CHAPTER VIII

GENERAL DISCUSSION

A number of studies have reported the effects of L-ascorbic acid (AA) on the function of diabetic renal cells, however a few studies during chronic diabetes mellitus are available. The present study demonstrated that the roles of AA on the renal pathophysiology in STZ-induced diabetic rats supplemented with AA for 4, 8, 16 and 24 weeks would relate to the oxidative stress. The effect of AA on the renal physiology particularly renal hemodynamics, renal mitochondrial activity and the effect on pathological changes, including the mass of kidney tissue and numbers of abnormal glomeruli, are shown in Chapter IV, V and VII. To investigate the mechanism of the effect of AA on the renal pathophysiology, the concentrations of MDA, TGF-β1 and Glut 1 in the renal cortex are also examined as shown in Chapter VI.

The experiment in Chapter IV illustrated the renal dysfunction of STZ-induced diabetic rats and the effect of AA to ameliorate the renal functions after AA supplementation for 16 weeks. The marked reductions of GFR and ERPF were apparent with the normal range of arterial blood pressure (90-180 mmHg) during the autoregulation of GFR and renal plasma flow, while the values of FF were increased in STZ-induced diabetic rats as shown in Table 4-4. The alterations of ERPF and GFR occurring in diabetic rats should be related to changes in intrarenal factors. With the evidences of that microvascular complications always occur in diabetes mellitus (Koh et al., 1986; Dedov et al., 2001; Du et al., 2003), the endothelial dysfunction resulting in the decreased nitric oxide in the diabetic glomeruli would be expected to occur in the present study. Therefore, the decrease in ERPF is possibly caused by the dysfunction of endothelial cells, which was associated with the decreased production of nitric oxide (Pflueger et al., 1999; Granstam et al., 2003). The decrease in ERPF is consented with the appearance of microvascular collapse in the glomerli (see Chapter VII) and with the decline in GFR in the diabetic rats. The marked elevation of the FF (GFR/ERPF) of the diabetic groups indicates more increases in vasoconstriction of the efferent arterioles than the afferent arterioles in the diabetic kidneys. The changes in renal hemodynamics, relating to marked elevations of efferent arteriolar

resistance and intraglomerular hydrostatic pressure, while marked decreases in renal plasma flow and peritubular capillary flow, has been noted in chronically severe glomerulonephritis (Ditzel et al., 1967; Hostetter et al., 1981; Christiansen et al., 1981; Hostetter et al., 1982; Futrakul et al., 2003). In the present study, glomerulosclerosis has been shown in diabetes mellitus at week 16 (Chapter VII). The development of renal arteriosclerosis of the small vessels (efferent, afferent arterioles and capillaries) in the diabetic groups may be assumed because RVR of diabetic rats elevated 6-7 folds over those of control groups. The increase in RVR led to the decrease in effective renal plasma flow, which caused the decrease in GFR in diabetic rats as compared with the normal rats (Table 4-4). Moreover, several factors affecting the renal hemodynamics for examples rennin-angiotensin system (Hollenberg et al., 2003), loss of body fluid, and antidiuretic hormone (Bardoux et al., 1999), and adrenomedulin (Hiragushi et al., 2004) are needed to consider in diabetes mellitus.

The roles of AA on the renal hemodynamics have been elucidated in the present study. After the supplementation of AA for 8 and 16 weeks, STZ-AA rats showed the decrease in RVR as compared with that of diabetic rats without AA treatment (STZ-rats). Accordingly, ERPF of STZ-AA was explicitly increased by 40 % of STZ, while GFR was concurrently increased by 50 % at week 16. The decrease in RVR and the increase in ERPF and GFR are possible due to the ability of AA to improve the vascular elasticity resulting in the increase in nitric oxide level (Taddei et al., 1998; Carr and Frei, 2000). AA has been reported to prevent the endothelial damage and the impaired nitric oxide production through anti-oxidant mechanisms (On et al., 2001; Chen et al, 2000). It is cleared that AA supplementation is effective to repair the renal hemodynamics in the diabetic rats. But it could not restore completely the renal hemodynamics impairment to achieve the normal range. It is probably due to other factors involved in the renal hemodynamics, in which role of AA is interesting to find out in diabetic rats. However, the beneficial effects of AA on the renal hemodynamics were not seen at week 24. It is expected that other factors involving in the renal hemodynamics might be more affect in the chronic diabetes mellitus.

The renal tubular function disturbances of diabetic rats were apparent in the present study. The decreases in fractional excretion of Na^+, K^+, and Cl^- were seen in diabetic rats at the early stage of diabetes (week 4 and 8). In addition, the decrease in

FE_{Cl} at week 16 and the increase in FE_K at week 24 were seen. A beneficial effect of AA supplementation on FE of the electrolytes was not seen in these 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 Chapter V, renal mitochondrial activity at week 8 and 16 were studied using glutamate and malate as the substrate in site I or succinate as the substrate in site II of electron transport chain. The present results showed no significant difference of RCI between the STZ-induced diabetic rats and the control rats using either glutamate plus malate or succinate alone as the substrates at week 8 and 16. It indicates that no significant different quality of renal mitochondria between the control rats and STZ-rats (Table 5-1 and 5-2). No significant difference of the mitochondrial activity for P/O ratio at week 8 was observed. This result agrees with the previous study reported that no alteration of renal and liver mitochondrial activity at 5 weeks of diabetes in STZ-induced diabetic rats (Rogers et al., 1986). However, it was found some different results at week 24. In the case of using glutamate plus malate as the substrate, although STZ and STZ-AA had higher oxygen consumption rates of mitochondria than that of CON in both Stage 3 and 4, P/O ratios were not markedly increased as compared with CON at week 24. It indicates that the increased oxygen consumption might not be used all for the phosphorylation of ADP; otherwise, there was partially uncoupling electron transport in diabetic rats. The increase in the oxidation of the substrates may occur to keep the electrochemical gradient or protonmotive force to maintain the electron transport chain. Differently, in the case of using succinate as the substrate, the oxygen consumption rate of STZ in Stage 3 was slightly increased. But it resulted in the significant increase in the P/O value of STZ as compared with those of CON and CON-AA. It indicates the increase in mitochondrial respiration in site II. In contrast, P/O ratio of STZ-AA was normalized. It was not different to that of either CON or STZ. It indicates the beneficial effect of AA supplentation to ameliorate the mitchondrial activity in site II in STZ-induced diabetic rats. The mechanism for the beneficial effect of AA supplementation to ameliorate the mitochondrial respiration in site II of STZ-induced diabetic rats need a further investigation.

In Chapter VI, the oxidative stress, the concentrations of Glut1 and TGF-β1 in the renal cortex were examined to investigate a mechanism of the effect of AA on renal pathophysiology. It has been noted that the increase in blood glucose concentration resulted in the occurrence of systemic oxidative stress which causes renal abnormalities in diabetes mellitus (Wohaieb et al., 1987; Hunt, 1991). In the present study, MDA concentrations in renal cortex in STZ-rats were markedly increased at week 8 and week 24 (Table 6-1). Interestingly, the MDA concentration was decreased in STZ-AA after AA supplementation for 16 weeks. The present result agrees with the previous study that found a decrease in renal MDA level in diabetic rats treated with vitamin C (Kedziora-Kornatowska et al., 2003). However, this effect was not apparent in STZ-AA at the severe stage of week 24, which a deficiency of α-tocopherol (vitamin E) would be expected. It has been known that vitamin E as a predominant antioxidant of lipid peroxidation accompanying with the action of AA to eliminate reactive oxygen species during the inhibition of lipid peroxidation (Je et al., 2001). Oxidative stress in diabetes is involved to an imbalance of antioxidants and other reactive oxygen species including oxygen free radicals, supernitrite, H_2O_2, $NO^{\cdot}$, $OH^{\cdot}$ etc. Those of ROS might be also affected by AA. The result of the decrease in MDA in the renal cortex agrees with the results of the renal functions for the increases in GFR and ERPF and normalizing the mitochondrial activity in site II. These results can conclude that oxidative stress causes the renal dysfunction and AA can improve the renal function via the decrease in the oxidative stress.

Moreover, in the present study, the increased TGF-β 1 production was demonstrated in STZ-induced diabetic rats which was similar to the previous study (Ziyadeh et al., 2000). There were the marked increases in TGF-β 1 of both diabetic groups over the control groups at week 8 after the diabetic induction and prolonged to week 16 and 24 (Table 6-2). These results agree with the evidences of the glomerular expansion and the thickening of the glomerular basement membrane, which occurred after 8 weeks of the diabetic induction (Osterby, 1992; Liu et al., 2003). In addition, it corresponds with the increase in percentage of KW/BW of STZ- rats (Table 4-2). The supplementation of AA could inhibit TGF-β 1 overexpression in the renal cortex of diabetic rats since week 8 until week 24. This finding is in the accordance with the previous study with Western blot technique, which demonstrated that AA could

decrease the overexpression of TGF-β in diabetic kidneys (Craven et al., 1997). The effect of AA on the suppression of TGF-β1 production concurs with the decrease in the MDA concentration and KW/BW. Furthermore, in the present study, it coincides with the amelioration of mitochondrial activity (Table 5-2) and renal functions, including the increases in GFR and ERPF, the decreased RVR (Table 4-4). This study indicates that AA plays a role in the inhibition of a linkage of oxidative stress and TGF-β_1 production.

The studies in cell culture have indicated that the high glucose concentration stimulated Glut 1 expression and glucose uptake (Mogyorosi et al., 1999; Inoki et al., 1999; Heilig et al., 2001). With the evidence of that high glucose melieu stimulates TGF-β 1 and Glut 1 overexpression and TGF-β 1 itself also stimulates Glut 1 overexpression in mesangial cell culture, Mogyorosi and Ziyadeh (1999) suggested that TGF-β 1 and Glut 1 might be a link between hyperglycemia and diabetic nephropathy. In the present study, oxidative stress and TGF-β 1 overexpression induced by hyperglycemia was suppressed by AA supplementation in diabetic rats. It indicates that AA plays a role in the inhibition of the linkage between oxidative stress and TGF-β, the mediator of diabetic nephropathy. Interestingly, in the present study, the overexpression of Glut 1 did not show in the diabetic rats without AA treatment at week 16 of the experimental period. In contrast, AA treated-diabetic rats showed an overexpression of TGF-β 1. It indicates that AA can stimulate Glut 1 production in diabetic rats. The increase in the uptake of AA by the renal cells would be expected to occur in the present study. Therefore, the increase in the antioxidant is invaluable to preserve the cells. This notion is supported by the attenuation of renal lipid peroxidation (Chapter VI), the amelioration of the renal dysfunction (Chapter IV), changing in renal mitochondrial activities (Chapter V) and renal pathology (Chapter VII).

In the present study, AA accomplished the decrease in glomerulosclerosis in diabetic rats. The number of abnormal glomeruli was decreased. More features of normal glomeruli with clear capillary lumen, no expansion of mesangial matrix and no glomerular adhesion to Bowman's capsule were apparent. This result indicates that AA supplementation has a role in the attenuation of the renal pathology in diabetic rats. In addition, the decline in hyperglycemia was seen in the diabetic rats

with AA supplementation at week 16 in the present study (Chapter IV). The pancreatic β-cells is possibly protected from the free radicals and could improve its function of insulin secretion (Bergsten et al., 1994; Kaneto et al., 1999; Steffner et al., 2004). These findings suggest that the amelioration of renal dysfunction and the attenuation of the renal pathology in diabetes are affected not only directly by AA supplementation but also possibly via the preservation of the pancreatic β-cell function in the decrease in oxidative stress resulted from the reduction of blood glucose concentration.