CHAPTER V
DISCUSSION

I. General Observation for Diabetic Animal Model Used in the Study

In this study, the experimental model of diabetes mellitus was induced by streptozotocin (STZ) with the dose of 70 mg/kg.BW.* β-cells were destroyed after a single dose of intraperitoneal STZ-injection. Hyperglycemia was shown within 24-48 hours. However, this model may retain sufficient β-cells to allow long-term survival despite a severely hyperglycemic condition. A modest improvement in glycemic control can occur if there is some islet regeneration. Thus, insulin concentration was able to examine as the result showed in Table 4. Therefore, this model was represented as hyperglycemia with hypoinsulinemia. Moreover, the results also indicated that the concentration of triglycerides and cholesterol were significantly increased in this diabetic rats (Table 5 and 6) while high-density lipoprotein was decreased (Table 7).The mechanism responsible for the development of these dyslipidemia may be due to the effect of insulin on lipoprotein lipase (LPL) and hepatic lipase activity (Dunn, 1992; Garg,1992). Acute insulin deficiency initially caused an increase in free fatty acid mobilization from adipose tissue, resulting in increased secretion of VLDL-TG from the liver. With long term insulin deficiency, the liver converts free fatty acids into ketone bodies, and VLDL-TG secretion diminish. At the same time, lipoprotein lipase and hepatic lipase activity falls resulting in impaired clearance of VLDL and chylomicrons from the plasma (Nikkila, Huttunen and Ehnholm,1977).The abnormalities of lipid metabolism could be reversed by insulin therapy (Abrams, Ginsberg and Grundy, 1982).

---

*In pilot study, the injection of STZ with the dose of 65 mg/kg.BW. into 100-150 g. Wistar rats has showed only 20-30% of animal used were become diabetic (with the criteria of > 400 mg/dl blood glucose). However, when the groups of rats that have body weight more than 130 g. were used, the numbers of diabetic rats were increased nearly 80%

Hypertension Observed in Diabetic Rats

The current experiment also exhibited hypertension, both systolic pressure (Table 8) and diastolic pressure (Table 9) including mean arterial pressure (Table 10). The presence of hypertension is a usual finding in long-term survivors of diabetes. One of the likely explanations for this deleterious association is the is the endothelial dysfunction (Pieper and Gross, 1991). The endothelium plays an important role in the regulation of vascular tone. The endothelial cells synthesize many active substances especially endothelium-derived relaxation factor or EDRF (Vane, Anggard and Botting,1990; Boulanger and Vanhoutte, 1994) whose biological activity is accounted for by nitric oxide (Palmer, Ferrige and Moncada, 1987). Several studies have demonstrated impaired endothelium-depending relaxation (Meraji et al.,1987; Durante, Sen and Sunahara, 1988; Mayhan, 1989; Tesfamariam, Brown and Cohen, 1991), increase in sensivity to noradrenaline in arteries from diabetic animals (Cohen et al., 1990; Taylor et al., 1992 and 1994), and augmented generation of vasoconstrictor prostanoid (Abebe and MacLeod, 1990). The impairment of endothelium-dependent relaxation in diabetes may be due to reduced production and release of EDRF (Calver, Collier and Vallance, 1992; Taylor et al., 1992; Tilton et al., 1993). It is possible that the mechanism of this function arise from either the hyperglycemia or the inadequate levels of circulating insulin as a result of the diabetic condition or both (Pieper, Meier and Hager, 1995). It has been proposed that hyperglycemia may impair nitric oxide production in vascular endothelium through the stimulation of the polyol pathway. The association with increased utilization of nicotinamide-adenine dinucleotide phosphate (NADPH),which may lead to reduce availability of cellular NADPH, an essential cofactor of nitric oxide synthase (Cameron and Colter,1992). Elevated glucose concentrations have also been found to impair endothelium-dependent relaxation through activation of protein kinase C (PKC) (Tesfamariam, Brown and Cohen, 1991).This activation leads to opening of Ca^{2+} channels, allowing the increased influx of extracellular Ca^{2+} into smooth muscle cells, thereby resulting in increased tension development (Abebe and MacLeod, 1990).

However, prolonged exposure of cell membrane protein to high glucose concentrations may result in the formation of advanced glycosylation end-products

(AGEs) by non enzymatic glycosylation (Cohen, 1993). AGEs have also been reported to decrease elasticity and increase fluid filtration in large arteries from diabetic rats (Brownlee, 1994). Moreover, the formation of AGEs may (Bucala, Tracey and Cerami, 1991),and may cause extensive damage of intracellular constituents by the generation of free radicals (Molinatti et al., 1990). The breakdown of nitric oxide by free radicals, generated by hyperglycemia-induced cyclo-oxygenase activity may also contribute to impair endothelium-dependent relaxation (Tesfameriam and Cohen, 1992). It is tempting to suggest that this endothelial abnormality contributes to the increased peripheral resistance characteristics of hypertension.

Diabetic Cardiovascular Complications

As the results shown in table 12 and 13, aortic flow rate and coronary flow rate was decreased in the diabetic induced rats in the experiment. It could be increase vascular resistance, resulting in hypertension. It may also be due to diabetic dyslipidemia that is normally counted as an important risk factor for generating atherosclerosis. The decreased cardiac out put and aortic flow rate in diabetes may cause diminished glucose conversion and depressed energy production from glucose (Rosen et al., 1986). Pogatsa et al.(1979) suggested that the decreased cardiac output is a result of diminished left ventricular compliance. As the result shown in Table 14, left ventricular contractility was significance decreased in diabetic rat. Fein and Sonnenblick (1985) reported that the diminished left ventricular compliance possibly reflecting a structural alteration in the interstitial compartment.

Atherosclerosis

The abnormal thickness of arterial wall was observed in this study. By SEM, the increment of size of artery and arteriolar wall in diabetic rats could be distinguished statistically from control rats, as shown in Table15 and 16. It was relatable to the duration of exposure to the hyperglycemia. Swelling of endothelial cell was observed in some case. The basement membrane thickening of capillaries in diabetic rats were more extensive than those in controls (Table 17). Some capillary demonstrated degenerative changes and duplication of basement membrane. This

morphology changes of these diabetic animals supported the idea that there is a disturbance of endothelial cell function. Penpargkul et al (1980) found that diabetes may be responsible for both structural and functional alterations of the coronary circulation. These alterations may contribute to the progressive deterioration of the myocardium further and to the pathogenesis of diabetic cardiomyopathy.

Proteinuria

Recent studies have shown that increased urinary albumin excretion rate is a predictor, not only of clinical diabetic nephropathy, but also of cardiovascular disease (Borch-Johnsen, K-Andersen, and Deckert, 1985; Jarett et al., 1984; An-kions and Firth, 1993). This is also in case of the present study. As shown in Table 17, the diabetic rats were significantly increased. The cause of increased cardiovascular mortality in diabetic with increase urinary albumin excretion rates remain unknown. The possiblibility that increased left ventricular contractibility and blood pressure may result in increased glomerular pressure and filtration rate, which over time, could have adverse consequences for renal structure and function, result in, first microalbuminuria and, later, proteinuria (Kimball et al., 1994). Also, there were the reports that diabetic and disturbances of insulin metabolism may precede the vascular abnormalities of increased vascular permeability (Patumraj, Ritter and Duran,1990; Yip et al., 1993). Williams (1996) concluded that hyperglycemia, hypertension and activation of the renin-angiotensin system could stimulate for increased vascular permeability factor (VPF) production. Increasing of renal permeability may be due to the binding between circulation LDL cholesterol and glucosaminoglycan of filtering membrane. Moreover, the filtered lipoprotein may accumulate in mesangial cells and stimulate them to proliferate and synthesize excess basement membrane material (Moorhead et al., 1982).

II. Studies of Garlic Extract of Diabetic Cardiovascular Complications

Hypoglycemic Effect of Garlic

In the present study, the daily oral feeding of crude garlic extract with the dose of 100 mg/kg.BW. could reduces the body weight loss in STZ-induced diabetic rats

and give the healthier appearance. Garlic extract also reduced blood glucose level in diabetic rats (Table 3) as the previous study in our laboratory group (Jetapai, 1994). The hyperglycemic effect of garlic was demonstrated by many workers and most of them attributed such effects to allicin type compounds. The possible mechanism of the hyperglycemic action of garlic products was explained by Mathew and Augusti (1973) that they may be potentiating the insulin effect of plasma by directly increasing the pancreatic secretion of insulin from some functional β-cells of the islets which escaped streptozotocinization. Another possibility may be to stimulate indirectly the pancreatic secretion of insulin by producing gastrointestinal hormones like pancreozymin as certain triglycerides have been known to act. However, a most probable mode of action of allicin may be to spare insulin from sulphydryl group inactivation as it can effectively combine with compounds like cysteine. Insulin inactivation by endogenous sources of cysteine, glutathione and serum albumin fractions rich in -SH groups ma be blocked by allicin and thus enhancing endogenous insulin effect to bring about a reduction in blood sugar. The results in Table 4 indicated that the serum insulin of both STZ-G rats and STZ-T rats increased significantly after 8,16 and 20 weeks. It is suggested that **the hypoglycemic effect of garlic was through the enhancement of insulin level in blood**. Jain and Vyas (1975) proposed that this hypoglycemic action inalloxan diabetic rabbits might be either by stimulating pancreatic secretion of insulin or by activating the inactive form. In their study, the hypoglycemic effects of ethyl alcohol, petroleum ether and ethyl ether extracts of garlic were percented equal to 64.5, 61.0 and 82.5 of tolbutamide effects. With yhe same standard dose of tolbutamide, this study using STZ-rats indicated that hypoglycemic activity of chloroform garlic extract were 74.22%, 96.23% and 93.27% as compared to tolbutamide at the weeks of 8,16 and 20 after STZ-injection. Moreover, hypoglycemic effect of garlic might be due to an increase in the insulin response during feeding, probably because the transport of blood glucose to the peripheral tissues was enhanced (Chang and Johnson, 1980). The increased insulin responsé also promotes to the conversion of the inactive form of glycogen synthetase to the active and enhances conversion of blood glucose into glycogen. The recent study, it is opined that the strong antidiabetic effect of garlic may also be due to the presence of S-allyl cystiene

sulphoxide (SACS), a sulphur containing amino acid of garlic which is the precursor of allicin and garlic oil (Sheela and Augusti, 1992).

Effect of Garlic on Dyslipidemia

As shown in Table 5, 6 and 7, garlic resulted in normalize dyslipidemia. The levels of triglycerides and cholesterol were decreased while HDL-levels was increased in diabetic animals, that fed oral garlic extract. Many reported obtained that **these effects were related with the activity of hydroxymethyglutaryl coenzyme A (HMG-CoA) reductase.** Sheela and Angusti (1992) shown that S-allyl cystiene sulphoxide (SACS) was also decreased significantly the concentration of serum lipids and decreased significantly liver and intestinal HMG-CoA reductase activity and liver hexokinase activity, leading to increase the excretion of fecal bile acids and neutral steroids. However, the administration of garlic resulted in inhibition of hepatic cholesterol biosynthesis (Sendl et al.,1992; Gebhardt, 1993). Chloroform extracts of garlic inhibited cholesterol synthesis 44-52% at a concentration of 166 mg/ml (Sendl et al.,1992). Garlic resulted in the concentration-dependent inhibition of cholesterolerogenesis at several different enzymatic steps. At low concentrations, sterol biosynthesis was decreased (Yeh and Yeh, 1994). This inhibition was exerted at the level of HMG-CoA reductase by formation of sulphide bridges with the HMG-CoA or the reductase molecules. The activity of HMG-CoA reductase is significantly reduced by garlic extracts (Brosche and Platt, 1991; Gebhardt, 1991; Warshafsky, Kamer and Sivak, 1993).At high concentrations, inhibition of cholesterol biosynthesis was not only seen at an early step, but also later steps resulting in slightly inhibition of cholesterol 7-alpha-hydroxylase and cholesterol acyltransferase (Gebhardt, 1991) included the accumulation of the precursors lanosterol and 7-dehydrocholeaterol (Gebhardt, 1993). At very high concentrations, fatty acid synthetase is inhibited by alliin leading to triacylglycerol lowering effect (Yeh and Yeh, 1994). Moreover, garlic could oxidized NADPH, which is a necessary cofactor for lipid synthesis, so lipid synthesis was decreased (Sheela and Augusti, 1992). However intensive insulin therapy resulted in improvement of dyslipidemia in IDDM patients (Abrams, Ginsberg and Grundy, 1982; Dunn, 1992). Thus, decreasing in serum

triglycerides and cholesterol level and increasing of HDL-level in STZ-G rats in this study may be due to enhancement of serum insulin level.

Hypotensive Effect of Garlic

In this study, the decrease of blood pressure (Table 8-10) and increase of cardiac out put (Table 12-13) were observed in STZ-G rats as compared to STZ-rats. The mechanism of hypotensive effect may be due to **garlic extract could increase nitric oxide synthase (NOS) activity** (Das, Khan and Sooranna, 1995) Some of the hypotensive action of garlic was attributed to histamine release which produced decrease in total peripheral resistance (Sial and Ahmed, 1982).Later, it has been suggested that garlic could lower blood pressure through its prostaglandin like mechanism (Rashid and Khan,1985).Aqel, Gharaibah and Salhab (1991) suggesting that the hypotensive action of garlic juice may be due to a direct relaxant effect on vascular smooth muscles.

In addition, the enhancement of insulin level in STZ-G rats may probably caused hypotensive action. The mechanism by which insulin causes vasodilation is still unknown. Yagi et al.(1988) reported that insulin has a direct effect on vascular smooth muscle. It could stimulate the production of endothelial relaxing factors (Baron, 1993; Wu et al., 1994). In addition, insulin could suppress endothelial production of potent vasoconstrictors such as endothelins and thromboxane. Alternatively, insulin could inhibit the action of pressor substance (Baron, 1993).

Effect of Garlic on Atherosclerosis

In this study, garlic extract could reduce the thickness of arterial wall, arteriolar wall and the basement membrane of capillaries, as shown in Tables15-17. These alteration might be due to the inhibiting effect of garlic on neointima formation. Heinle and Betz (1994) reported that garlic could decrease DNA content of the vessel wall and reduction of collagen type I and IV including arrange diffusely in the extracellular matrix. This reduction could attenuate impairment of endothelium dysfunction. The ability of endothelial cells to generate vasoactive substances has become. The study by using garlic also inhibition the contraction of rabbit and quinea pig aorta rings induced

by norepinephrine (Aquel et al., 1991). Especially, the garlic could increase nitric oxide synthase activity (Das, Khan and Sooranna, 1995).

The vascular action of garlic indicating that garlic administration retards the development of atherosclerosis risk factors such as hyperglycemia, hypertension, dyslipidemia. It effects include inhibited platelet aggregation and increase of fibrinolytic activity and clotting time (Gadkari and Joshi, 1991; Lawson, Ransom and Hughes, 1992; Lengnani et al., 1993). Orekhov et al. (1995) indicated that garlic extract caused not only directly anti-atherosclerosis-related (therapeutic) but also anti-atherogenic-related (preventive) action, reducing atherogenic manifestations at the cellular level.

Garlic and Antioxidant

There is growing evidence that oxygen-derived free radicals are implicated in the endothelial dysfunction and cardiovascular morbidity of diabetes (Cohen, 1993). A possible source of oxygen-free radicals in diabetes is auto-oxidant of glucose, which results in the generation of reactive ketoaldehydes and subsequent formation of advanced glycosylation end-products (AGEs). Impaired generation of naturally occurring antioxidants in diabetes can also expected result in increased oxidative cell damage. Enhanced metabolism of glucose via the polyol pathway may be expected to deplete NADPH, which is required for nitric oxide. Thus it may interfere with endothelium-dependent vasorelaxation. Treatment with antioxidants improves many metabolic abnormalities associated with diabetes (Giugliano, Cariello and Paolisso, 1995). It has been reported that garlic powder and alliin have antioxidant and free radical scavenging activity (Kourounakis and Rekka, 1991). Garlic's antioxidant action can actually prevent fats from being oxidised and deposited in the tissue (Phelps and Harris, 1993).

Garlic and Proteinuria

The vascular effect of garlic may also occur in kidney vasculature and glomerular basement membrane. The endothelial permeability to macromolecules was likely decreased. Although proteinuria of STZ-G rats in this study were less than those of STZ-rats, but it still has not significantly different (Table 18). The reason might be

due to the intensive diabetes therapy would not decrease proteinuria and no intervention has been demonstrated to halt the progression of nephropathy (Nathan, 1993).

Overall, garlic extract could prevent or delay the diabetic cardiovascular abnormalities by improving the endothelial dysfunction both morphology and function. The possible hypothesis was that garlic extract can normalize dyslipidemia, hypertension and, especially, hyperglycemia. It has been showed in this study that garlic could reduced blood glucose level resemble to tolbutamide. But tolbutamide has many adverse side effects such as nausea, vomitting, hypersensitivity (fever, rash, photosensitivity), hypoglycemia, anti-lipocytic action (obesity), antithyroid action, hemopoietic system effect and, importantly, cardiovascular effect. Moreover, long term tolbutamide treatment could cause coronary occlusion (Oates and Wood, 1989). Therefore, I believed that garlic might be a great benifit agent to prevent the development of atherosclerosis including the incidence of cardiovascular disorder in diabetes in the future.