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AST-120 Improves Microvascular Endothelial Dysfunction in End-Stage Renal Disease Patients Receiving Hemodialysis

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Purpose: Endothelial dysfunction (ED) is a pivotal phenomenon in the development of cardiovascular disease (CVD) in patients receiving hemodialysis (HD). Indoxyl sulfate (IS) is a known uremic toxin that induces ED in patients with chronic kidney disease. The aim of this study was to investigate whether AST-120, an absorbent of IS, improves microvascular or macrovascular ED in HD patients.

Materials and Methods: We conducted a prospective, case-controlled trial. Fourteen patients each were enrolled in respective AST-120 and control groups. The subjects in the AST-120 group were treated with AST-120 (6 g/day) for 6 months. Microvascular function was assessed by laser Doppler flowmetry using iontophoresis of acetylcholine (Ach) and sodium nitroprusside (SNP) at baseline and again at 3 and 6 months. Carotid arterial intima-media thickness (cIMT) and flow-mediated vasodilation were measured at baseline and 6 months. The Wilcoxon rank test was used to compare values before and after AST-120 treatment.

Results: Ach-induced iontophoresis (endothelium-dependent response) was dramatically ameliorated at 3 months and 6 months in the AST-120 group. SNP-induced response showed delayed improvement only at 6 months in the AST-120 group. The IS level was decreased at 3 months in the AST-120 group, but remained stable thereafter. cIMT was significantly reduced after AST-120 treatment. No significant complications in patients taking AST-120 were reported.

Conclusion: AST-120 ameliorated microvascular ED and cIMT in HD patients. A randomized study including a larger population will be required to establish a definitive role of AST-120 as a preventive medication for CVD in HD patients.

Key Words: AST-120, hemodialysis, microvascular circulation, endothelial dysfunction, laser-Doppler flowmetry, indoxyl sulfate

INTRODUCTION

End-stage renal disease (ESRD) patients have higher cardiovascular morbidity and mortality compared to the general population.[1] In addition to the well-known cardiovascular risk factors such as hypertension, diabetes, and dyslipidemia that are usually present in this population, endothelial dysfunction (ED) induced by acute and chronic inflammatory status in patients with chronic kidney disease (CKD) contributes to overt cardiovascular disease (CVD).[2,3] ED is a systemic pathologic condition that is defined by the inability of vessels to optimally dilate in response to vasodilators acting on the endothelium,[4] and precedes overt atherosclerosis. Because ED commonly occurs during the early stage of potential CVD such as diabetes mellitus, hypercholesterolemia, and hypertension, it is considered an excellent prognostic marker of cardiovascular complications in patients with established CVD as well as patients carrying significant CVD risk factors.[4,5] Furthermore, because ED begins early in the progression of CKD, early detection of ED in these patients is important to predict CVD.

Although various methods for assessing ED have been developed over the years, flow-mediated vasodilation (FMD) by ultrasound is the most widely used noninvasive method to eval-

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uate macrovascular ED. However, recent studies have suggested that characterization of microvascular reactivity in superficial vessels is a sensitive marker for ED in ESRD patients.[6] Indeed, microcirculation is the initial site of injured endothelial change in subjects at risk of CVD, and microvascular damage precedes systemic ED leading to further CVD complications.[7,8] Thus, early detection for microvascular dysfunction may be important for preventing progression of systemic ED.

Indoxyl sulfate (IS) is a well-known uremic toxin, the concentration of which is inversely correlated with residual renal function in CKD patients.[9] IS inhibits endothelial proliferation, and repair via generation of oxidative stress and induces impaired endothelial function.[10-12] AST-120 is an oral activated charcoal adsorbent used to reduce plasma IS and ameliorate ED in pre-dialysis CKD patients.[12] However, there are currently no reports of the usefulness or effects of AST-120 in ESRD patients.

The aim of this study was to investigate whether AST-120 ameliorates ED of micro- or macro-circulation in ESRD patients. In addition, we also aimed to determine whether AST-120 might be useful as a preventative agent for development of CVD in an at-risk ESRD population.

MATERIALS AND METHODS

Subjects and study design

This was a prospective case-control study of ESRD patients receiving hemodialysis (HD) in Ewha Womans University Mokdong Hospital between March 2011 and February 2013. A total of 28 clinically stable patients 19–64 years of age receiving maintenance HD for more than 3 months were enrolled. Exclusion criteria were as follows: severe constipation, known gastrointestinal motility disorder, difficulty in stool passage, acute gastric ulcer, liver cirrhosis, allergy to AST-120 (Kremezin®, Kureha Pharmaceuticals, Tokyo, Japan), confirmed peripheral neuropathy, dermatologic problems, and history of arteriovenous fistula operation on both arms. None of the patients had acute diseases such as myocardial infarction, unstable angina, acute pulmonary embolism, acute neurologic disease, malignancies, or overt systemic infections within 6 months prior to enrolling in the study. Control subjects were enrolled using the same exclusion criteria as the study group.

Patients were divided into two groups consisting of the AST-120 treatment group and a control group. Subjects in the AST-120 group received AST-120 (6 g) per day for 6 months. To evaluate ED and atherosclerosis, FMD, iontophoresis with laser Doppler flowmetry (LDF), and carotid intima-media thickness (cIMT) were measured at baseline and again 3 and 6 months after initiation of the study. To examine how CKD per se affects vascular function, iontophoresis with LDF, FMD, and cIMT were measured in 31 pre-dialysis CKD patients. The patients with estimated glomerular filtration rate (eGFR) under 60 mL/min/1.73 m^2 (CKD stage 3, 4, and 5) were enrolled. eGFR was calculated with Modification of Diet in Renal Disease (MDRD) equation. CKD stage was determined according to Kidney Diseases Outcomes Quality Initiative (KDOQI) CKD classification as followings; CKD stage 3 was defined as GFR in 30–59 mL/min/1.73 m^2, stage 4 was between 15–29 mL/min/1.73 m^2, and stage 5 was under 15 mL/min/1.73 m^2. The study protocol was approved by the Ewha Institutional Review Board and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Assessment of endothelial dysfunction: flow-mediated vasodilation and iontophoresis with laser Doppler flowmeter

All tests were performed prior to starting a dialysis session and fasting after midnight for at least 8 hours.

Flow-mediated vasodilation (FMD)

Brachial FMD was assessed using the method of Celermajer, et al.[13] Briefly, after 20 minutes of rest in a supine position in a temperature-controlled room (22–24°C), the study arm of each patient was comfortably immobilized in the extended position throughout the measurement. The change in the luminal diameter of the brachial artery during the reactive hyperemia phase was used for assessing endothelium-dependent vasodilation (EDV). After keeping the subjects lying down for 15 minutes, endothelium-independent vasodilation (EIDV) was measured based on the change in the luminal diameter of the brachial artery in response to 0.6 mg nitroglycerine. The lag times from the baseline to initial reaction and peak reaction were defined as the initial reaction time (IRT) and peak reaction time (PRT), respectively. For additional details on measurements, please refer to the supplementary methods (Supplementary Method 1, only online).

Iontophoresis with laser Doppler flowmetry

Iontophoresis employs electrically repulsive forces to deliver a locally applied drug across the skin for therapeutic and diagnostic purposes. Iontophoresis with charged vasoactive substances across the skin through small electric current has been suggested as a non-invasive and proper tool to determine ED.[14] Likewise, LDF is used to measure cutaneous blood perfusion using the principle of the Doppler shift for lasers, and provides a linear relationship with the velocity of red blood cells (RBCs).[15-17]

All subjects sat in a comfortable chair and had a 20-min acclimatization period in a temperature-controlled room (22–24°C). After an arm with no graft or fistula was selected, the skin of forearm was carefully cleaned using alcohol. A drug delivery chamber electrode (PF 383; Perimed, Järfälla, Sweden) linked with a different electrode (PF 384; Perimed) was attached to the volar surface of the forearm at a distance of 10-mm. Next, 1% solutions of acetylcholine (Ach) and sodium nitroprusside (SNP) were introduced into the anodal and cathodal chambers for measuring endothelial-dependent and -inde-

pendent responses, respectively.[18] The laser Doppler probe connected to the LDF (Periflux PF4001, standard probe PF408, Perimed) was fixed within the drug chamber to explore the same small area of the skin. The laser Doppler outputs were recorded continuously as arbitrary units (perfusion units, PU) using an interfaced computer equipped with acquisition software. After recording 5-min of stable baseline perfusion, dose-response curves to Ach and SNP were obtained by step-wise application of currents.[19] Ach was delivered with six doses (0.1 mA for 20 sec each) followed by another two doses (0.2 mA for 20 sec each) with a 60-sec interval between each single dose. After a 1-min recovery period, SNP was delivered with two doses (0.1 mA for 20 sec each) followed by one dose (0.2 mA for 20 sec) with a 180-sec interval between the two successive doses. The absolute maximal response was defined as the flow rate that was reached after the last drug delivery. In order to eliminate baseline variability, the blood flow responses to locally delivered Ach and SNP were expressed as a ratio of changed PUs to baseline PU.[20,21] Detailed information and descriptions of the measurement protocol are described in the supplementary data (Supplementary Method 2, only online).

Intima-media thickness of the common carotid artery

Carotid ultrasonography was performed using a 10-MHz scanning frequency in B mode. We analyzed cIMT using the computer-based software Intimascope® (Media Cross Co. Ltd., Tokyo, Japan).[22] One skilled technician scanned the right and left carotid arteries in transverse planes while the subject was in the supine position. Images were obtained 20 mm proximal to the origin of the bulb at the far wall of the common carotid artery (CCA). In this region, computer-based IMT was evaluated by three methods: 3-point, maximal, and average evaluations. Three-point evaluation refers to the average value of 3-point IMT, including two end points and the middle point in the >2 cm region. Maximal (max) evaluation was obtained by the IMT value at a maximal point of the region. Lastly, average IMT (aver-IMT) was the average value of 250 computer-based points in the region. Because the computer-automated aver-IMT evaluations by 3-point or aver-IMT methods have been suggested to be more reliable indices for atherosclerosis, we used aver-IMT in this study. We also measured cIMT for each patient at baseline and again at 6 months.

Blood sampling

Venous blood samples were collected for each patient before starting dialysis session after overnight fasting (at least 8 hours) at baseline and at 3 and 6 months. Samples were obtained from the HD-needle puncture site and analyzed immediately. Standard laboratory tests included complete blood cell count and routine blood chemistry including serum albumin, protein, blood urea nitrogen (BUN), creatinine, cholesterol, low density lipoprotein (LDL), and uric acid. High sensitive C-reactive protein (hsCRP) was measured using a nephelometric immunoassay (Handok Pharm., Seoul, Korea).

Plasma levels of IS were measured at baseline and again at 3 and 6 months prior to a dialysis session. IS concentrations were measured by HPLC (Agilent Technologies, Santa Clara, CA, USA) using integration software (Hewlett-Packard Chemstation; Hewlett-Packard Inc., Waldbronn, Germany).[15] Plasma samples were immediately stored at -70°C, thawed at room temperature for analysis; they were transferred to an autosampler and injected onto an HPLC system. The separation was performed using a SHISEIDO Capcell Park MF Ph-1 SC80 column (150×4.6 mm, 5 μm; Shiseido Co., Ltd., Tokyo, Japan). The flow rate was set at 1.0 mL/min, and the wavelengths of the fluorescence detector were set at 295 nm for excitation and 390 nm for emission. A calibration curve was constructed for the range of 0.1 to 10.0 mg/dL.

Statistical analysis

Statistical analysis was performed using SPSS software for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean±SD or number (%) unless otherwise specified. Student's t-test was used to compare continuous variables between two groups, and the chi-square test was used for comparing two categorical values. The Wilcoxon signed rank test was used to compare the time-dependent changes in FMD, iontophoresis with LDF, cIMT, and other values as a function of AST-120 treatment in each patient. p values<0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Patient characteristics at baseline did not differ significantly between the control and AST-120 groups (Table 1). The mean age was similar in both groups: 50.7 years in the control group and 50.0 years in the AST-120 group. The incidence of diabetes was similar: 5 of 14 subjects (35.7%) in the control group and 3 of 14 (21.4%) in AST-120 group. There were no significant differences in laboratory findings between the two groups, except higher phosphorus levels in the AST-120 group. The baseline characteristic of pre-dialysis patients was described in the Supplementary Table 1 (only online). In brief, total 31 patients were included in analysis, and the numbers of patient according to CKD stage were 12 at stage 3, 11 at stage 4, and 8 at stage 5. There was no difference between mean age and gender ratio between pre-dialysis CKD patients and HD patients groups. Comorbidity condition such as hypertension, diabetes, and CVD was similarly observed between two groups. Both endothelium-dependent and EIDV assessed by FMD and iontophoresis with LDF were less impaired in pre-dialysis CKD patients than HD patients. Furthermore, cIMT was more preserved in pre-dialysis CKD patients compared to HD patients (Supplementary Table 2, only online).

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Indoxyl sulfate

The baseline IS level in the AST-120 group was slightly less than that of the control group, although the difference was not statistically significant. After 3 months of AST-120 (6 g/d) treatment, serum IS levels were significantly decreased compared to baseline (3.24 mg/dL vs. 1.88 mg/dL, p=0.033). Likewise, IS levels remained lower than baseline in the AST-120 group after treatment for 6 months, although the difference was not statistically significant. On the other hand, the IS level in the control group did not change significantly from baseline during the course of the study. The IS level was significantly lower in the AST-120 group than control at 3 month's comparison (4.14 mg/dL vs. 1.88 mg/dL, p=0.001) (Table 2).

Table 1. Baseline Characteristics

Variables	Control (n=14)	AST-120 (n=14)	*p* value
Age (yrs)	50.7±9.5	50.0±9.3	0.352
Gender (F/M)	8/6	8/6	1.000
Dialysis duration (months)	67.7±13.0	67.5±12.9	0.991
Comorbidity, n (%)			
Hypertension	14	14	1.000
Diabetes	5 (35.7)	3 (21.4)	0.678
CVD	2 (14.3)	3 (21.4)	1.000
Medication, n (%)			
ACEi or AT II blocker	10 (71.4)	11 (78.6)	1.000
CCB	10 (71.4)	11 (78.6)	1.000
β-blocker	6 (42.9)	5 (35.7)	1.000
Anti-platelet agent	6 (42.9)	5 (35.7)	1.000
Laboratory findings			
Hemoglobin (g/dL)	10.2±0.8	10.7±0.9	0.227
Hematocrit (%)	30.7±2.9	32.7±2.8	0.104
BUN (mg/dL)	73.2±14.6	81.4±15.3	0.265
Creatinine (mg/dL)	10.7±2.8	11.3±3.0	0.511
Calcium (mg/dL)	8.7±0.5	8.6±0.6	0.376
Phosphorus (mg/dL)	5.0±0.9	5.9±1.2	0.024
Albumin (g/dL)	3.9±0.2	3.9±0.3	0.701
Total cholesterol (mg/dL)	136.8±37.1	140.6±24.8	0.410
LDL cholesterol (mg/dL)	74.1±28.9	68.3±21.9	0.928
hsCRP (mg/dL)	0.27±0.33	0.22±0.33	0.804

CVD, cardiovascular disease; ACEi, angiotensin converting enzyme inhibitor; AT II blocker, angiotensin II receptor blcoker; CCB, calcium channel blocker; BUN, blood urea nitrogen; LDL, low-density lipoprotein; hsCRP, high-sensitivity C-reactive protein.
All results were given as mean±SD or number (%).

Table 2. Changes in Serum Level of Indoxyl Sulfate

Variables	Control (n=14)	AST-120 (n=14)	*p* value*
Baseline	4.22 (1.00–10.36)	3.24 (0.12–8.14)	0.246
3 months	4.14 (2.06–12.41)	1.88 (0.05–9.09)	0.001
6 months	3.92 (0.95–7.95)	2.53 (0.14–5.87)	0.302

*p value represents a difference between control and AST-120 groups.

Endothelial dysfunction: micro-, macro-circulatory endothelial dysfunction

Baseline microvascular endothelial function, assessed by iontophoresis with LDF, was significantly impaired in HD patients groups. However, impaired microvascular function in HD patients showed a significant amelioration after AST-120 treatment. Specifically, the patients in the AST-120 group exhibited an increased endothelium-dependent Ach-induced response 3 months after AST-120 treatment [6.24 (3.11–14.63) PU vs. 10.40 (4.28–19.98) PU, p=0.005] (Fig. 1A), and this improvement of Ach-induced response was preserved through 6 months [9.91 (7.29–19.32) PU]. Conversely, the control group exhibited decreased Ach-induced vasodilation at both 3 and 6 months compared to baseline [8.49 (3.91–21.76) PU vs. 5.94 (3.78–16.29) PU vs. 6.36 (3.45–10.69) PU]. SNP-induced response was significantly increased at 6 months after AST-120 treatment [5.27 (2.84–13.10) PU vs. 7.29 (3.36–16.44) PU, p=0.004] (Fig. 1B). However, the control group showed decreased SNP-induced perfusion at 6 months compared with baseline [7.02 (2.77–22.48) PU vs. 4.91 (1.94–11.09) PU, p=0.004]. The SNP-induced responses at 3 months showed no significant changes compared to baseline in both the control and AST-120 groups. Similar results were found in the analysis of non-diabetic patients (n=9 in control vs. n=11 in AST-120, data not shown).

Macrovascular ED, assessed by FMD at baseline and 6 months, was not consistent with that of microvascular function. Specifically, baseline EDV was severely impaired in all subjects and there was no significant difference between the control and AST-120 groups (4.64±2.87% vs. 4.73±2.55%, p=0.930). EIDV was also similar between the two groups (12.08±6.52% in control vs. 13.56±8.26% in AST-120, p=0.568). After 6 months, both EDV and EIDV failed to show significant changes compared to baseline (Fig. 2). However, the control group showed a tendency by which both EDV and EIDV were reduced more than baseline (EDV; 3.76±2.99%, p=0.096 vs. EIDV; 11.71±6.51%, p=0.363).

Common carotid arterial intima-media thickness

Baseline carotid IMT in ESRD patients was thicker than the general population and similar among study groups [1.01 (0.9–1.42) mm in control vs. 0.96 (0.79–1.11) mm in AST-120, p=0.077]. After 6 months, the cIMT was significantly reduced in the AST-120 group [0.90 (0.81–1.08) mm vs. 0.96 (0.79–1.11) mm, p=0.006]. However, the 6th month cIMT in the control group was significantly thicker [1.03 (0.90–2.18) mm vs. 1.01 (0.9–1.42) mm, p=0.002] (Fig. 3).

Correlation among iontophoresis with LDF, FMD, cIMT and indoxyl sulfate level

The Ach-induced endothelium-dependent response was positively correlated with SNP-induced response (β=0.782, p=0.000). With respect to brachial FMD assessment, EDV also positively correlated with EIDV (β=0.552, p=0.000). There was

no significant correlation between microvascular (Ach-induced iontophoresis) and macrovascular endothelium-dependent responses (EDV). However, the endothelium-independent microvascular (SNP-induced iontophoresis) and macrovascular (EIDV) responses exhibited a close relationship (β=0.293, p=0.032). Neither micro- nor macro-endothelium-dependent function significantly correlated with cIMT or IS levels, respectively. However, EIDV inversely correlated with cIMT and serum IS level (β=-0.339, p=0.012 vs. β=-0.328, p=0.016). In addition, neither hsCRP nor hemoglobin levels significantly correlated with ED or cIMT (data not shown).

Compliance

The mean compliance for AST-120 over the 6 month study was 75.5±22.3% (77.1±22.7% during 1–3 months vs. 73.1±23.1% during 4–6 months). During the medication period no significant side effects such as constipation, gastrointestinal irritability, and hypersensitivity were noted. None of the enrolled 14 patients dropped out of the study, and all of the patients successfully completed the 6-month medication regimen.

DISCUSSION

In the present study, we showed that microvascular ED was improved after AST-120 treatment in HD patients. Likewise, microvascular ED was significantly improved at 3 months after AST-120 treatment, and this effect was preserved during continued treatment for 6 months. The degree of change of the Ach-induced response was greatest during the first 3 months

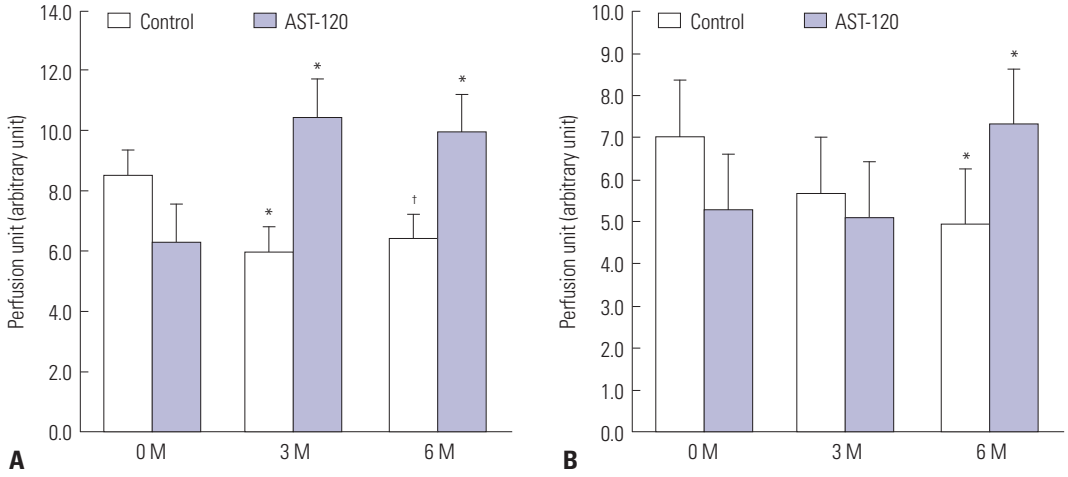

Fig. 1. Changes in microcirculatory vascular dysfunction assessed by iontophoresis with LDF between control and AST-120 groups. (A) The acetylcholine (Ach)-induced response was significantly improved after both 3 months (M) and 6 M of AST-120 treatment compared to baseline, but was worsened in the control group. (B) The nitroprusside (NSP)-induced response was not different for either 3 M or 6 M of AST-120 treatment. Vascular response was described as the ratio of perfusion units of responsive flow compared with basal flow. *p=0.005 vs. iontophoresis of 0 M, †p=0.001 vs. iontophoresis of 0 M.

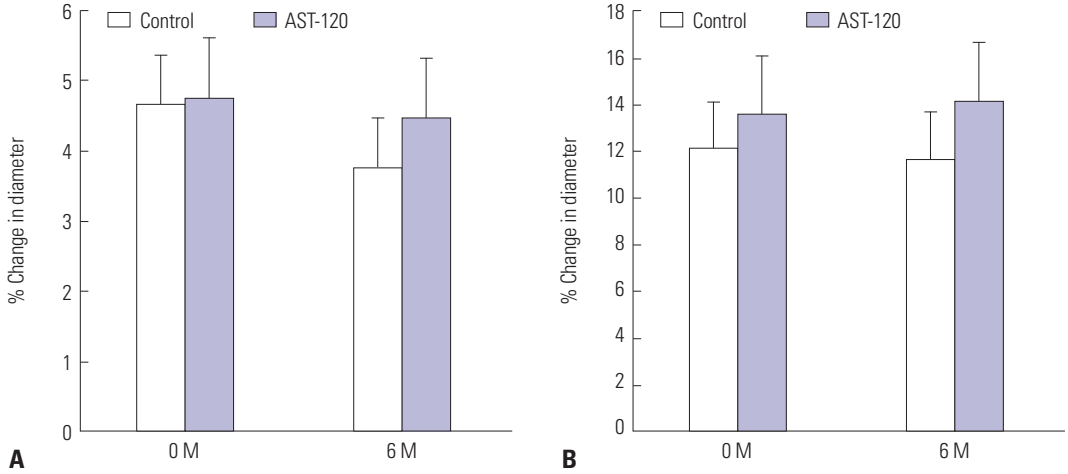

Fig. 2. Comparison of macrocirculatory vascular dysfunction assessed by brachial flow-mediated vasodilation between the control and AST-120 groups. Both endothelium-dependent vasodilation (A) and endothelium-independent vasodilation (B) were not significantly changed in either group after 6 months compared to baseline.

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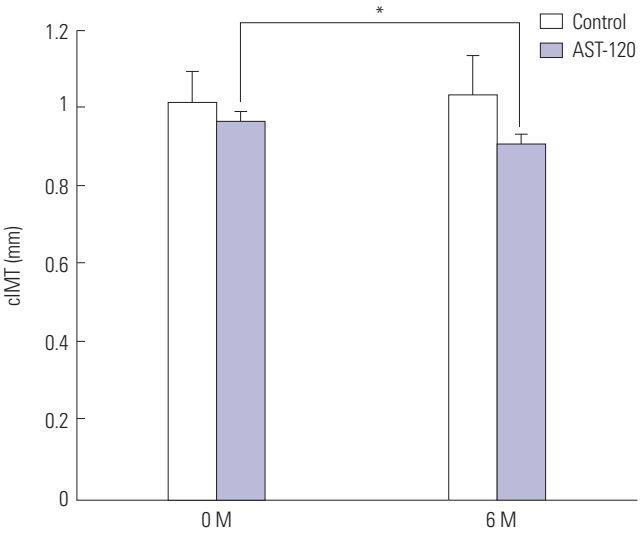

Fig. 3. Changes in carotid intima-media thickness (cIMT) between the control and AST-120 groups. cIMT was significantly reduced after 6 months in the AST-120 treatment group. *p<0.01 vs. cIMT at baseline.

of AST-120 treatment. Conversely, SNP-induced EIDV showed a delayed improvement compared with the Ach-induced response, as it was increased at only 6 months after AST-120 treatment.

There are many reports regarding the beneficial role of AST-120 on pre-dialysis CKD patients. AST-120 reduces kidney fibrosis and slows progression of CKD in human and rats by reducing levels of IS and other uremic toxins.[23,24] Apart from renoprotective effect, AST-120 improves vascular function in various pre-dialysis CKD cases. Treatment with AST-120 for 24 weeks ameliorates FMD in pre-dialysis CKD patients,[12] ED in CKD rat model,[25] and reduces the extent and instability of atherosclerosis in mouse renal injury models.[26] Recently, treatment with AST-120 for 3 months decreased serum cardiovascular biomarkers like malondialdehyde and interleukin-6 in chronic dialysis patients.[27] Likewise, the present study showed that AST-120 ameliorated vascular function apart from renal protective effect. However, there have been no reports dealing with the effects of AST-120 on vascular function in ESRD patient on dialysis, especially microvascular function. Thus, to the best of our knowledge, our present study is the first to evaluate the effect of AST-120 on ED in dialysis patients.

Interestingly, the degree of improvement in Ach-induced endothelium-dependent response after AST-120 treatment for 3 months was preserved through 6 months, although the IS levels at 6 months were not significantly decreased compared with baseline. The more significant decrease in IS levels during the first 3 months might have been due to higher patient compliance over the first 3 months of the study. Specifically, although the mean compliance was similar between 1–3 months and 4–6 months, individual dose rates were lower for months 4 to 5. While altered compliance may explain the differences in IS levels, the overall results suggest that the effects obtained during the initial 3 month of treatment persisted through 6 months.

The SNP-induced response was improved, albeit delayed, compared to Ach-induced response in patients receiving AST-120. This result may suggest a possible serial improvement in microvascular function. In other words, an initial recovery of productive function of nitric oxide (NO) might induce a serial improvement in dilatory response of vascular smooth muscle cells (VSMCs). Although more evidence is required, our findings may suggest the possibility that pre-existing structural and functional impairment in VSMCs can be recovered.

Unlike microvascular function, macrovascular ED, assessed by FMD, was not improved by AST-120 treatment. In addition, both EDV and EIDV in the control group showed a tendency to worsen over the 6 month study period. On the other hand, however, initial EDV and EIDV were either preserved or slightly increased after 6 months in the AST-120 treatment group. This observation has two possible explanations. First, more extended treatment with AST-120 might have improved macrovascular ED. Secondly, there are different mechanisms that determine endothelial function according to micro- or macrocirculations; previous reports support the latter possibility.

The term 'endothelial function' widely refers to the ability of the endothelium to maintain vascular homeostasis by releasing compounds that induce direct relaxation of VSMCs, namely NO, prostacyclin, and endothelium-derived hyperpolarizing factors (EDHFs).[28] An important feature of ED is the inability of arteries and arterioles to optimally dilate in response to appropriate stimulation by vasodilators acting on the endothelium.[29,30] In conduit vessels, ED is notoriously associated with impaired NO production from endothelium, decreased NO bioavailability, and inactivation of NO by reactive oxygen species.[4] However, in peripheral microcirculation, the role of the non-NO dependent vasodilatory pathway appears to be more important than in macrocirculation. Indeed, the contributions of recovered EDHFs or compensatory enhanced prostanoid-dependent vasodilatory response are suggested as the determining factors for the preservation of microvascular endothelial function. EDHF is a non-characterized endothelial-factor and plays a role in non-NO, non-prostaglandin-mediated EDV, ultimately causing hyperpolarization and relaxation of VSMCs.[31] The contribution of EDHFs inversely correlated with vessel size, with predominant EDHF activity occurring in resistance vessels and the most compensatory upregulating EDHFs under the conditions of reduced NO bioavailability in smaller vessels.[31-34] Especially, exogenous Ach-mediated peripheral vasodilation acts through a non-NO dependent pathway. Holowatz, et al.[35] suggested that Ach-mediated vasodilation is induced through both prostanoid and non-NO-, non-prostanoid dependent pathways. Furthermore, the remaining vasodilation in response to Ach infusion could be attributed to EDHFs. Durand, et al.[36] also suggested the limited contribution of NO in peripheral microvascular homeostasis by showing that the Ach-induced response is mediated by prostanoids. Uremia

per se increases oxidative stress and decreases the function of NO such that EDHFs may be upregulated in early staged CKD patients. This compensatory vasodilatory response in residence vessels must be impaired in ESRD, and a recovery of EDHF by elimination of uremic toxins could be responsible for amelioration in microcirculatory endothelial function.

It is interesting to note that carotid IMT was significantly reduced in patients treated with AST-120 for 6 months, whereas no similar improvement in FMD. Increased cIMT, which is a well-known marker for atherosclerosis and arterial stiffness, is also an independent risk factor for death from CVDs. Previous studies have shown that AST-120 treatment for 24 months reduces arterial stiffness and IMT in non-diabetic CKD patients before dialysis.[37] Although FMD and IMT are closely correlated with each other, the reason why only IMT was improved should be the focus of future work. In the present study, if the same assessments for ED and IMT after a washout period of drug effect medication had been performed, the vascular protective effect of AST-120 might have been demonstrated more clearly.

Iontophoresis using Ach and SNP with LDF has been developed for appropriate assessment of function.[38,39] Although many investigations on the effects of vasoactive drugs on local skin blood flow contributed to further understanding of microvascular reactivity, they were complicated by relatively low sensitivity of laser Doppler technique.[40] There are two factors for explanation of low sensitivity of lase Doppler technique when vasoconstrictors are applied in the capillary bed: 1) the laser light, instead of being absorbed by RBC's in the capillaries, penetrates to deeper areas such as the subdermal plexus, where the changes of flow may be smaller;[41] 2) laser Doppler detect decreased perfusion values within the perfusion range of unprovoked skin.[41] However, this tool is non-invasive and the reproducibility of LDF is good, with an intra-assay variation coefficient of less than 6%.[17] Thus, this method can be considered appropriate to evaluate microvascular function and to develop new therapeutic pharmacology.

This study has some limitations. First, this study utilized a relatively small patient population. Second, an objective reference to define ED, assessed by iontophoresis, has not yet been established. In addition, we used a relative perfusion unit to determine the volume change of blood in comparison to the perfusion unit during the resting status. However, there are no reference values according to renal function. Thus, there might have been inter-study bias. However, despite these limitations, ours is the first study to investigate the beneficial effects of AST-120 on vascular function in HD patients.

In conclusion, AST-120 ameliorated microvascular ED and cIMT in HD patients. With improved compliance, AST-120 may be safely applied to dialysis patients to improve vascular function. A randomized, case-control study comprising a larger population is required to establish a definitive role of AST-120 as a preventive medication for CVD in HD patients. Furthermore, a mechanism to improve microvascular function by IS and a role of EDHF should be more clearly defined.

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REFERENCES

1. Parfrey PS, Foley RN. The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol 1999;10:1606-15.
2. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int 1999;55:648-58.
3. Hogas SM, Voroneanu L, Serban DN, Segall L, Hogas MM, Serban IL, et al. Methods and potential biomarkers for the evaluation of endothelial dysfunction in chronic kidney disease: a critical approach. J Am Soc Hypertens 2010;4:116-27.
4. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. Int J Vasc Med 2012;2012:918267.
5. Corrado E, Rizzo M, Coppola G, Muratori I, Carella M, Novo S. Endothelial dysfunction and carotid lesions are strong predictors of clinical events in patients with early stages of atherosclerosis: a 24-month follow-up study. Coron Artery Dis 2008;19:139-44.
6. Kruger A, Stewart J, Sahityani R, O'Riordan E, Thompson C, Adler S, et al. Laser Doppler flowmetry detection of endothelial dysfunction in end-stage renal disease patients: correlation with cardiovascular risk. Kidney Int 2006;70:157-64.
7. Elherik K, Khan F, McLaren M, Kennedy G, Belch JJ. Circadian variation in vascular tone and endothelial cell function in normal males. Clin Sci (Lond) 2002;102:547-52.
8. Brodsky SV, Gealekman O, Chen J, Zhang F, Togashi N, Crabtree M, et al. Prevention and reversal of premature endothelial cell senescence and vasculopathy in obesity-induced diabetes by ebselen. Circ Res 2004;94:377-84.
9. Aoyama I, Niwa T. An oral adsorbent ameliorates renal overload of indoxyl sulfate and progression of renal failure in diabetic rats. Am J Kidney Dis 2001;37(1 Suppl 2):S7-12.
10. Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, et al. The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int 2004;65:442-51.
11. Barreto FC, Barreto DV, Liabeuf S, Meert N, Glorieux G, Temmar M, et al. Serum indoxyl sulfate is associated with vascular disease and mortality in chronic kidney disease patients. Clin J Am Soc Nephrol 2009;4:1551-8.
12. Yu M, Kim YJ, Kang DH. Indoxyl sulfate-induced endothelial dysfunction in patients with chronic kidney disease via an induction of oxidative stress. Clin J Am Soc Nephrol 2011;6:30-9.
13. Celermajer DS, Sorensen K, Ryalls M, Robinson J, Thomas O, Leonard JV, et al. Impaired endothelial function occurs in the systemic arteries of children with homozygous homocystinuria but not in their heterozygous parents. J Am Coll Cardiol 1993;22:854-8.
14. Cupisti A, Rossi M, Placidi S, Fabbri A, Morelli E, Vagheggini G, et al. Responses of the skin microcirculation to acetylcholine in patients with essential hypertension and in normotensive patients with chronic renal failure. Nephron 2000;85:114-9.
15. Niwayama J, Sanaka T. Development of a new method for monitoring blood purification: the blood flow analysis of the head and foot by laser Doppler blood flowmeter during hemodialysis. Hemodial Int 2005;9:56-62.

http://dx.doi.org/10.3349/ymj.2016.57.4.942

16. Nilsson GE, Tenland T, Oberg PA. Evaluation of a laser Doppler flowmeter for measurement of tissue blood flow. IEEE Trans Biomed Eng 1980;27:597-604.
17. Tenland T, Salerud EG, Nilsson GE, Oberg PA. Spatial and temporal variations in human skin blood flow. Int J Microcirc Clin Exp 1983;2:81-90.
18. Morris SJ, Shore AC, Tooke JE. Responses of the skin microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. Diabetologia 1995;38:1337-44.
19. Davis KR, Ponnampalam J, Hayman R, Baker PN, Arulkumaran S, Donnelly R. Microvascular vasodilator response to acetylcholine is increased in women with pre-eclampsia. BJOG 2001;108:610-4.
20. Schabauer AM, Rooke TW. Cutaneous laser Doppler flowmetry: applications and findings. Mayo Clin Proc 1994;69:564-74.
21. Silverman DG, Jotkowitz AB, Freemer M, Gutter V, O'Connor TZ, Braverman IM. Peripheral assessment of phenylephrine-induced vasoconstriction by laser Doppler flowmetry and its potential relevance to homeostatic mechanisms. Circulation 1994;90:23-6.
22. Yanase T, Nasu S, Mukuta Y, Shimizu Y, Nishihara T, Okabe T, et al. Evaluation of a new carotid intima-media thickness measurement by B-mode ultrasonography using an innovative measurement software, intimascope. Am J Hypertens 2006;19:1206-12.
23. Owada A, Nakao M, Koike J, Ujiie K, Tomita K, Shiigai T. Effects of oral adsorbent AST-120 on the progression of chronic renal failure: a randomized controlled study. Kidney Int Suppl 1997;63:S188-90.
24. Aoyama I, Miyazaki T, Niwa T. Preventive effects of an oral sorbent on nephropathy in rats. Miner Electrolyte Metab 1999;25:365-72.
25. Namikoshi T, Tomita N, Satoh M, Sakuta T, Kuwabara A, Kobayashi S, et al. Oral adsorbent AST-120 ameliorates endothelial dysfunction independent of renal function in rats with subtotal nephrectomy. Hypertens Res 2009;32:194-200.
26. Yamamoto S, Zuo Y, Ma J, Yancey PG, Hunley TE, Motojima M, et al. Oral activated charcoal adsorbent (AST-120) ameliorates extent and instability of atherosclerosis accelerated by kidney disease in apolipoprotein E-deficient mice. Nephrol Dial Transplant 2011;26:2491-7.
27. Lee CT, Hsu CY, Tain YL, Ng HY, Cheng BC, Yang CC, et al. Effects of AST-120 on blood concentrations of protein-bound uremic toxins and biomarkers of cardiovascular risk in chronic dialysis patients. Blood Purif 2014;37:76-83.
28. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment of skin microvascular endothelial function in humans. Trends Pharmacol Sci 2006;27:503-8.
29. Kaneto H, Katakami N, Matsuhisa M, Matsuoka TA. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediators Inflamm 2010;2010:453892.
30. Witting PK, Rayner BS, Wu BJ, Ellis NA, Stocker R. Hydrogen peroxide promotes endothelial dysfunction by stimulating multiple sources of superoxide anion radical production and decreasing nitric oxide bioavailability. Cell Physiol Biochem 2007;20:255-68.
31. Ozkor MA, Quyyumi AA. Endothelium-derived hyperpolarizing factor and vascular function. Cardiol Res Pract 2011;2011:156146.
32. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A. Importance of endothelium-derived hyperpolarizing factor in human arteries. J Clin Invest 1997;100:2793-9.
33. Coats P, Johnston F, MacDonald J, McMurray JJ, Hillier C. Endothelium-derived hyperpolarizing factor: identification and mechanisms of action in human subcutaneous resistance arteries. Circulation 2001;103:1702-8.
34. Scotland RS, Madhani M, Chauhan S, Moncada S, Andresen J, Nilsson H, et al. Investigation of vascular responses in endothelial nitric oxide synthase/cyclooxygenase-1 double-knockout mice: key role for endothelium-derived hyperpolarizing factor in the regulation of blood pressure in vivo. Circulation 2005;111:796-803.
35. Holowatz LA, Thompson CS, Minson CT, Kenney WL. Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. J Physiol 2005;563(Pt 3):965-73.
36. Durand S, Tartas M, Bouyé P, Koïtka A, Saumet JL, Abraham P. Prostaglandins participate in the late phase of the vascular response to acetylcholine iontophoresis in humans. J Physiol 2004;561(Pt 3):811-9.
37. Nakamura T, Kawagoe Y, Matsuda T, Ueda Y, Shimada N, Ebihara I, et al. Oral ADSORBENT AST-120 decreases carotid intima-media thickness and arterial stiffness in patients with chronic renal failure. Kidney Blood Press Res 2004;27:121-6.
38. Morris SJ, Shore AC. Skin blood flow responses to the iontophoresis of acetylcholine and sodium nitroprusside in man: possible mechanisms. J Physiol 1996;496(Pt 2):531-42.
39. Farkas K, Kolossváry E, Járai Z, Nemcsik J, Farsang C. Non-invasive assessment of microvascular endothelial function by laser Doppler flowmetry in patients with essential hypertension. Atherosclerosis 2004;173:97-102.
40. Brown H, Moppett IK, Mahajan RP. Transient hyperaemic response to assess vascular reactivity of skin: effect of locally iontophoresed acetylcholine, bradykinin, epinephrine and phenylephrine. Br J Anaesth 2003;90:446-51.
41. Fredriksson I, Larsson M, Strömberg T. Measurement depth and volume in laser Doppler flowmetry. Microvasc Res 2009;78:4-13.