CHAPTER I

GENERAL INTRODUCTION

Hyperglycemia has been demonstrated to induce the renal pathophysiology in diabetes mellitus (Osterby, 1983; Mauer, 1984). The high glucose milieu effects the renal cells such as mesangial cells (Rasch, 1979; Ayo, 1990), tubular cells (Remuzzi, 1998). Mesangial cells are directly affected by high blood glucose concentration and play an important role in the development of glomerulopathy (Rasch, 1979). In addition, the thickening of glomerular basement membrane as well as glomerular expansion with an increase in matrix material leading to the development of glomerulosclerosis (Mauer et al., 1984; Osterby et al., 1992). Hyperglycemia induces the sclerosis of both glomerular and tubular parts (Wang et al., 2001; Morcos et al., 2002) of the kidney in diabetes mellitus, called the diabetic nephropathy. The abnormalities induced by hyperglycemia not only occur in the renal morphology but in the renal functions. An increase in the degree of interstitial fibrosis coinciding with mesangial expansion has been noted (Mauer, 1992). In the early stage of diabetic nephropathy, the glomerular filtration rate (GFR) is increased due to increasing in the filtration surface area (Kroustrup, 1977). With the advancing renal disease, the decline in GFR and renal plasma flow is associated with an increase in the mesangial volume and glomerular occlusion, which lead to a decrease in the capillary filtering surface area (Mauer, 1994). In addition, metabolic and hemodynamic disturbance are closely related in altering the glomerular microcirculation (Baynes et al., 1991; Dedov II et al., 2001), and hypertension development is predicted in diabetic nephropathy (Cediel et al., 2002).

Hyperglycaemia causes renal damage through several mechanisms including non-enzymatic glycation of protein, activation of the polyol pathway (Dunlop, 2000) activation of the hexosamine pathway (Schieicher, 2000) and increased intracellular accumulation of reactive oxygen species. Oxidative stress is enhanced by high blood glucose concentration has been demonstrated as the cause of diabetic nephropathy (Zhang et al., 1997; Salahudeen et al., 1997; Heidland et al., 2001). With many evidences both *in vivo* and *in vitro* demonstrate high glucose and mechanical stretch

stimulate the production of transforming growth factor-β1 (TGF-β1) via a PKC-dependent mechanism (Koya, et al., 1997; Koya, et al., 2000). TGF-β1, which favours extracellutar matrix accumulation, is an important mediator in the development of diabetic nephropathy involving in the production of glomerular matrix materials (Ziyadeh, 1994; Ziyadeh et al., 2000). It has been demonstrated *in vitro* study that high glucose concentration-induced TGF-β1 overexpression resulting in the increase in extracellular matrix protein in the glomeruli (Sharma et al., 1997; Zhang et al., 1999; Heidland et al., 2001). It also has been demonstrated that glucose itself (Heilig et al., 1997), and TGF-β1 overexpression (Inoki et al., 1999) induced the glucose transporter-1 (Glut 1) upregulation in mesangial cells which can enhance the glucosetoxicity.

Mitochondrias, which are rich in the cells to generate energy, can be affected by oxidative stress (Rogers et al., 1986). The oxidative stress-induced mitochondrial disturbances have been demonstrated in various tissues of diabetic animals (Asayama et al., 1989; Bastar et al., 1998; Jang et al., 2000; Rosca et al., 2002). Studies of mitochondrial activity represent the renal cell function, especially in the tubular cells. Decreasing in the oxidative stress with some antioxidants can improve the mitochondrial activity (Shamrai et al., 1978; Thakran et al., 2004).

L-ascorbic acid (vitamin C) is a powerful antioxidant that has been shown to prevent the oxidative stress in rats treated with toxic agents (Appenroth et al., 1998; Greggi et al., 2000). The turnover rate of ascorbic acid in diabetic patients was higher than that in the normal volunteers. Experiments in diabetic rats indicated that the increased turnover of ascorbic acid was probably due to the increase in the oxidation of ascorbate to dehydroascorbate in mitochondria. Ascorbic acid supplementation at a dose of 500 mg per day for a short period of 15 days resulted in a temporary increase in the plasma ascorbate level (Som et al., 1981). The decrease in L-ascorbic acid concentration in serum and tissues always occurs in diabetes mellitus (Seghieri et al., 1994; Lindsay et al., 1998). The *in vitro* study of the effect of L-ascorbic acid on the nephrotoxic injury indicated that L-ascorbic acid promoted recovery of the renal cell functions and mitochondrial function (Nowak et al., 2000). Supplementation of L-ascorbic acid to streptozotocin-induced diabetic rats has been shown to markedly increase in the concentration of L-ascorbic acid in both plasma and renal cortex (Lindsay et al., 1998). There was an evidence of the reduction of the increase in

albumin clearance and the level of glomerular TGF-beta in streptozotocin-induced diabetic rats supplemented with L-ascorbic acid (Craven et al., 1997). In addition, the preservation of β-cell function in diabetes mellitus during giving ascorbic acid has been noted (Kaneto et al., 1999).

Dehydroascorbic acid (DHA), the oxidized form of L-ascorbic acid, is transported into cells by the same transporter as that of glucose (Glut 1) (Zuniga et al., 2001). Competition for membrane transport between glucose and L-ascorbic acid has been shown in human lymphocytes, granulocytes and fibroblasts (Pecorado and Chen, 1987). Exclusion of DHA uptake by Glut 1 in hyperglycemic condition worsens the hyperglycemia-induced oxidative stress level. These effects, which are relevant clinically, has been debated and variations with the stage of clinical hyperglycemia-induced renal dysfunction are being considered. It is possible that L-ascorbic acid has a beneficial effect on diabetes mellitus. However, a few data is available regarding the effects of L-ascorbic acid on the renal functions and pathology and a mechanism of L-ascorbic acid in the prevention of renal changes in diabetes mellitus. Therefore, this study is performed to investigate the roles of supplemental L-ascorbic acid in the renal hemodynamics, functions and mitochondrial activity and in the glomerular pathology in streptozotocin-induced diabetic rats. In addition, the mechanism of L-ascorbic acid action on the changes in the renal pathophysiology via oxidative stress, TGF-β1 and Glut 1 expression was investigated.