria is not merely a biomarker for the progression of CKD, but also a mediator of this devastating disorder [8,9]. Indeed, albumin, one of the major components found in proteinuria, is reported to cause proinflammatory and profibrotic changes in cultured proximal tubular cells [8,9]. In addition, proteinuria is shown to be correlated with the severity of tubulointerstitial damage in CKD patients [8,9]. These observations suggest that AST-120 treatment could improve tubular damage in CRF patients partly by reducing proteinuria.

We found here that AST-120 treatment reduced urinary excretion levels of 8-OHdG in CRF patients. The finding was consistent with the previous observation of Nakagawa et al [20] showing that AST-120 suppressed oxidative stress in uremic rats. In their study, AST-120 treatment was reported to inhibit tubulointerstitial fibrosis and prevent the progression of renal dysfunction in uremic rats, which was associated with reduced tubular and urinary excretion levels of 8-OHdG [20]. Since, in this study, urinary 8-OHdG levels were also independently related to L-FABP levels, AST-120 could ameliorate tubular damage in CRF patients via antioxidative properties as well.

In the present study, we did not clarify how AST-120 treatment decreased proteinuria and oxidative stress generation in our CRF patients. However, uremic toxins-lowering capacity may be involved in these beneficial effects of AST-120 because (1) uremic toxins such as IS and AGEs caused proximal tubular cell injury via oxidative stress generation in vitro [10,21,22], (2) administration of IS progressed glomerular sclerosis in uremic rats [23], (3) AGE injection to normal rats caused tubulointerstitial injury [24], (4) administration of AST-120 decreased urinary levels of IS and 8-OHdG and ameliorated renal function in CKD rats [20,25], and (5) AST-120 treatment decreased serum or urinary levels of IS and AGEs in CKD patients [2,3,26,27]. Taken together, the present observations suggest that AST-120 may exert beneficial effects in CRF patients by protecting tubular damage partly via reduction of proteinuria and oxidative stress generation. Furthermore, in this study, AST-120 treatment decreased IL-6 levels as well. Because IL-6 is a well-recognized marker of generalized inflammation and inflammatory reaction elicits oxidative stress generation [17,28], anti-inflammatory effects of AST-120 could contribute to its antioxidative properties in CRF patients.

4.1. Limitations

The number of subjects in this study is relatively small; therefore, it does not have enough statistical power to draw a definite conclusion that AST-120 treatment improved tubular damage partly via reduction of proteinuria and oxidative stress generation. Furthermore, we were not able to measure IS or AGE levels in blood or urine during experimental periods because of the lack of samples. Examining the relationship between these uremic toxin levels and L-FABP levels would be helpful in understanding the protective properties of AST-120 against proximal tubular cells injury in CRF patients. Further large clinical study is needed to elucidate whether reduction of circulating or urinary levels of uremic toxins such as IS and AGEs by AST-120 could be mechanistically related to renal protection in our nondiabetic CRF patients.

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