Oral charcoal adsorbent (AST-120) prevents progression of cardiac damage in chronic kidney disease through suppression of oxidative stress

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Abstract

Background. Chronic kidney disease (CKD) is an important risk factor for cardiovascular disease (CVD). Increased oxidative stress plays a role in the pathogenesis of CVD in CKD patients. The oral charcoal adsorbent AST-120 attenuates the progression of CKD possibly by removing uraemic toxins such as indoxyl sulfate (IS), and reduces oxidative stress. We investigated the relationship between oxidative stress and cardiac damage in CKD and its prevention by AST-120.

Methods. Male Lewis rats were administered adriamycin at 8 weeks of age, and the right kidney was removed at 12 weeks of age. From 14 weeks of age, the rats were treated daily with AST-120 (n=8) or were untreated (control group, n=8). At 34 weeks of age, the rats were killed and urinary and blood biochemical tests as well as cardiac histological analyses were performed.

Results. At 14 weeks of age, there were no significant differences in blood pressure, renal function (creatinine clearance: 1.54 ± 0.28 mL/min versus 1.60 ± 0.22 mL/min), oxidative stress markers or other biochemical data between the control and AST-120 groups. At 34 weeks, despite similar blood pressure and renal function (creatinine clearance: 0.78 ± 0.46 mL/min versus 0.75 ± 0.54 mL/min), serum concentrations of IS and urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG), acrolein and IS were significantly lower in the AST-120 group than in the control group. Heart volume, left ventricular volume and cardiac fibrosis were significantly smaller in the experimental AST-120 group than in the control group. Heart volume, left ventricular volume and cardiac fibrosis were significantly smaller in the experimental AST-120 group than in the control group. Immuno-histological analysis revealed that the numbers of 8-OHdG- and acrolein-positive cardiomyocytes and the degrees of myocardial and perivascular fibrosis were ameliorated by AST-120 administration. The myocardial fibrosis score was significantly associated with the 8-OHdG- (r = 0.848, P < 0.001) and acrolein-positive (r = 0.812, P < 0.001) cell scores. The perivascular fibrosis score was also significantly associated with the 8-OHdG- (r = 0.906, P < 0.0001) and acrolein-positive (r = 0.789, P < 0.001) cell scores.

Conclusions. Oxidative stress is suggested to play a key role in the development of cardiac hypertrophy and fibrosis in CKD. AST-120 may suppress oxidative stress and reduce cardiac damage in CKD.

Keywords: AST-120; cardiac damage; cardiac hypertrophy; chronic kidney disease; oxidative stress

Introduction

Accumulated evidence has shown that not only end-stage renal disease but also mild renal dysfunction and/or the presence of albuminuria are powerful cardiovascular risk factors [1–4]. Patients with chronic kidney disease (CKD) have a higher risk of developing cardiovascular disease (CVD) than the general population [5]. An American Heart Association statement published in 2003 recommended that patients with CKD be considered as members of the highest risk group for subsequent CVD events [6]. Thus, although CKD has been shown to have an important role in CVD, the details of interactions between the kidneys and cardiovascular system remain unclear. As one of the several risk factors for CVD, oxidative stress plays a key role in its pathogenesis [7]. Oxidative stress is also increased in CKD patients [8].

AST-120 (Kremezin®; Daiichi-Sankyo Industry Co., Tokyo, Japan) is an oral charcoal adsorbent that reduces the levels of circulating uraemic toxins such as indoxyl sulfate (IS) and indole acetic acid. AST-120 prevents the progression of renal insufficiency through the reduction of uraemic toxins. Several reports have demonstrated that it...
potentially prevents histological and functional aggravation of CKD in human patients and an animal model of CKD [9,10]. In addition, a recent report has shown that AST-120 attenuates oxidative stress produced by uremic toxins in CKD [11,12].

We, therefore, speculated that oxidative stress may be an important factor in the progression of CVD in CKD. In the present study, we investigated whether AST-120 suppresses oxidative stress and the progression of cardiac damage in a rat model of CKD.

Materials and methods

Animals
Six-week-old male Lewis rats were purchased from Charles River Japan Inc. (Tokyo, Japan). The rats were housed with food and water available ad libitum in light- and temperature-controlled environments.

Experiment 1: chemical and surgical model. At 8 weeks of age, 16 rats were administered 3 mg of adriamycin (Kyowa Hakko Co., Tokyo, Japan) by injection into the tail vein. It has been reported that adriamycin generates free radicals and increases oxidative stress. Since we have focused on the role of oxidative stress in the progression of CKD and CVD, we used this chemical and surgical model in this study. At 12 weeks of age, the right kidney was removed. At 14 weeks of age, 8 of the 16 rats were assigned to a group treated with AST-120, and matched for body weight, blood pressure and renal function with the remaining 6 rats, which constituted the control group. In addition, at 8 weeks of age, 6 rats were administered saline and at 12 weeks of age, sham operation was performed (sham group). Control, AST-120 and sham group rats were fed a high protein diet including 30% protein. Rats in the AST-120-treated group were fed a high protein diet containing 8% AST-120.

Experiment 2: surgical model. Right nephrectomy was performed on 12 rats at 7 weeks of age. One week later, the left kidney was partially resected, removing a quantity corresponding to 2/3 of the weight of the previously resected right kidney. At 14 weeks of age, 6 of the 12 rats were assigned to a group treated with AST-120, and matched for characteristics with the remaining 6 rats, which constituted the control group. As well as in experiment 1, these rats were fed a high protein diet and rats in the treated group were administered AST-120.

At 34 weeks of age, the rats were killed under ether anaesthesia, after a 24-h urine sample had been collected from each rat using a metabolic cage. Blood samples were collected from the abdominal aorta for serum measurements, and the heart was removed for measurement of volume and histological analysis. All animal care and procedures were approved by the Animal Care and Use Committee of our institution.

Biochemical analysis
After centrifugation for 5 min at 3000 r.p.m., serum samples were stored at −80°C until analysis. Urine samples were also stored at −80°C for later analysis. Serum creatinine, urea nitrogen, total cholesterol, triglyceride and high-density lipoprotein cholesterol levels were measured using a Fuji Dri-Chem 3500 analyser (Fujifilm Japan, Tokyo, Japan). Urinary creatinine levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Nephrat; Exocell, Philadelphia, PA, USA). Serum and urinary IS were measured by a novel high-performance liquid chromatography method. The urinary excretion of 8-hydroxydeoxyguanosine (8-OHdG) and acrolein, which are sensitive markers of oxidative stress in the cardiac tissue, was assessed with anti-8-OHdG and acrolein monoclonal antibodies raised in rats (NOF Corporation, Tokyo, Japan). In brief, heart slices were pre-incubated with blocking agents (Simple Stain Rat; Nichirei, Tokyo, Japan) and then incubated with the primary antibodies mentioned above for 60 min at room temperature. A universal immunoperoxidase polymer (Histofine Simple Stain MAX PO, Nichirei) was used in immunostaining. The 8-OHdG- and acrolein-positive cells in the cardiac tissue were counted in 20 random microscopic fields to give 8-OHdG- and acrolein-positive cell scores. All evaluations were performed in a blinded manner.

Statistical analysis
We used StatView 5.0 (SAS Institute Inc., Cary, NC, USA) for all statistical analyses. Values are presented as mean ± SD. The significance of differences between the two groups was analysed by Student’s t-test. Relationships between variables were assessed using univariate linear regression analysis. A P-value of <0.05 was considered to represent statistical significance.

Results

Experiment 1: chemical + surgical model

Animal characteristics and biochemical measurements. At 14 weeks of age, body weight, blood pressure and biochemical data were similar in the control and AST-120 groups (Table 1). Characteristics and biochemical data at 34 weeks of age are shown in Table 2. Body weight decreased slightly in the AST-120 group compared with the control group. Blood pressure measurements and lipid profiles were similar between the two groups at 20 and 34 weeks of age. In contrast, at 34 weeks of age, urinary protein excretion tended to decrease by AST-120 administration.

Assessment of IS and systemic oxidative stress. Elevated serum IS levels and urinary IS excretion were significantly ameliorated by administration of AST-120 (Figure 1).
Table 2. Animal characteristics at 34 weeks of age

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>AST-120 (n = 8)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>541.6 ± 77.9</td>
<td>529.8 ± 59.9</td>
<td>0.738</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>152.6 ± 8.7</td>
<td>153.4 ± 11.0</td>
<td>0.882</td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.38 ± 1.73</td>
<td>2.59 ± 2.05</td>
<td>0.664</td>
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<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>145.1 ± 90.3</td>
<td>162.0 ± 112.8</td>
<td>0.746</td>
</tr>
<tr>
<td>T-cho (mg/dL)</td>
<td>391.9 ± 113.8</td>
<td>392.4 ± 182.1</td>
<td>0.695</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>669.1 ± 418.2</td>
<td>528.8 ± 593.1</td>
<td>0.593</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>101.9 ± 21.5</td>
<td>93.4 ± 24.9</td>
<td>0.477</td>
</tr>
<tr>
<td>Ccr (mL/min)</td>
<td>0.78 ± 0.46</td>
<td>0.75 ± 0.54</td>
<td>0.765</td>
</tr>
<tr>
<td>U-protein (mg/day)</td>
<td>432.0 ± 147.0</td>
<td>314.6 ± 149.6</td>
<td>0.136</td>
</tr>
<tr>
<td>U-protein (mg/day)</td>
<td>432.0 ± 147.0</td>
<td>314.6 ± 149.6</td>
<td>0.136</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; T-cho, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein cholesterol; Ccr, creatinine clearance; U-protein, urinary protein excretion. Values are mean ± SD.

We also measured the urinary excretion of 8-OHdG and acrolein, which are systemic oxidative stress markers. At 14 weeks of age, the urinary excretion of 8-OHdG and acrolein were similar between the control and AST-120 groups (Table 1). However, at 34 weeks of age, 24-h urinary 8-OHdG and acrolein excretion were also markedly lower in the AST-120 group than in the control group (Figure 2; 8-OHdG: control group 200.7 ± 42.7 ng/day; AST-120 group 147.3 ± 37.8 ng/day; P < 0.05. Urinary acrolein excretion was significantly reduced by AST-120 administration, although baseline data were similar in the control and AST-120 groups.

Evaluation of cardiac abnormalities and oxidative stress in cardiac tissue. To confirm the effect of AST-120 administration on the heart more precisely, we also performed morphological and histological analysis in sham-operated rats.

At 34 weeks of age, relative cardiac and left ventricular weights were significantly lower in the AST-120 group than in the control group despite similar blood pressures in the two groups (Figure 3; relative cardiac weight −13.3%, relative left ventricular weight −15.1%). Myocardial and perivascular fibrosis was significantly more severe in the control group than in the AST-120 group (Figure 4A and B).

To evaluate oxidative stress in the cardiac tissue, we performed immunohistological analysis. The numbers of 8-OHdG- and acrolein-positive cardiomyocytes were significantly lower in the AST-120 group than in the control group (Figure 5A and B). As shown in Figure 7A, the myocardial fibrosis score was significantly associated with the 8-OHdG- and acrolein-positive cell scores: control group versus Sham group, P < 0.05. The relative left ventricular weight was also significantly associated with the 8-OHdG- and acrolein-positive cell scores: control group versus Sham group, P < 0.05.

Relationship between IS, oxidative stress and cardiac damage in the control and AST-120 groups. Urinary IS excretion was significantly correlated with urinary 8-OHdG excretion (r = 0.680, P = 0.005) and acrolein excretion (r = 0.699, P = 0.036) (Figure 6A) and 8-OHdG- (r = 0.852, P < 0.001) and acrolein-positive (r = 0.792, P = 0.001) cell scores (Figure 6B). As shown in Figure 7A, the myocardial fibrosis score was significantly associated with the 8-OHdG- (r = 0.848, P < 0.001) and acrolein-positive (r = 0.812, P < 0.001) cell scores. The perivascular fibrosis score was also significantly associated with
Experiment 2: surgical model

At the 34th week, deterioration of renal function was suppressed (Ccr: control group 0.90 ± 0.18 mL/min, AST-120 group: 1.11 ± 0.22 mL/min, \( P = 0.07 \)) and urinary excretion of IS and 8-OHdG significantly reduced by administration of AST-120 (U-IS: control group 4.40 ± 0.77 ng/day, AST-120 group 1.70 ± 0.62 ng/day, \( P < 0.0001 \); U-8-OHdG: control group 693.8 ± 205.7 ng/day, AST-120 group 372.2 ± 124.6 ng/day, \( P < 0.005 \)). Furthermore, relative cardiac weight was significantly lower (relative cardiac weight: control group 0.292 ± 0.034, AST-120 group 0.250 ± 0.019, \( P < 0.02 \)) and myocardial and perivascular fibrosis were significantly reduced in the AST-120 group than in the control group (myocardial fibrosis score: control group 4.0 ± 1.0, AST-120 group 1.4 ± 0.5, \( P < 0.001 \); perivascular fibrosis score: control group 2.8 ± 0.8, AST-120 group 1.8 ± 0.8, \( P = 0.09 \)). As for oxidative stress in the cardiac tissue, the numbers of 8-OHdG- and acrolein-positive cardiomyocytes were significantly lower in the AST-120 group than in the control group (8-OHdG-positive cell score: control group 43.6 ± 5.3, AST-120 group 17.6 ± 2.1, \( P < 0.001 \); acrolein-positive cell score: control group 32.8 ± 3.9, AST-120 group 14.4 ± 2.3, \( P < 0.001 \)).

Discussion

Our study demonstrated that (1) serum and urinary IS, urinary 8-OHdG and urinary acrolein excretion were significantly reduced in the AST-120 group, (2) oxidative stress in the cardiac tissue were significantly lower in the AST-120 group and (3) administration of AST-120 ameliorated cardiac fibrosis and left ventricular hypertrophy (LVH).

Oxidative stress is believed to be a risk factor not only for cardiovascular but also for renal disease [7,8]. In our study, urinary 8-OHdG and acrolein excretion increased together with reduction of renal function, and the association of uraemic toxins such as IS with increased oxidative stress is speculated to be one of the mechanisms. IS induced the production of reactive oxygen species (ROS) in in vitro studies [11–13]. Such increased ROS production is considered to occur through a pathway involving NADPH oxidase or NADPH-like oxidase. In addition, it has been reported that IS strongly decreased total glutathione levels,
which is the most active non-enzymatic antioxidant, in endothelial cells [13]. Nakagawa et al. reported that IS increased oxidative stress in experimental uraemic rats [14]. They found that urinary IS had positive linear correlations with urinary acrolein and 8-OHdG levels. Such increases in oxidative stress were reduced by administration of AST-120 [11,14]. Similarly, in our study, urinary IS excretion and oxidative stress markers such as 8-OHdG and acrolein in both urine and the cardiac tissue were significantly suppressed by the administration of AST-120. Furthermore, positive linear relationships between urinary IS excretion and 8-OHdG as well as acrolein were observed in both urine and the cardiac tissue in the present study. In light of these findings, IS might induce oxidative stress, and hence, lowering the IS level might lead to a reduction in oxidative stress in CKD.

Many studies have reported that oxidative stress plays an important role in the pathogenesis of CVD in CKD [7]. Conti et al. reported an increased antioxidant response and the progression of cardiac abnormalities, including LVH, in CKD model rats [15]. Furthermore, Amann et al. reported that cardiac abnormalities were observed in CKD model rats with mild to moderate renal dysfunction [16,17]. They also demonstrated that antioxidant therapy with dl-α-tocopherol improved LVH and typical changes of the myocardium in experimental CKD model rats [18].

In the present study, we also analysed cardiac abnormalities histomorphologically in CKD model rats. LVH and fibrosis in both cardiomyocytes and the perivascular territory were significantly more severe in the control group than in the AST-120 group. Using immunohistochemistry, we observed reduced oxidative stress in the cardiac tissue in the AST-120 group. Furthermore, oxidative stress and IS were significantly ameliorated by AST-120 administration, and indicators of oxidative stress in both urine and the cardiac tissue were significantly correlated with the intracardiac damage. These findings suggest that oxidative stress and IS might play a role in the progression of cardiac abnormalities in CKD. However, detailed mechanisms of the progression of these cardiac abnormalities in CKD were not clarified in this study.

LVH is a relatively common finding in CKD and has been regarded as an important predictor of mortality from CVD. In addition, >70% of dialysis patients have LVH, LV dilatation or low fractional shortening at the initiation of dialysis treatment, and LVH has been found in >50% of CKD patients prior to the requirement for dialysis [19–21]. LVH is a crucial determinant of prognosis [22]. Therefore, preventing the progression of LVH is a very important strategy in the management of CKD patients, and for this purpose, it is necessary to control blood pressure. However, as mentioned above, suppression of oxidative stress is also crucial in reducing mortality from CVD combined with LVH. Our study demonstrated that AST-120 successfully prevented the progression of LVH suggesting that treatment with AST-120 might delay not only the worsening of renal...
function but also the progression of LVH. Consequently, we speculate that this intervention could successfully lead to a reduction in mortality from CVD.

Previous reports demonstrated that IS has been implicated in the pathogenesis of renal disease [23–26]. Therefore, we also examined oxidative stress, IS and renal damage in the present study. However, we did not find significant improvement of renal damage and oxidative stress in the renal tissue after treatment with AST-120, despite lowered oxidative stress and IS levels. We also performed an experiment using 5/6 nephrectomized rats. In preliminary study using this model, renal damage and oxidative stress were improved at 30 weeks of age (data not shown). Therefore, we thought that the reason for this inconsistency with previous studies might be that we observed the rats for a longer time and that differences in the type of underlying kidney pathology may be associated with the result.

Though adriamycin is cardiotoxic by itself, it has been reported that the cardiotoxicity of adriamycin is mainly due to oxidative stress. Thioredoxin is well known as one of the anti-oxidative substances. Shioji et al. reported that thioredoxin transgenic mice attenuates adriamycin-induced cardiotoxicity [27]. In addition, they have demonstrated that the change of cardiomyocytes were not apparent in treated groups with 6 or 15 mg/kg adriamycin. In the present study, we used a much smaller dose of adriamycin (3 mg/kg) than they did. Therefore, we believe that the direct effect of adriamycin on cardiomyocytes was trivial. Furthermore, we also performed another experiment using a surgical model of CKD. As for the effect of oral charcoal adsorbent on heart in this model, similar results were observed.

Thus, although we did not clarify the detailed mechanisms of cardiac and renal damage in this study, our findings suggest that increased oxidative stress as a result of the action of IS may make cardiac abnormalities worse. The detailed laboratory process requires further study, which is now under way in our laboratory.

Conclusions

Our data indicate that oxidative stress plays a key role in the development of cardiac abnormalities in CKD. Furthermore, it is suggested that AST-120 suppresses oxidative stress and prevents the progression of cardiac damage in CKD.

Conflict of interest statement. None declared.

References

Association of kidney function and uncarboxylated MGP protein: Data from the Heart and Soul Study

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Abstract

Background. Vascular calcification is highly prevalent in persons with chronic kidney disease (CKD) and predicts cardiovascular disease (CVD) events. Matrix Gla protein (MGP) is a potent inhibitor of vascular calcification, and lower levels of its precursor—uncarboxylated MGP (ucMGP)—are associated with vascular calcification and atherosclerosis. Whether mild to moderate decrements in kidney function are associated with lower serum ucMGP is unknown.

Methods. In a cross-sectional study among 842 outpatients with stable CVD, estimated glomerular filtration rate (eGFR), serum cystatin-C and urine albumin-to-creatinine ratio (ACR) were measured and serum ucMGP levels were determined by ELISA. Multivariate linear regression evaluated the association of each kidney function measure with serum ucMGP levels.

Results. The mean eGFR was 76 ± 23 mL/min/1.73 m², and 186 subjects (22%) had moderate CKD (eGFR < 60 mL/min/1.73 m²). The mean ± SD ucMGP level was 3289 ± 1177 nM. In unadjusted analysis, each 10 mL/min/1.73 m² lower eGFR was associated with 101 nM lower ucMGP level. Among subjects with or without diabetes, each 0.1 mg/L higher cystatin-C was associated with 39 nM lower ucMGP (95% CI 23 to 55; P < 0.001). In contrast, no significant association was observed between ACR and ucMGP in either unadjusted or adjusted analyses (adjusted P = 0.17). All associations were similar among subjects with or without diabetes (P-values for interaction > 0.50).

Conclusions. Among outpatients with stable CVD, a reduced glomerular filtration rate is associated with a decreased serum ucMGP level. In contrast, ACR is not associated with ucMGP levels. Whether ucMGP is a useful marker of vascular calcification and CVD event risk in persons with CKD deserves future study.

Keywords: atherosclerosis; chronic kidney disease; matrix Gla protein; vascular calcification

Introduction

Chronic kidney disease (CKD) is a strong risk factor for cardiovascular disease (CVD) mortality [1–4] and affects ~13% of the US population [5]. At each stage of CKD, the risk of CVD mortality is several times higher than the risk of progression to end-stage renal disease (ESRD) [1,6,7]. Despite intensive investigation, the mechanisms responsible for this strong association remain unknown [8]. One candidate mechanism may be accelerated vascular calcification, which is highly prevalent in CKD and independently predicts CVD events [9–17]. Recent research