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Original Article

Effect of Continuous Supplementation of L-ascorbic Acid on Renal Functions in Streptozotocin-induced Diabetic Rats

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ABSTRACT **Nephropathy is a crucial complication in diabetes mellitus. The renal pathophysiological changes are caused by hyperglycemia, which generates more reactive oxygen species and impaires antioxidant mechanisms in diabetes mellitus. The oxidative stress activates the metabolic pathways that contribute to diabetes-induced microvascular damage. L-ascorbic acid (AA), a powerful antioxidant, is decreased in plasma and tissue concentrations in diabetes mellitus. With the evidences of antioxidant effects of AA, this study was performed to investigate effects of the continuous supplementation of AA on the renal function in streptozotocin (STZ)-induced diabetic rats. Male Sprague-Dawley rats weighing 180-220 g were induced diabetes mellitus with a tail vein injection of STZ dissolving in citrate buffer, at a dose of 55 mg/kg while the control rats were injected with the citrate buffer. The diabetic stage was verified with the fasting blood glucose concentration >200 mg/dl. The rats were divided into 4 groups as follows CON: the control rats given tap water, CON-AA: the control rats supplemented with AA, STZ: the diabetic rats given tap water, STZ-AA: the diabetic rats supplemented with AA. AA solution was supplemented to the rats for 4, 8 and 16 weeks. The renal hemodynamics and functions were studied at the end of experimental periods. Sixteen weeks after the AA supplementation, high blood glucose concentration of STZ-AA was significantly reduced as compared with STZ. GFR and ERPF of STZ and STZ-AA were significantly decreased but increased in RVR as compared with CON and CON-AA. STZ-AA was significantly increased in glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) but decreased in renal vascular resistance (RVR) as compared with STZ. The ratio of urine flow rate to GFR (V/GFR) of STZ-AA was non-significantly different from either CON or STZ. In STZ-AA, urinary excretion of Na^+, K^+ and Cl^- ($UVNa^+$, UVK^+ and $UVCl^-$) was significantly decreased as compared with CON but non-significantly different from STZ. Fractional excretion of Na^+ and K^+ ($FE_{Na}{}^+$ and $FE_K{}^+$) in STZ-AA were significantly decreased as compared with STZ. This study indicated that AA had some beneficial effects on the renal functions in the STZ-induced diabetic rats after the continuous supplementation of AA for 16 weeks.**

Key words: **continuous supplementation, L-ascorbic acid, renal function, streptozotocin-induced diabetic rtas**

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INTRODUCTION

Diabetic nephropathy is one of the major causes of end- stage renal disease. It has been noted that hyperglycemia stimulates the development of diabetic nephropathy. Hyperglycemia does not only generate more reactive oxygen species but also impaires antioxidant mechanisms leading to the nephropathy in diabetes mellitus[1-6]. Hyperglycemia activates the metabolic pathways that contribute to diabetes-induced microvascular damage. In the normal physiological glucose concentrations, the activation of PKC occurs via the cell signal transductions, which is associated with a complex sequence of biochemical interactions through G-protein coupled receptor-mediated pathway[7,8]. Diacylglycerol (DAG) is the intermediate substance of glucose metabolism, which is the important mediator to signal the activation of PKC[9]. Other pathways, including glycation, polyol pathway and superoxide overproduction also play the role of diabetic microvascular complications. The activation of PKC β also accounts for the pathophysiology associated with diabetic nephropathy. The diabetic microvascular complications including an initial increase in glomerular filtration rate or creatinine clearance, mesangial matrix expansion, glomerular capillary crowding, and glomerular occlusion result in the subsequently decreased renal function[10,11].

Normally, glucose undergoes oxidation, producing protein reactive ketoaldehyde, hydrogenperoxide and highly reactive oxidant[12]. The increased oxidative stress in uncontrolled diabetes manifests the marked alterations in tissue antioxidant status and causes the renal pathophysiology in diabetes mellitus[2]. Attachment of these reactive oxygen species to proteins contributes to protein fragmentation and cross-linking in diabetes mellitus[13-14]. Furthermore, increased free radicals can induce apoptosis, which also contributes to the development of diabetic nephropathy[15,16].

Renal hemodynamics and function alteration has been found in patients with early diabetes mellitus. The elevated glomerular filtration rate (GFR) is a frequent finding in early diabetes mellitus. Vascular endothelium dysfunction has been elucidated in diabetes mellitus. The changes of endothelial vasoactive factors, endothelial and leukocyte adhesion molecules in diabetes mellitus were involved in the development of diabetes complications such as nephropathy and retinopathy[17]. Angiotensin II plays an important role on the renal injuries in STZ-induced diabetic rats[18]. It produces the increased renal perfusion pressure in the kidneys where as thromboxane A2 was reduced in STZ-induced diabetic rats[19]. Renal micropuncture techniques have shown that single-nephron GFR is elevated in moderately hyperglycemic diabetic rats and possibly elevated glomerular capillary pressure. Several potential mediators of increased SNGFR have been examined, including hyperglycemia, increased glomerular prostaglandin production, and decreased sensitivity of the tubuloglomerular feedback mechanism[20].

In the early stage of Type I diabetes mellitus, HbA_{1c} is increased and correlated to the increase in glomerular filtration rate or effective renal plasma flow. That reflected the correlation of hyperfiltration or renal hyperperfusion and hyperglycemia in diabetes[21]. No reduction in basal vasodilating NO in the circulation was found at the early onset of diabetic rats. The sensitivity to vasodilating effects of acetylcholine at the level of small resistance was not impaired in the diabetic rats. In addition, a normal action of NO in the kidney, heart and muscle was found in the early STZ-diabetic rat[22]. However, it has been noted that renal hyperfiltration and hyperperfusion may be caused by the increase in nitric oxide production in early diabetic stage[23-24].

In chronic diabetes mellitus, the mechanisms of the elevated GFR in insulin-dependent diabetics are enhanced RPF, increased transglomerular hydrostatic pressure gradient and increased glomerular ultrafiltration coefficient[25]. The renal pathologic changes of diabetes include thickening of renal extracellular basement membranes and mesangial matrix is closely and inversely related to peripheral capillary wall filtration surface and to proteinuria, hypertension, and decreasing glomerular filtration rate. These lesions are markedly advanced by the time of renal dysfunction[26]. A local inhibition of nitric oxide in the kidney associated with renal hyperfiltration, an increase in glomerular filtration rate, renal plasma flow was reported[27]. This evidence is according to the evidence of endothelium dysfunction in diabetes mellitus.

L-ascorbic acid (vitamin C) is a powerful antioxidant, which can prevent and inhibit the renal damage induced by cytotoxic agents in rats[28,29]. It has a stimulatory effect on the sulphate incorporation into mesangial cells and matrix proteoglycan leading to the negatively charged extracellular matrix. High glucose is capable to

inhibit the effect of ascorbic acid on the sulphation stimulation of proteoglycan molecules[30]. This is the particularly important cause of nephropathy in diabetes mellitus, which the low AA level is apparent. Serum AA is reduced in diabetic patients because of high urinary excretion of ascorbic acid, impaired tubular reabsorption of filtered ascorbic acid and increased AA clearance[31-32]. Moreover, the maximal uptake rate (V_{max}) is significantly lower in the diabetic nephropathy cells compared to the normal[33]. Supplementation of AA can increase the AA concentration in the kidneys of diabetic rats[34]. A marked increase in both plasma and renal cortical AA level in diabetic rats supplemented with ascorbic acid is greater than those in un-supplemented diabetic rats[35]. It can reduce kidney weight, and prevent the increase in glomerular volume in diabetic rats. In addition, the increase in glomerular TGF-β and albumin clearance in diabetic rats can be prevented by AA treatment.

The evidences indicated some beneficial effects of AA on diabetes mellitus; however, the knowledge of AA effect on the renal physiology is not much available. Therefore, this study was performed to investigate effects of the continuous supplementation of AA on the renal function in STZ-induced diabetic rats.

MATERIALS AND METHODS

Animal preparation

Eighty-four male Sprague-Dawley rats weighing 180-220 grams from The National Animal Center of Mahidol University were carried out in this study. To induce diabetes mellitus, STZ dissolved in citrate buffer, pH 4.5 was injected via tail vein to the rats at a dose of 55 mg/kg BW. The control rats were injected with the citrate buffer without STZ as a placebo. The blood glucose concentration higher than 200 mg/dl was verified for hyperglycemic state. Fasting blood samples were obtained from the tail vein two days after the injections for measuring glucose concentration by using glucometer (Advance Glucometer, Boehringer Mannheim, Germany). The animals did not exhibit hyperglycemia at 48 hours after STZ injection would be excluded from the study. The control and diabetic rats were divided randomly into four groups as following:

CON is the control rats given with tap water.

CON-AA is the control rats supplemented with AA in tap water.

STZ is the diabetic rats given with tap water.

STZ-AA is the diabetic rats supplemented with AA in tap water.

All animals were fed with standard rat chow and tap water or AA solution *ad libitum* throughout the experimental period of 4, 8 and 16 weeks.

L-ascorbic acid supplementation

AA (BDH, VWR International Ltd., England) solution was prepared freshly by dissolving in tap water at the concentration of 1 g/L[36] and was supplemented to the rats in both **CON-AA** and **STZ-AA** groups after confirming the hyperglycemic state. The solution was continuously supplemented *ad libitum* to the rats and was changed daily. Instead of AA solution, tap water was given *ad libitum* to the rats in **CON** and **STZ** groups.

Clearance studies

The studies of renal hemodynamics and functions were performed at the end of the experimental periods. Blood glucose concentration of the rats was measured by a glucometer (Accue-CHEK Advantage, Roche) at the specified period. The animal was fasted for 9-10 hours before performing the clearance study. The rat was weighed and anesthetized with pentobarbital sodium (60 mg/kg BW). A tracheostomy was performed to insert a tracheal tube to open the air way and to remove some secretion. Right common carotid artery was canulated to collect blood samples and recorded blood pressure with a computerized polygraph (McLab System, ADInstruments). Femoral vein was inserted with polyethylene tube for infusion of normal saline or the solution of inulin and para-aminohippuric acid (PAH).

Both ureters were canulated for urine sample collections by the operation at linea alba. Normal saline was infused at the rate of 10 ml/kg BW/hr by a syringe pump (Harvard syringe pump 21) to replace the body fluid loss during the surgery. After the surgery, the priming dose of inulin (20 mg/kg) and PAH (4 mg/kg) was i.v. administrated to the rats and sustained with the mixture of 1 g/dL inulin and 0.2 g/dL PAH in normal saline at the rate of 10 ml/kg BW/hr instead of normal saline throughout the experiments. A period of 45 minutes was allowed to elapse for stabilizing the plasma concentration of the substances. Following that, urine

samples were collected two periods of 30 minutes consecutively. The rate of the urine flow was measured. To study the renal hemodynamics, clearances of inulin and PAH were determined to represent GFR and ERPF, respectively.

Blood sample was collected at the mid-point of each urine collection. Total volume of 0.8 ml of replacing blood was performed immediately to the rat after each blood sampling. To prepare the replacing blood, red cells from another rat were washed 3 times and mixed with 6 percent bovine serum albumin in normal saline to access the same total volume[37,38]. The blood samples were determined for the values of hematocrit by a microhema-tocrit centrifuge (model Z230H,BHG HERMLE) and measured by micro-capillary reader (I.E.C. Cat No. 2201, DAMON/IEC DIVISION). At the end of the experiments, both kidneys were immediately excised, stripped the surrounding tissue, blotted and weighed so that RBF and GFR could be expressed as millilitres per minute per gram of kidney weight.

The renal hemodynamics and functions were evaluated by determining of GFR, ERPF, RVR, FF, UV_{Na}, UV_K, UV_{Cl}, FE_{Na}, FE_K, FE_{Cl}, V and V/GFR. Urine and plasma samples were analyzed for the concentration of inulin by anthrone method and for the concentration of PAH by the modified method of Smith (1962) to determine GFR and ERPF, respectively[39]. The concentrations of electrolytes in both urine and plasma for sodium, potassium and chloride ions were determined to calculate the fractional excretion of the electrolytes. The concentrations of sodium and potassium were measured by flame photometer (Flame photometer 410C, Ciba Corning, Inc.) and the concentration of chloride by chloridometer (Chloride analyzer 925, Ciba Corning Inc.).

Calculation for the measurements of renal hemodynamics and function

The calculated values are obtained using the following equations:

GFR = Cin = Uin V/Pin
Cin = clearance of inulin (ml/min)
V = urine flow rate (ml/min)
Uin = urinary inulin concentration (mg/ml)
Pin = plasma inulin concentration (mg/ml)

$$\mathbf{ERPF} = \mathbf{C_{PAH}} = \mathbf{U_{PAH}\ V/P_{PAH}}$$

ERPF = effective renal plasma flow (ml/min)
CPAH = clearance of PAH (ml/min)
V = urine flow rate (ml/min)
UPAH = urinary PAH concentration (mg/ml)
PPAH = plasma PAH concentration (mg/ml)

$$\mathbf{ERBF} = \frac{\mathbf{RPF}}{\mathbf{1\text{-}(Hct/100)}}$$

ERBF = effective renal blood flow (ml/min)
Hct = hematocrit value (%)

$$\mathbf{FF} = \frac{\mathbf{GFR \times 100}}{\mathbf{ERPF}}$$

FF = filtration fraction (%)

$$\mathbf{V/GFR} = \frac{\mathbf{V \times 100}}{\mathbf{GFR}}$$

V/GFR = fractional excretion of urine

$$\mathbf{FE} = \frac{\mathbf{(U_E\ V/P_E) \times 100}}{\mathbf{GFR}}$$

FE = fractional excretion of electrolytes (%)
UE = concentration of urinary electrolytes (μEq/μl)
PE = concentration of plasma electrolytes (μEq/μl)

$$\mathbf{RVR} = \frac{\mathbf{MAP}}{\mathbf{ERBF}}$$

RVR = renal vascular resistance (mmHg/ml/min/g KW)
MAP = mean arterial pressure (mmHg)
ERBF = effective renal blood flow (ml/min/g KW)

Statistical analyses

The results were statistically analyzed by analysis of variance (ANOVA) using Least significant difference test (LSD) as the post hoct tests. The significant comparisons were indicated at *p*-value lower than 0.05.

RESULTS

Blood glucose concentration

The mean values of blood glucose concentrations

Table 1 Comparison of blood glucose concentration, body weight, kidney weight or percentage of kidney weight to body weight among groups of streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 4, 8, 16 and 24 of the experimental periods (n = 7-8).

Groups		Week 4	Week 8	Week 16
Blood glucose concentration (mg/dl)	CON	82.4 ± 11.8 (n = 9)	80.1 ± 8.1 (n = 7)	75.3 ± 5.2 (n = 10)
	CON-AA	74.5 ± 12.1 (n = 8)	81.6 ± 12.2 (n = 10)	77.7 ± 5.8 (n = 10)
	STZ	457.87 ± 65.49[ab] (n = 15)	438.5 ± 57.1[ab] (n = 11)	413.3 ± 49.0[ab] (n = 13)
	STZ-AA	494.5 ± 72.4[ab] (n = 14)	436.5 ± 82.2[ab] (n = 13)	367.1 ± 96.5[abc] (n = 9)
BW (g)	CON	366.73 ± 16.13	419.50 ± 27.61	482.20 ± 33.94
	CON-AA	374.94 ± 16.48	419.13 ± 33.16	460.58 ± 18.0
	STZ	161.13 ± 16.30[a,b]	139.98 ± 23.53[a,b]	180.79 ± 51.23[a]
	STZ-AA	152.78 ± 22.14[a,b]	141.61 ± 39.83[a,b]	216.91 ± 18.10[a]
KW (g)	CON	2.85 ± 0.33	2.96 ± 0.39	3.10 ± 0.21
	CON-AA	3.00 ± 0.38	2.91 ± 0.18	3.31 ± 0.45
	STZ	2.27 ± 0.17[a,b]	2.51 ± 1.08	3.15 ± 0.74
	STZ-AA	2.17 ± 0.27[a,b]	2.22 ± 0.23[a,b]	2.80 ± 0.15

Mean ± SD

[a]compared with CON, [b]compared with CON-AA and [c]compared with STZ at the specified periods; $p < 0.05$

of STZ and STZ-AA were about 4-5 folds higher than those of CON and CON-AA at the specified experimental periods (Table 1). The significant decrease in blood glucose concentration of STZ-AA as compared with that of STZ was seen at week 16. There was no significant difference of blood glucose level between CON and CON-AA at the specified periods.

Changes of renal glomerular functions and hemodynamics

Renal hemodynamics of CON and CON-AA was no significantly different at the specified periods unless GFR of CON-AA was significantly higher than that of CON at week 4 (Figure1). GFR and ERPF of STZ and STZ-AA were significantly decreased as compared with those of CON and CON-AA at the specified periods. At week 16, GFR and ERPF of STZ-AA was 64 and 59 percent higher than that of STZ. FF of STZ and STZ-AA was markedly increased as compared with CON and CON-AA at the specified periods. No significant difference of FF was seen between STZ and STZ-AA. RVR of all diabetic rats were much higher than those of CON and CON-AA at the specified periods. The significant decreases in RVR of STZ-AA comparing with STZ were seen at week 8 and 16.

Changes of renal tubular functions

Urine flow rates of the diabetic rats seem to be decreased as compared with those of CON and CON-AA at the specified periods (Figure 2). However, V/GFR of the diabetic rats was significantly increased as compared with those of CON and CON-AA at week 8. At week 16, V/GFR of STZ-AA was non-significantly decreased as compared with that of STZ. It was simultaneously not significantly different with those of CON and CON-AA. No significant difference of V and V/GFR were shown between CON and CON-AA at the specified periods.

$U_{Na}V$ and UKV of STZ and STZ-AA were significantly decreased as compared with those of CON and

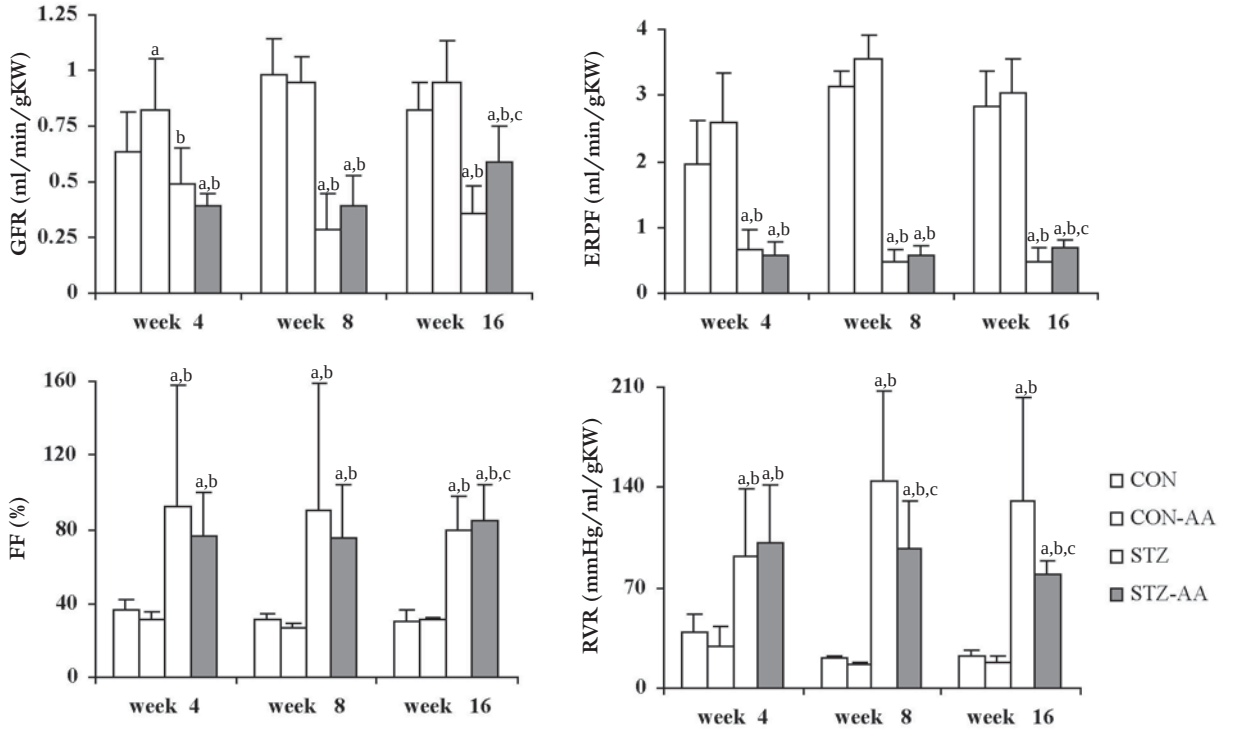


Figure 1 Comparison of GFR, ERPF, FF or RVR of the STZ-induced diabetic rats and the control rats with or without AA supplementation at the specified periods. All values are means + SD. Statistically significant differences are indicated by a comparing with CON, b comparing with CON-AA and c comparing with STZ at $p < 0.05$.

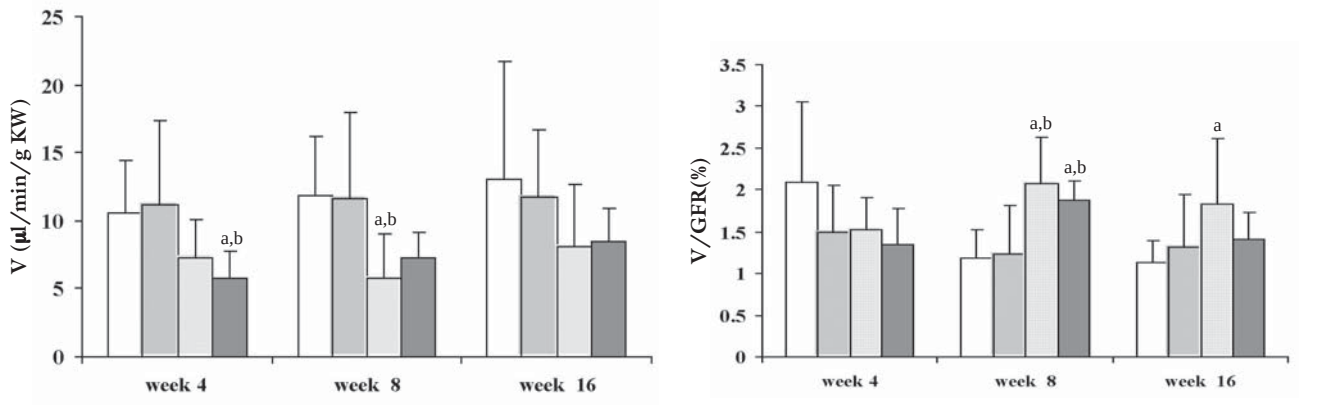


Figure 2 Comparison of V or V/GFR of the STZ-induced diabetic rats and the control rats with or without AA supplementation at the specified periods. All values are means + SD. Statistically significant differences are indicated by a comparing with CON and b comparing with CON-AA at $p < 0.05$.

CON-AA at the specified periods (Figure 4). While the plasma concentrations of Na^+ and K^+ were not different among groups (Figure 3). $U_{Cl}V$ of STZ and STZ-AA were significantly decreased as compared with CON and CON-AA at week 4 and 8. At week 16, only $U_{Cl}V$ of STZ-AA was significantly decreased as compared with CON while PCl of STZ and STZ-AA were significantly decreased as compared with CON-AA.

FE_{Na} of STZ and STZ-AA were decreased as compared with CON and CON-AA at the specified periods

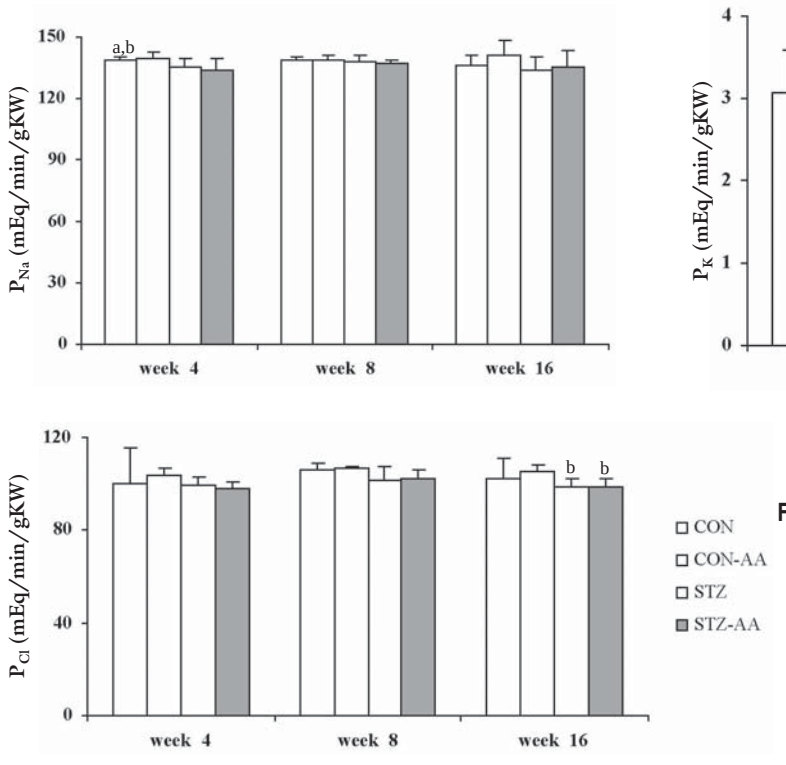


Figure 3 Comparison of PNa, PK, or PCl of the STZ-induced diabetic rats and the control rats with or without AA supplementation at the specified periods. All values are means + SD. Statistically significant differences are indicated by a comparing with CON and b comparing with CON-AA at $p < 0.05$.

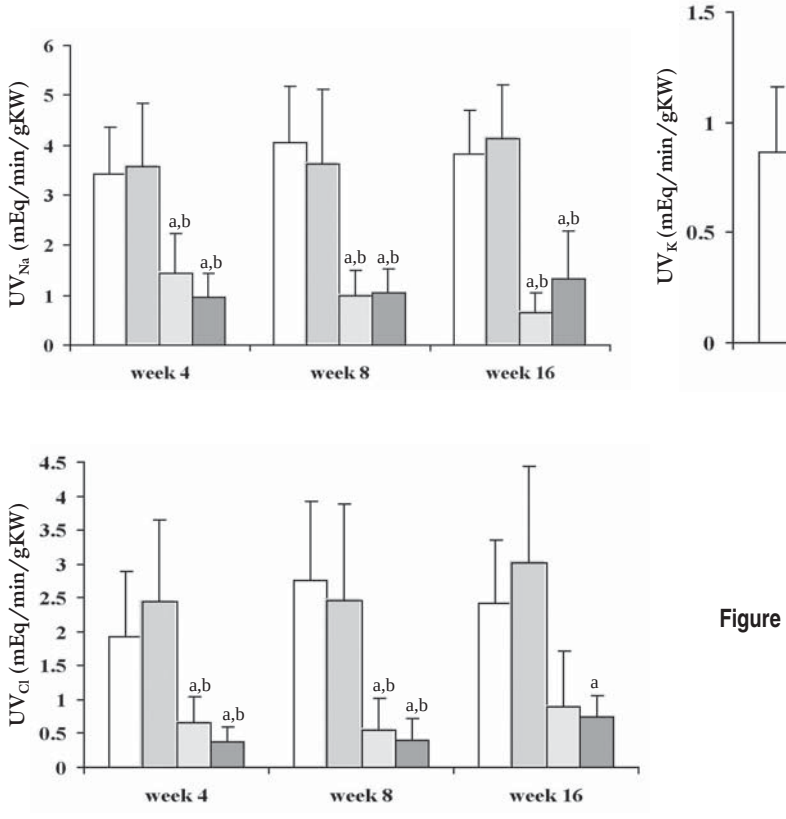
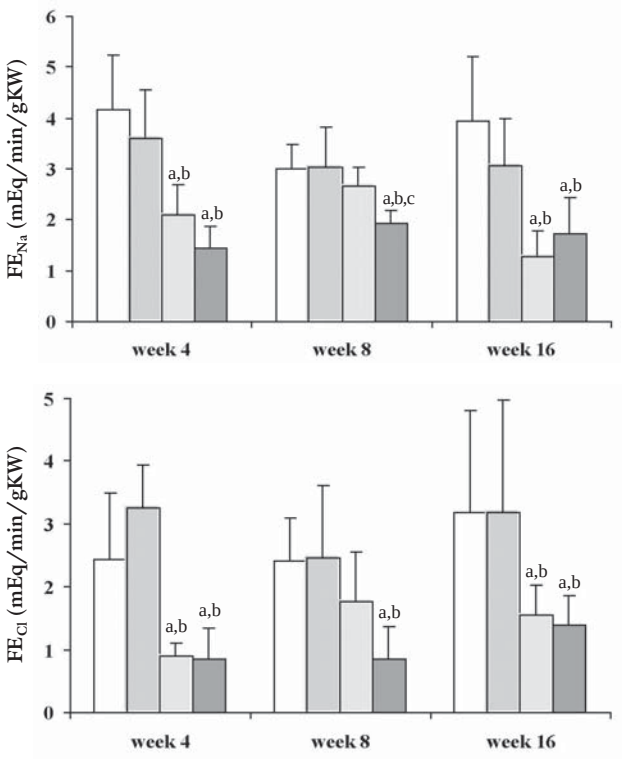


Figure 4 Comparison of UVNa, UVK, or UVCl of the STZ-induced diabetic rats and the control rats with or without AA supplementation at the specified periods. All values are means ± SD. Statistically significant differences are indicated by a comparing with CON and b comparing with CON-AA at $p < 0.05$.



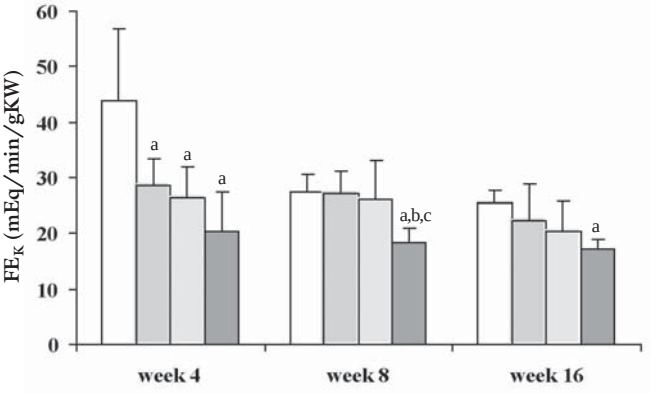


Figure 5 Comparison of FENa, FEK, or FECl of the STZ-induced diabetic rats and the control rats with or without AA supplementation at the specified periods. All values are means ± SD. Statistically significant differences are indicated by [a]comparing with CON, [b]comparing with CON-AA and [c]comparing with STZ at $p < 0.05$.

(Figure 5). STZ-AA was significantly decreased in FE_{Na} as compared with STZ at week 8. There was no significant difference of FE_{Na} between CON and CON-AA at the specified periods.

FE_K of CON-AA, STZ and STZ-AA were significantly decreased as compared with CON at week 4. At week 8, FE_K of STZ-AA was significantly decreased as compared with STZ. At week 16, it showed the significant decrease of FE_K only when compared with CON. There was no significant difference of FE_K between CON and CON-AA at the specified periods of week 8 and 16. The significant decreases in FE_{Cl} were found in STZ and STZ-AA as compared with CON and CON-AA at the specified periods. There was no significant difference of FE_{Na} between CON and CON-AA at the specified periods.

DISCUSSION

The present study was performed to clarify effects of the continuous supplementation of AA on the renal functions in STZ-induced diabetic rats. It has been noted that increased blood glucose concentration resulted in occurrence of systemic oxidative stress and caused renal abnormalities in diabetes mellitus[12,40-41]. Type I diabetes mellitus eventually lose the insulin secretion and accompany by the increases in glucagons, catecholamines and cortisol. The changes in those hormones lead to the hepatic glucose production and the consequent increase in the blood glucose level. Namely, the excess of glucagon exacerbates the hyperglycemia by increasing the hepatic glucose release and decreasing the hepatic glucose uptake[42,43]. Epinephrine and norepinephrine concentrations, which might be elevated in type I diabetes mellitus could impair the insulin action at the hepatic and extrahepatic tissues and stimulate the secretion of glucagon, leading to the further increase in the endogenous glucose production[44-46]. In the present study, it demonstrated that the continuous supplementation of AA could reduce the high blood glucose concentration after AA supplementation for 16 weeks even though it did not reach the normal level. This result may be the effect of AA on the decrease in cathecolamine level in diabetes mellitus, which possibly results in the decrease in endogenous glucose production, leading to the attenuation of

hyperglycemia. Furthermore, AA may preserve some living pancreatic β-cells, which are also affected by the oxidative stress[47].

The normal range of the arterial blood pressure (90-180 mmHg) would be expected to maintain the renal autoregulation of GFR and RBF throughout the periods of the clearance study. Any alterations of ERPF and GFR occurring in diabetic animal should be caused by some changes in intrarenal factors. In diabetes mellitus, the significant increases in GFR and blood glucose concentration in spite of no increase in the renal blood flow have been noted[48]. Normally, higher GFR as the value of body weight is found in diabetes mellitus However, in the present study, a marked reduction of the GFR as the value per gram of kidney weight is apparent. The increase in RVR leads to the decrease in ERPF and GFR in the diabetic rats as compared with that of CON and CON-AA. After the supplementation of AA for 16 weeks, STZ-AA elucidates the increase in ERPF by 59 percent of the value of STZ. GFR of STZ-AA is concurrently increased by 64 percent of the value of STZ. These results correspond with the decrease in RVR of STZ-AA about 33 percent from the values of STZ at week 8 and 43 percent at week 16. The increases in ERPF and GFR are possible due to the ability of AA to ameliorate the endothelial dysfunction. In the present study, AA administration might affect on the increase in the nitric oxide level in blood vessels resulting in the reactivation of NO-dependent vascular elasticity in diabetic animals[49]. In addition, changing of various K^+ channels on smooth muscle affecting the biosynthesis of cyclooxygenase products such as 6-keto-prostaglandin F1α and thromboxane A2 and endothelium-dependent relaxation may contribute to the development of vascular complications in streptozotocin-induced diabetic rats[50]. AA supplementation to the diabetic rats might also have a beneficial effect involing in the vasodilator production. A normal action of NO in the kidney was found, and no impairment of vasodilation effect has been reported in the early STZ-diabetic rat[22]. However, changes of renal hemodynamics in the diabetic rats were found since week 4 to week 16. There is a wide variation of the stages and progression of diabetes mellitus. Moreover, several factors affecting the renal hemodynamics for examples rennin-angiotensin system, loss of body fluid, and antidiuretic hormone and adrenomedulin are needed to consider in diabetes mellitus[51-53].

In the present study, the ratios of V/GFR in diabetic animals were markedly higher than those of the control animals at week 8 and 16. It indicated that there was an increase in fractional delivery of filtrate to the diluting sites of ascending limb of Henle's loop in diabetic animals. However, it is not necessary to assume that this increased fractional delivery during diabetes comes from an effect in the anatomic proximal tubule. A marked decrease in GFR in STZ-treated animals would reduce filtered load of sodium ion, which transported to the inner medullary segment of the ascending limb resulting in a reduction of the concentration of sodium ion in the medullary interstitium. In consequence, less water would be absorbed from the descending limb. Therefore, increased filtrate would be delivered to the medullary and cortical diluting segments. In addition, glucose reabsorption was decreased which was consistent with a fall in proximal tubular reabsorption in nephrotic syndrome[54]. A severe hyperglycemia (over 400 mg/dL) in the present study would induce osmotic diuresis resulting in the polyuria, which is commonly found in diabetes mellitus[55]. The body fluid loss could stimulate renin-angiotensin system and antidiuretic hormone (ADH). The increase in water reabsorption in distal and collecting tubule would be occurred. These changes are amplified in diabetes mellitus[56]. In the present study, the slightly decrease in V/GFR of STZ-AA as compared with STZ, simultaneously without a significant difference from that of CON implied that AA was able to improve the polyuria in diabetic rats at week 16. The knowledge of the effect of AA related to RAS on the decrease in polyuria is not available. The role of AA administration on RAS in diabetic animals is needed to investigate. The decreases in urinary excretion and fractional excretion of Na^+, K^+, and Cl^- were seen in diabetic rats throughout 16 weeks. A marked decreased in GFR in the diabetic animals would reduce filtered load of the sodium. The decrease in filtered load of sodium ion resulted in the decrease in FE_{Na} as compared with CON or CON-AA. This result was similar to a previous study in short-term diabetes patients who had no change in GFR and RPF, but lowered fractional excretion of sodium as compared with control subjects[57]. Since polyuria occurred in the diabetic rats, loss of sodium ion into the urine would be expected and resulted in a reduction of so-

dium concentration in the medullary interstitium. Therefore, the increase in sodium reabsorption via the passive diffusion was probably occurred in the ascending limb. In addition, the increased luminal glucose could stimulate the proximal sodium reabsorption[58]. Other factors that can affect the sodium reabsorption should be considered as well. ADH and aldosterone might be indirectly and directly involved in the sodium reabsorption in the collecting duct. In addition, acidosis, which was expected in the chronic diabetic rats in the present study, was likely to result in the increase in sodium reabsorption in renal tubules for the regulation of acid-base balance and lead to the decrease in FE_{Na}. Since ketoacidosis usually occurs in IDDM, H+ may be eliminated in the form of titrable acid with sodium salt into the urine. The decrease in FE_{Na} of STZ-AA as compared with STZ at week 8 might be due to an increase in the co-transport of AA with sodium through the proximal cells.

The decrease in FE_K caused by the acutely-induced hyperglycemia has been reported[57]. Potassium excretion by the kidneys is determined by the rate of potassium secretion by the distal tubule and collecting duct. Potassium secretion by these tubule segments is stimulated by a rise in tubular flow rate. The decrease in U_KV of the diabetic rats resulted from a marked decrease in GFR and/or urine flow rate. In addition, the reduction of potassium excretion shown as the decreases in U_KV and FE_K of the diabetic rats during 16 weeks of diabetes mellitus indicated the appearance of acute metabolic acidosis. A beneficial effect of AA supplementation on the renal tubular function was not seen in the experiments. Many possible factors may affect the changes in FE of the electrolytes in diabetes mellitus, including RAS, acid-base imbalance, diuresis, natriuresis and loss of body fluid and electrolytes.

In conclusion, the 16 week-continuous supplementation of AA to the STZ-induced diabetic rats had the beneficial effects on renal hemodynamics. The renal vascular resistance augmentation in the same as the reduction of GFR and ERPF were attenuated although it could neither attain the normal level nor prevent the diabetic complication. Furthermore, the 16 week-continuous supplementation of AA also could reduce the high blood glucose level in the STZ-induced diabetic rats. However, more prolonged supplementation of AA is needed to investigate.

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