EFFECT OF AN ORAL ADSORBENT AST-120 ON GLOMERULAR AND TUBULAR INJURY IN METABOLIC SYNDROME/DIABETES RATS

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OBJECTIVES

- Metabolic syndrome is known to be an important risk factor involved in the development of diabetic nephropathy.
  Diabetes Care. 2008; 31(12):2357-61

- AST-120 is an oral carbon adsorbent consisting of porous carbon particles.
- It has been used clinically for treatment of chronic kidney disease (CKD) patients to slow the progression of CKD
  Nephron Clin Pract. 2007; 105(3):c99–c107

- There is little evidence that AST-120 should be prescribed for subjects with early stage overt diabetic nephropathy.

Therefore, we investigated whether AST-120 has an effect on the early stage of nephropathy using SHR/NDmc-cp (SHR/ND), a rat model of metabolic syndrome/ type 2 diabetes. .

METHODS

Animal study

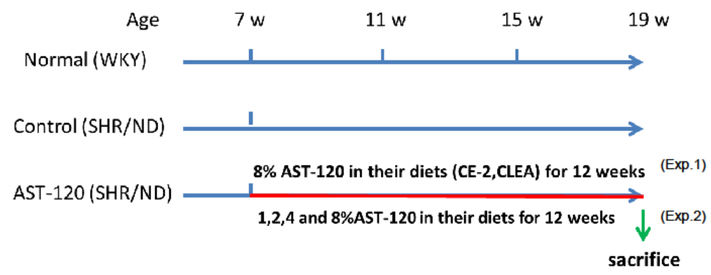
SHR/NDmcr-cp/cp (SHR/ND) rats (7w age, male) were divided into each group.
AST-120 was administered for 12 weeks containing with their diets.
The systolic blood pressure, the levels of total cholesterol, triglycerides , blood urea nitrogen, serum creatinine , Creatinine clearance, and urinary protein, were measured.
At 19 weeks age all rats were sacrificed.

Experiment 1 (n =5)

Normal (Wistar-Kyoto (WKY) rats)
Control (SHR/ND)
AST-120 (SHR/ND, 8%AST-120)

Experiment 2 (n = 6)
(investigation of the effect on urinary protein in dose-dependent manner)

Normal	2%AST-120
Control	4%AST-120
1%AST-120	8%AST-120



Podocyte injury

Podocyte foot process width (FPW) was measured by Transmission Electron Microscopy.
The glomerular basement membrane (GBM) length was measured and the number of podocyte foot processes along the GBM was counted in each picture. They were calculated for 30 randomly selected fields of each kidney section. The foot process width was calculated by the following formula.

$$FPW = \pi/4 \times \frac{\Sigma \text{ GBM length}}{\Sigma \text{ foot process}}$$

Kidney International 2004; 66, 1901-06

Glomerular hypertrophy

Glomeruli size was measured in 100 glomeruli per individuals using Qwin3 soft (Leica).

Monocyte/macrophage infiltration

Monocyte/macrophage infiltration in renal tissues was investigated by CD68 staining.
An evaluation method of the section stained by anti- CD68 antibody (ED-1) was to count the number of CD68 positive cells in 20 randomly selected fields in the cortex.

Tubular injury

Tubular injury was investigated by kidney injury molecule-1 (KIM-1) staining and evaluating the mRNA expression of Kim-1 by real-time PCR.
An evaluation method of the section stained by anti- KIM-1 was to measure the KIM-1 positive area in 20 randomly selected fields .

RESULTS

Tabele1. Biochemical parameters at 19 weeks of age

	WKY	SHR/NDmcr-cp	
	Normal	Control	AST-120
Body weight (g)	409.9 ± 13.5	563.9 ± 15.8*	563.5 ± 12.8*
Systolic blood pressure (mmHg)	134.9 ± 8.1	189.9 ± 11.1*	177.0 ± 5.3*†
Total cholesterol (mg/dL)	80.4 ± 1.8	101.6 ± 8.7*	93.2 ± 8.3*
Triglyceride (mg/dL)	56.2 ± 12.2	753.4 ± 86.2*	774.8 ± 135.2*
HbA1c (%)	4.0 ± 0.1	5.3 ± 0.1*	5.5 ± 0.2*
Blood urea nitrogen (mg/dL)	23.3 ± 0.73	33.4 ± 1.72*	33.4 ± 2.60*
Serum creatinine (mg/dL)	0.35 ± 0.03	0.29 ± 0.02	0.30 ± 0.02
Creatinine clearance (mL/min)	3.34 ± 0.18	3.13 ± 0.25	3.08 ± 0.16*

Mean ± SD * p < 0.01 vs. Normal, † p < 0.05 vs. Control, t-test

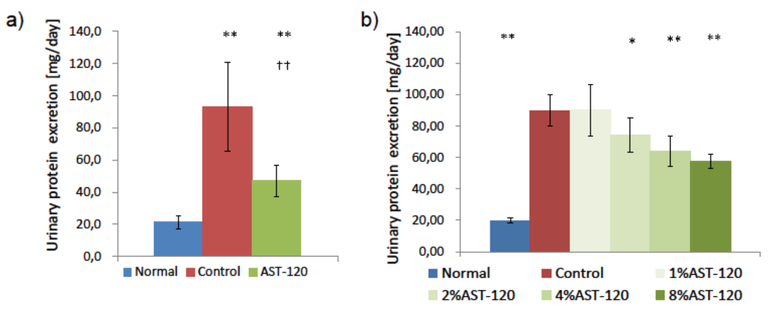


Fig. 1 Urinary protein excretion at 19 weeks age,
a) Experiment 1 Mean ± SD, ** p < 0.01 vs. Normal. †† p < 0.01 vs. Control, t-test
b) Experiment 2 Mean ± SD, *p < 0.05, ** p < 0.01 vs. Control, Dunnett's test

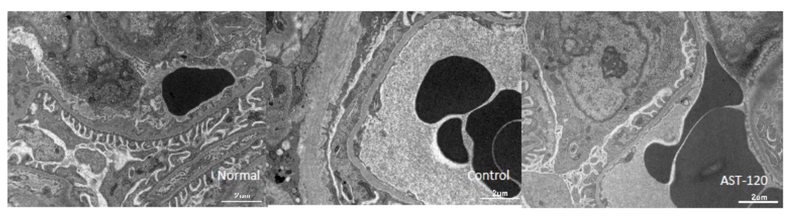


Fig. 2 Representative electron microscopy images

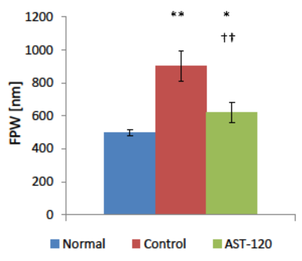


Fig. 3 Podocyte foot process width (FPW)
Mean ± SD, ** p < 0.01, * p < 0.05 vs. Normal,
†† p < 0.01 vs. Control, t-test

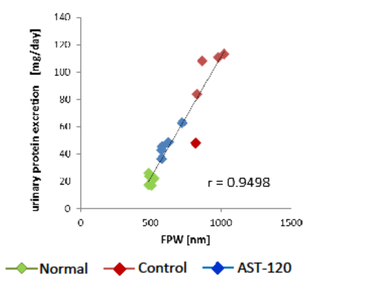


Fig. 4 Correlation between FPW and the levels of urinary protein excretion

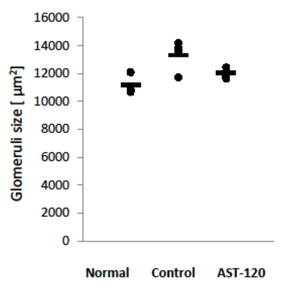


Fig. 5 Glomeruli size
●; individual ─ ; mean

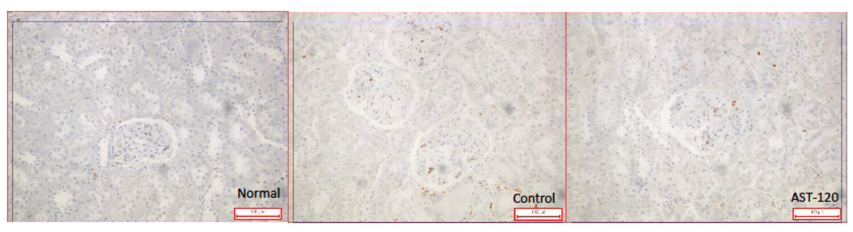


Fig. 6 Representative images of sections stained for CD68 by immunohistochemistry

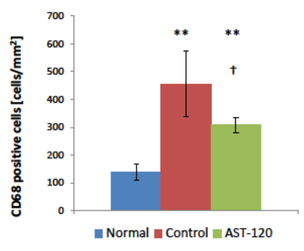


Fig. 7 CD68 positive cells
Mean ± SD, ** p < 0.01, vs. Normal,
† p < 0.05 vs. Control, t-test

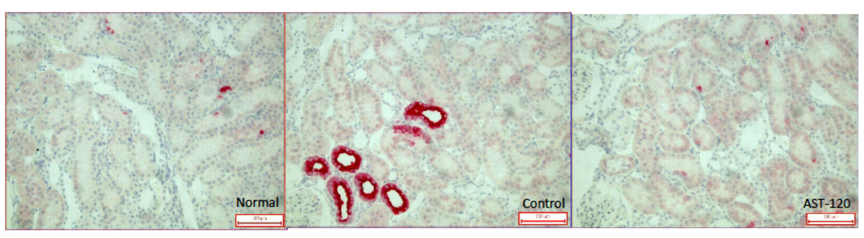


Fig. 8 Representative images of sections stained for KIM-1 by immunohistochemistry

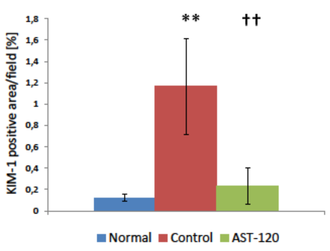


Fig. 9 Kim-1 expression
Mean ± SD, n=5 , ** p < 0.01, vs. Normal,
†† p < 0.01 versus Control, t-test

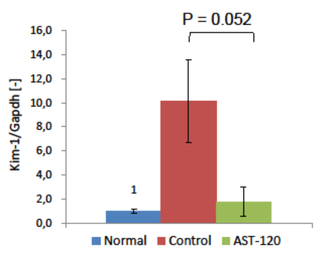


Fig. 10 Kim-1 gene expression
Mean ± SE, n=5

SHR/ND rats (Control and AST-120 groups) showed obesity, hypertension, hyperlipidemia and hyperglycemia as compared to WKY rats (Normal group) (Table1).

8% AST-120-administered SHR/ND rats showed significantly lower levels of urinary protein excretion (Fig. 1), the FPW (Fig. 3) and glomeruli size (Fig. 5) as compared with SHR/ND rats.

AST-120 also reduced the urinary protein excretion in a dose-dependent manner (Fig.1).

The FPW was significantly correlated with the levels of urinary protein excretion (r = 0.9498) (Fig.7)

The number of CD68 positive cells and KIM-1 positive area in renal tissues were reduced by the administration of AST-120 (Fig. 6, 7, 8, 9 and 10).

CONCLUSIONS

These results indicate that the administration of AST-120 at an early stage of diabetic nephropathy has a protective effect on the disease progression.

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