CHAPTER VII

PATHOLOGICAL STUDY OF GLOMERULAR PART IN STZ-INDUCED DIABETIC RATS WITH L-ASCORBIC ACID SUPPLEMENTATION

Introduction

Hyperglycemia has been demonstrated to induce the renal pathophysiology in diabetes mellitus (Osterby, 1983; Mauer, 1984). The apparent of sclerosis occurs both in the glomeruli and renal tubules in diabetes mellitus called the diabetic nephropathy (Wang et al., 2001; Morcos et al., 2002). Mesangial cells are directly affected by high blood glucose concentration and play an important role in the development of glomerulopathy (Rasch., 1979). Glomerulosclerosis is a conspicuous morphological change in diabetes (Osterby et al., 1992). The development of glomerulosclerosis is characterized by vascular injury and the glomerular expansion, mesangial proliferation and expansion with an increase in matrix material (Mauer et al., 1984; Osterby et al., 1992), which is associated with an increase in TGF-β_1 expression (Sharma et al., 1995). Ascorbic acid has been tested for the effect on the apoptotic response of cells treated with cytotoxic agent in rats (Appenroth et al., 1998, Greggi et al., 2000). It has a stimulatory effect on the sulphate incorporation into mesangial cells and matrix proteoglycan leading to the negatively charged extracellular matrix (McAuliffe et al., 1997). With the beneficial effect of L-ascorbic acid, the present study was performed to investigate whether the supplementation of L-ascorbic acid is able to prevent the glomerular pathology in STZ-induced diabetic mellitus.

Materials and methods

The percentage of kidney weight respected to body weight was considered as a factor to determine the renal pathology. The kidneys from the experiment in 3.1 were crossly sectioned at the middle part into a 2 mm-thickness piece and preserved in 10 % formalin. The renal tissues were processed by paraffin embedding and periodic

acid-Schiff staining (Chapter III issue 3.6). The sections were examined under light microscope for the glomerular pathology which was characterized by vascular injury, regional adhesion of the glomerular tuft to Bowman's capsule and expansion of the mesangial matrix (Reyes et al., 1992 and Fornoni et al., 2003). Of one hundred glomeruli, numbers of glomeruli which exhibit glomerulosclerosis were counted.

Statistics

All values were expressed as means with standard deviations. Statistical comparisons among groups in the same observation periods were analyzed by ANOVA and using Least significant difference (LSD) as the post hoct tests. The significant difference was indicated at p-value < 0.05.

Results

These experiments were carried out to study glomerular pathology. The numbers of the glomeruli which express the sclerosis at the experimental periods are shown in Table 7-1, Figure 7-1and Figure 7-2.

Normal glomeruli were characterized by well visualized capillary lumens, normal mesangial cells without hyperproliferation and no mesangial material matrix expansion. Glomerulosclerosis was characterized by hypercellular glomeruli with obliteration of the capillary lumens, glomerular mesangial expansion with either diffuse or nodular lesions (Figure 7-1). At all of the experimental periods, the numbers of normal glomeruli of CON were about 70 glomeruli / 100 glomeruli. At week 4 and 8 of the experimental periods, no significant difference of the numbers of abnormal glomeruli was seen among groups. The abnormal glomeruli of STZ was about 35.8 $\pm$ 3.8 glomeruli / 100 glomeruli at week 4 and 32.2 $\pm$ 7.8 glomeruli / 100 glomeruli at week 8 while Those of STZ-AA were 33.8 $\pm$ 7.1 glomeruli / 100 glomeruli at week 4 and 35.7 $\pm$ 14.0 glomeruli / 100 glomeruli at week 8 (Table 7-1).

At week 16 of the experimental periods, the number of abnormal glomeruli was significantly increased ($p < 0.05$) in STZ (55.3 $\pm$ 3.2 glomeruli / 100 glomeruli) as compared with those of CON (27.3 $\pm$ 8.9 glomeruli / 100 glomeruli) and CON-AA (34.3 $\pm$ 14.2 glomeruli / 100 glomeruli). While that of STZ-AA (46.8 $\pm$ 4.0) was significantly increased ($p < 0.05$) as compared with that of CON (27.3 $\pm$ 8.9 glomeruli

/ 100 glomeruli) but significantly decreased as compared with that of STZ (55.3 ± 3.2). At week 24, the number of abnormal glomeruli of STZ (54.3 ± 7.1 glomeruli / 100 glomeruli) was significantly increased ($p < 0.05$) as compared with those of CON (30.3 ± 8.1 glomeruli / 100 glomeruli) and CON-AA (37.3 ± 3.4 glomeruli / 100 glomeruli). While that of STZ-AA (39.0 ± 3.9) was significantly increased ($p < 0.05$) as compared with that of CON (30.3 ± 8.1 glomeruli / 100 glomeruli) but significantly decreased ($p < 0.05$) as compared with that of STZ (54.3 ± 7.1 glomeruli / 100 glomeruli).

Table 7-1 Changes in the number of abnormal glomeruli 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.

Groups	Numbers of abnormal glomeruli per 100 glomeruli			
	week 4	**week 8**	**week 16**	**week 24**
CON	31.4 ± 7.6 n=5	30.8 ± 5.4 n=4	27.3 ± 8.9 n=4	30.3 ± 8.1 n=4
CON-AA	31.6 ± 10.0 n=5	32.2 ± 7.8 n=5	34.3± 14.2 n=4	37.3 ± 3.4 n=4
STZ	35.8 ± 3.8 n=5	32.2 ± 7.8 n=5	55.3 ± 3.2[a, b] n=3	54.3 ± 7.1[a, b] n=4
STZ-AA	33.8 ± 7.1 n=5	35.7 ± 14.0 n=3	46.8 ± 4.0[a, c] n=4	39.0 ± 3.9[a, c] n=5

Mean ± SD

[a] compared with CON at the same column, $p < 0.05$

[b] compared with CON-AA at the same column, $p < 0.05$

[c] compared with STZ at the same column, $p < 0.05$

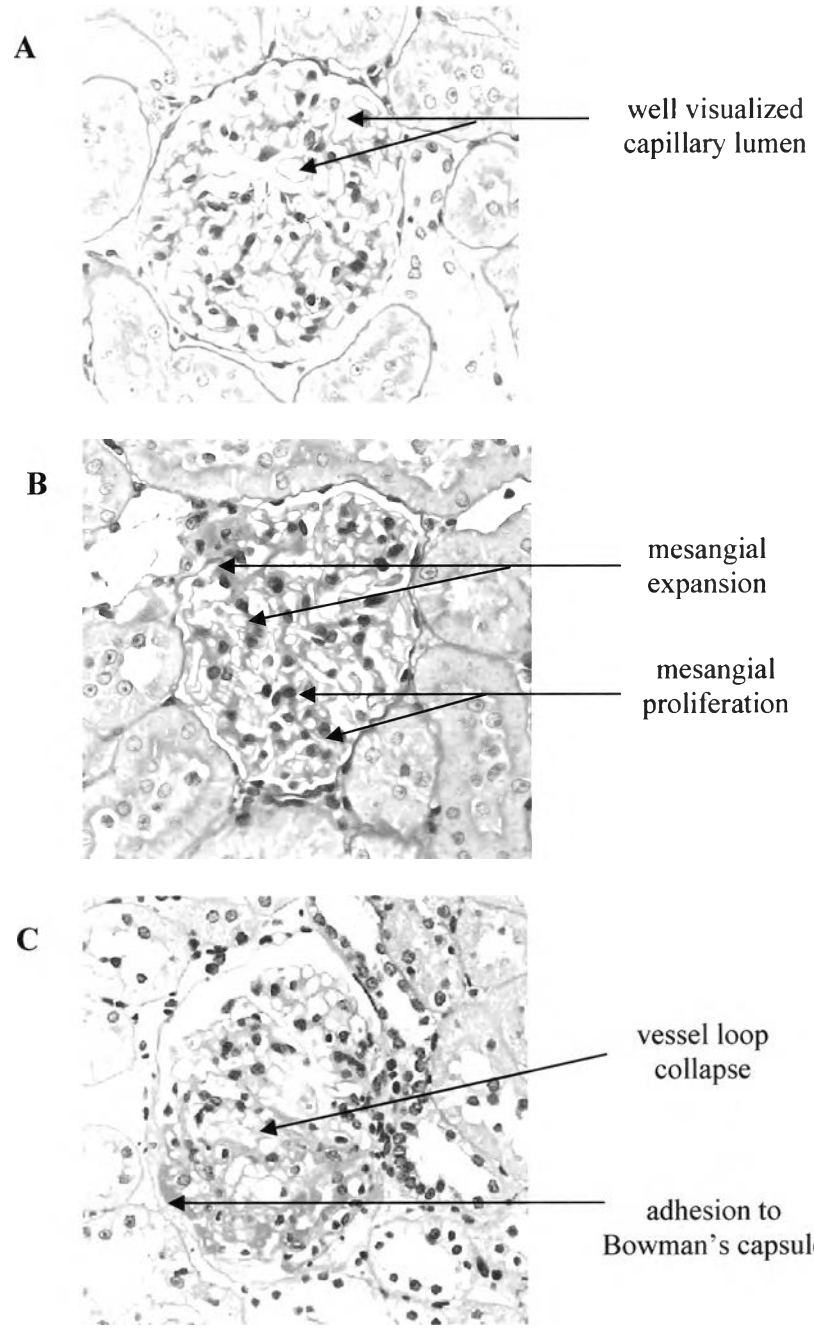

Figure 7-1 Glomerular pathology of streptozotocin-induced diabetic rats, comparing with normal glomeruli. **A** represents a normal glomerulus, characterized by the well-visualized capillary lumen, no mesangial proliferation and expansion. **B** and **C** represent abnormal glomeruli (glomerulosclerosis), characterized by mesangial proliferation, expansion of matrix material and glomerular tuft adhesion to the Bowman's capsule.

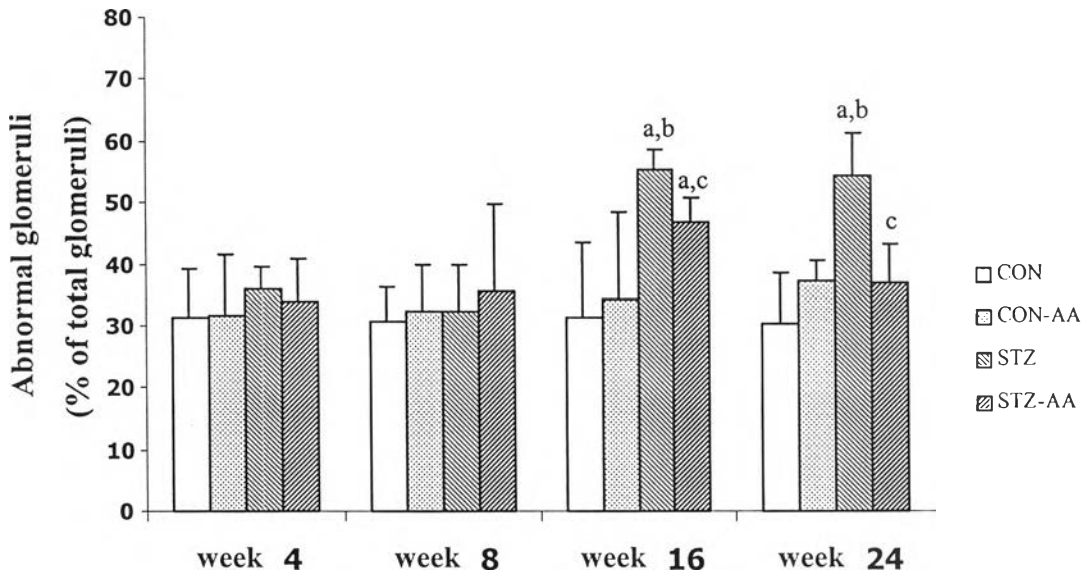

Figure 7-2 Alterations of the numbers of abnormal glomeruli in streptozotocin-induced diabetic rats and control rats with or without L-ascorbic acid supplementation at week 8 and 24 of the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON and [b] compared with CON-AA and [c] compared with STZ at each period, $p < 0.05$. the experimental periods. All values are means ± SD. Statistically significant differences are indicated by [a] compared with CON and [b] compared with CON-AA at each period, $p < 0.05$.

Discussion

The present study was performed to investigate whether L-ascorbic acid supplementation could ameliorate renal pathology in STZ-induced diabetic rats. In the present study, STZ show the significantly increase in the number of abnormal glomeruli at week 16 after the diabetic induction. After the supplementation of L-ascorbic acid for 16 weeks, STZ-AA showed the significantly decrease in the number of abnormal glomerul. This result coincides with the decrease in renal cortex concentration of MDA and TGF-β1 (Chapter VI). In addition, the amelioration of the renal pathology also concurs with the amelioration of the renal physiological defects of diabetic rats after the supplementation of L-ascorbic acid. Diabetic microvascular complications has been noted (Bohle et al., 1991; Dedov et al., 2001). In the present study, glomerulosclerosis were seen more increase in STZ-induced diabetic rats at week 16 of the experimental periods. The glomerular lesions were characterized by loss of vascular tuft, destruction of vascular lumen, adhesion of the glomerular tuft to Bowman's capsule were seen concomitantly the increase in MDA and TGF-β1. The supplementation of L-ascorbic acid could decrease the renal cortical concentrations of MDA as the diabetic vascular injury cause, and TGF-β1as the biomarker of diabetic nephropathy, in STZ-induced diabetic rats (Table 6-1 and 6-2). The improvement of microvascular injury was seen in the diabetic rats supplemented with L-ascorbic acid as shown in the amelioration of renal function and hemodynamics including the decrease in renal vascular resistance, increase in renal plasma flow and glomerular filtration rate (Table 4-4), and concurred with the decrease in the number of injuried glomeruli (Table 7-1).

In conclusion, glomerulosclerosis occuring in STZ-induced diabetic rats is reduced by supplemental L-ascorbic acid. The beneficial effect of L-ascorbic acid supplementation on the prevention of renal pathology should be considered as the therapeutic supplemental agent in diabetes mellitus.