CHAPTER I

INTRODUCTION

Diabetes mellitus is a multifactorial disease associated with high risk for vascular complications. The morbidity associated with long-standing diabetes mostly results by microangiopathy, retinopathy, nephropathy and neuropathy. Increasing evidences have suggested that vascular endothelial dysfunction may play a major role in particular on diabetic cardiovascular complications (Virsaladze D. et al, 2001). The long-term hyperglycemia associated with both type 1 and type 2 diabetes mellitus in the slow development of multiple secondary complications.

Diabetic retinopathy is one of major complications that caused most suffering in diabetes patients. Clinically, preproliferative diabetic retinopathy is characterized by tortuousity of vessel, micro-and macro-aneurysms, and vascular nonperfusion, which results in areas of ischemic retina (Yanoff M, 1969). The nonperfusion, as demonstrated by fluorescein angiography, has been presumably involved by vaso-occlusions (Bresnick GH et al, 1975). The increased leukocytes are moderate in nature and precede any overt clinical evidence of retinopathy. The leukocytes actively tether themselves to the endothelial cell lining via classic adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) on the vasculature (Miyamoto K et al, 1999) and β_2 integrins on the leukocytes (Canas-Barouch F et al, 2000). The expression of these adhesive molecules increase in early diabetes and correlate with the increase leukocytes (Canas-Barouch F et al, 2000; Miyamoto K et al, 1999). Additional adhesive molecules, including vascular cell adhesion molecule-1 (VCAM-

1) may also be involved. The increased leukocytes coincides with the onset of diabetic vascular dysfunction. At first, the dysfunction is subclinical in nature, probably because of the lack of sensitivity of current clinical detection methods. However, when more powerful experimental techniques are applied, the early alterations uncovered include a subtle breakdown of the blood-retinal barrier, premature endothelial cell injury and death, and capillary ischemia/reperfusion.

Hemodynamic alterations in retinal vessels are involved in the development of diabetic retinopathy (Blair NP et al, 1982; Kohner EM et al, 1975). Therefore, an understanding of retinal blood flow changes in insulin-dependent diabetes mellitus (IDDM) patients would help clarify the pathophysiology of the disease. Kawagishi T et al (1995) showed that changes in retinal hemodynamics were present before the clinical detection of overt diabetic retinopathy and suggest that the presence of short-term hyperglycemia partly contributes to impaire retinal circulation.

Normally, the iris blood-flow perfusion depends on hemodynamic and vascular factors in the carotid arteries. A direct comparison of measurment may reflect the changes that occur in the iris blood-flow perfusion. Therefore, nonperfusion of iris vessel could result in functional visual loss. It has been recently observed substantial capillary in iris vessel of diabetic subjects, and suggested that vaso-occlusive processes in the diabetic eye were not limited only to retina (Bandello F et al, 1994).

The association between diabetic complications and oxidative stress has been fairly well supported. Currently, the potential contribution of increased oxidative stress to the development of endothelial dysfunction in diabetes has received much of interest. Enhanced oxidative stress in the blood and tissue is thought to play an important role in the onset and

progression of microvascular complications in diabetic patients. Particularly, the molecular mechanisms of the enhancement of oxidative stress in diabetes have been characterised by two dependable domains which 1) increased production of reactive oxygen species (ROS) and 2) impaired endogenous antioxidant defenses. Several suggestions regarding the origins of oxidative stress in diabetes, are free radical generated by glycation of proteins, consumption of NADPH through the polyol pathway, glucose autoxidation, hyperglycemia-induced pseudohypoxia, and activation of protein kinase C (Vander Jagt DJ, 2000). Normally oxidative level controlled by a variety of cellular antioxidant defense mechanisms consisting of enzymatic and nonenzymatic scavengers (Aydin A, 2001). Such enzymes include: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Such those lay an important role in protecting cell and tissue from oxidative stress. And the endogenous nonenzymatic antioxidants including vitamin C, vitamin E, reduced glutathione, β-carotene, various amino acids, proteins, uric acid and bilirubin, etc. They all can directly scavenge reactive oxygen spieces (Kashiwagi A, 2001). Under hyperglycemic conditions, the intracellular concentrations of reduced vitamin C, reduced glutathione and vitamin E are reported to be decreased (Giugliano D et al, 1996). Accordingly, it can be said that antioxidant supplementation is disirable in patients with diabetes mellitus.

Vitamin C, or namely ascorbic acid, is an antioxidant agent, two major properties of vitamin C make it an ideal antioxidant. First is the low one-electron reduction potentials of both ascorbate (282 mV) and it's one-electron oxidation product, the ascorbyl radical (-174 mV), which is derived from the one-diol functional group in the molecule (Halliwell B,

1996). These low reduction potentials enable ascorbate and the ascorbyl radical to react with the reduce basically all physiologically relevant radical and oxidants. The second major property that make vitamin C such as effective antioxidant is the stability and low reactivity of the ascorbyl radical formed when ascorbate scarvenges a reactive oxygen or nitrogen species (Carr AC and Frei B, 1999). Levels of vitamin C in plasma and in various tissues are decreased in diabetic patients and in animals with experimentally induced diabetes. Cellular deficiency of vitamin C has been implicated in some of the cellular pathology and complications of diabetes mellitus such as angiopathy. It has been suggested that vitamin C supplementation may help to prevent the development of some diabetic complications as well (Dai S and Mc Neill JH, 1995).

Previous studies performed by our colleagues (Jariyapongskul A et al, 2002; Sridulyakul P et al, 2003) demonstrated that daily supplementation of vitamin C could improve the diabetic-induced endothelial dysfunction in STZ-rats. In particular, Jariyapongskul et al has showed that long term supplementation of vitamin C could decrease leukocyte adhesion to the cerebral postcapillary venules in diabetic-rats. However, there is no direct evidence showing the protective role of vitamin C supplementation on diabetic induced changes of iris blood-flow perfusion and number of leukocyte adhesion.

In accordance with the previous concept, therefore, we design the experiment in order to study effects of vitamin C supplementation in particular on the relationship between leukocyte adhesion and iris blood-flow perfusion in diabetic rats.

The purposes of present study are to determine

1. whether vitamin C supplementation reduces leukocyte adhesion
2. whether vitamin C supplementation increase iris blood-flow perfusion, and
3. the possible relationship on leukocyte adhesion and iris blood-flow perfusion.