AN ORAL ADSORBENT AST-120 REDUCED OXIDATIVE STRESS, PROTEINURIA AND ALBUMINURIA 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.
- An oral adsorbent AST-120 has been used clinically as a drug for treatment of chronic kidney disease (CKD) patients to slow the progression of CKD.
- There is little evidence when AST-120 should be prescribed for subjects with early stage overt diabetic nephropathy.
- In this study, we aimed to assess the effect of AST-120 on the early stage of nephropathy using SHR/NDmc-cp（SHR/ND), a rat model of metabolic syndrome/ type 2 diabetes.

・Effect of treatment with AST-120 in SHR/ND

Method

Seven-week-old male SHR/ND (n=12) rats were divided into two groups (n=6 each) : AST-120 treated group (AST-120 group) and control group.
AST-120 group was administered AST-120 with the chow containing 8% for 16 weeks.
Wistar-Kyoto (n=8) rats were used as a normal.

Results

・ Biochemical parameters

Control group showed obesity, hyperlipidemia, hyperglycemia (as evidenced by HbA1c levels) and hyperinsulinemia as compared with Normal group. AST-120 had no effect on these abnormalities (data not shown) .

Table. 1. Biochemical parameters in Normal and Control groups at 23 weeks of age.

	Body weight (g)	Dietary intake (g/day)	T-cho (mg/dL)	TG (mg/dL)	HbA1c (%)	Ins (ng/mL)
Normal (n=8)	442 ± 4	17.7 ± 0.4	86 ± 1	95 ± 6	4.3 ± 0.1	1.9 ± 0.2
Control (n-6)	627 ± 9***	26.1 ± 0.6***	104 ± 4***	1074 ± 62***	5.8 ± 0.1***	12.8 ± 0.5***

Mean ± SE. *** p < 0.001 versus Normal.

・ Urinary albumin and protein

Excretions of albumin and protein in the urine were significantly increased with time as compared with normal group. AST-120 significantly suppressed their increases.

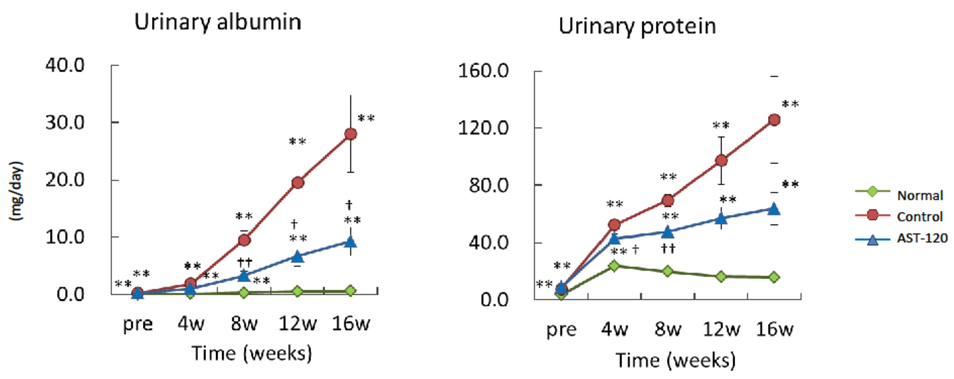

Fig. 1. Excretions of albumin and protein in the urine of Normal, Control and AST-120 groups.
Mean ± SE, n=8(Normal), n=6(Control and AST-120).
* p < 0.05, ** p < 0.01 versus Normal. †p < 0.05 , †† p < 0.01 versus Control

・Metabolomic analysis

Method

Serum samples of three groups (n=5 each) at 8 weeks after administration of AST-120 were analyzed by capillary electrophoresis mass spectrometry with time-of-flight (CE-TOFMS) and applied CE-TOFMS data to principal component analysis (PCA).
To validate these metabolites for the markers, we measured the serum levels of them by selected reaction monitoring method of LC/ESI-MS/MS.

Results

Serum samples of three groups (n=5 each) at 8 weeks after administration of AST-120 were analyzed by CE-TOFMS and 113 anions and 119 cations were identified.
PCA score plot showed clear separation among three groups.
By hierarchical cluster analysis, these metabolites were divided into 16 clusters. Among them, we focused on 40 metabolites in cluster 8 of which serum levels were increased in control group as compared with normal group and were reduced in AST-120 group.

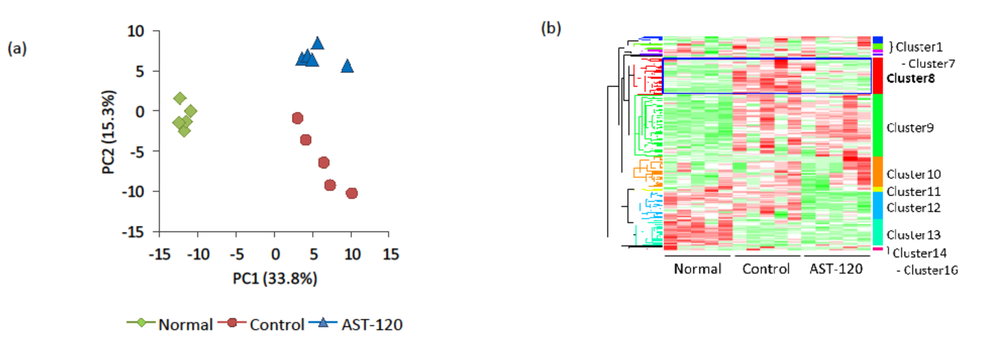

Fig. 2. The difference in the serum metabolites among three groups.
(a) PCA score plot of the CE-TOFMS data of serum samples.
(b) Hierarchical clustering showing patterns of metabolites. Red and green indicate high and low concentration of metabolites, respectively.

・ Top 3 peaks having the highest loading on PC2.

We confirmed that their serum levels were significantly increased in control group as compared with normal group and were significantly reduced in AST-120 group.

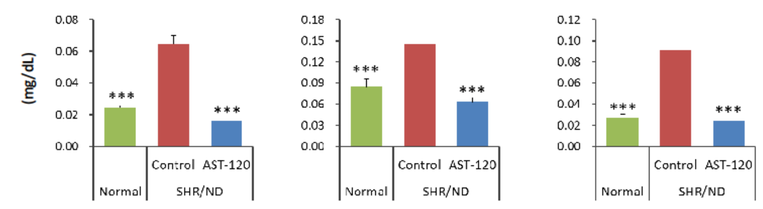

Fig. 3. Serum levels of *o*-hydroxybenzoic acid, hippuric acid and indole-3-acetic acid in 3 groups.
Mean ± SE, n=8, *** p < 0.001 versus control.

・Analysis of oxidative stress maker

Method

Seven-week-old male SHR/ND (n=10) rats were divided into two groups (n=5 each) : AST-120 group and control group.
AST-120 group was administered AST-120 with the chow containing 8% for 4 or 12 weeks. Wistar-Kyoto (n=5) rats were used as a normal.
Gene expression of oxidative stress markers in renal tissues treated with or without AST-120 for 4weeks was analyzed by real-time PCR. Urinary 8-OHdG was measured by ELISA.

Results

・ Urinary albumin and protein

Excretions of albumin and protein in the urine were significantly increased with time as compared with normal group. AST-120 significantly suppressed their increases (same as Fig.1).

・ Systolic BP （SBP）

SBP was significantly increased with time as compared with normal group. AST-120 significantly suppressed their increases.

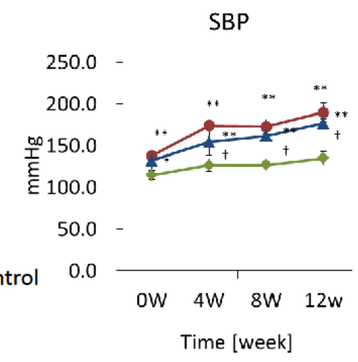

Fig. 4. SBP in Normal, Control and AST-120 groups.
Mean ± SD, n=5
* p < 0.05, ** p < 0.01 versus Normal. †p < 0.05 , †† p < 0.01 versus Control

・ Gene expression

The expression of two subunits of NADPH oxidase, Nox2 and p47^{phox}, measured in renal tissues was lower in AST-120 Group than in control Group.

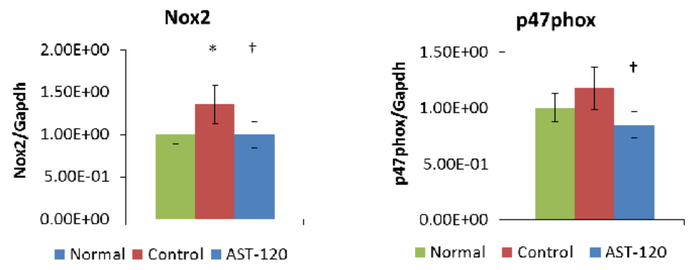

Fig. 5 Gene expression of Nox2 and p47^{phox} in 3 Groups
Mean ± SD, n=5 * p < 0.05 versus Normal. †p < 0.05 versus Control

・ Urinary 8-OHdG

Urinary 8-OHdG was significantly increased as compared with normal group. AST-120 significantly suppressed their increases.

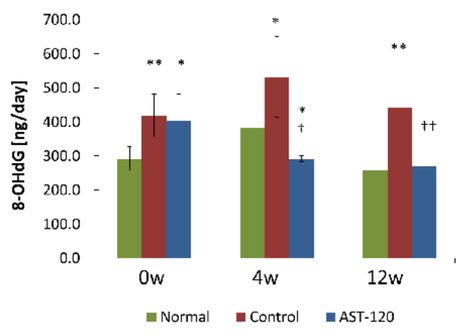

Fig. 6 Urinary 8-hydroxy-2'-deoxyguanosine (8-OhdG) excretion of 3 Groups.
Mean ± SD, n=5 , * p < 0.05, ** p < 0.01 versus Normal. †p < 0.05 , †† p < 0.01 versus Control

CONCLUSIONS

AST-120 administration decreased proteinuria, albuminuria and SBP in SHR/ND rats, and also reduced the expression of oxidative stress makers in renal tissues and Urinary 8-OHdG.
We detected 40 metabolites of which serum levels were increased in SHR/ND rats as compared with normal rats, and were reduced by administration of AST-120.
It indicated that the administration of AST-120 at an early stage has a protective effect on the progression of diabetic nephropathy.

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