The Korean Journal of Internal Medicine
Vol. 16, No. 2, June, 2001

The Effect of Cilostazol on Glucose Tolerance and Insulin Resistance in a Rat Model of Non-insulin Dependent Diabetes Mellitus

Sang Ah Chang, M.D., Bong Yun Cha, M.D.*, Soon Jib Yoo, M.D., Yoo Bae Ahn, M.D., Ki Ho Song, M.D., Je Ho Han, M.D., Jong Min Lee, M.D., Hyun Sik Son, M.D., Kun Ho Yoon, M.D., Moo IL Kang, M.D., Kwang Woo Lee, M.D., Ho Young Son, M.D., and Sung Ku Kang, M.D.

Department of Internal Medicine, Catholic University Medical College, Seoul, Korea

Background : *It has been reported that many peripheral vasodilating drugs might improve insulin resistance. Cilostazol, a antithrombotic agent, increases peripheral blood flow in non-insulin dependent diabetic patients. The effect of cilostazol treatment on insulin resistance in streptozotocin (STZ)-induced non-insulin dependent diabetic Wistar rats was examined.*

Methods : *About a half of two-day old neonate siblings were injected intraperitoneally with STZ and maintained for six months, at which time they were compared with age-matched control rats for intraperitoneal glucose tolerance test (IPGTT) and for glucose infusion rate (GINF) in a euglycemic hyperinsulinemic glucose-clamp study. After that, these studies were also performed after feeding rat chow containing cilostazol (100 mg/kg/day) to rats with STZ-induced non-insulin dependent diabetes mellitus for four-weeks and compared with those of age-matched control rats.*

Results : *In the intraperitoneal glucose tolerance test studies, plasma glucose levels of STZ-induced non-insulin dependent diabetic rats were significantly higher and plasma insulin levels significantly lower than those of age-matched control rats in the age of six months. Glucose infusion rate was lower in STZ-induced non-insulin dependent diabetic rats than those of age-matched control rats. However, after a four-week cilostazol treatment, glucose infusion rate of STZ-induced non-insulin dependent diabetic rats was not significantly different from that of control rats.*

Conclusion : *These findings suggested that cilostazol may improve insulin resistance in STZ-induced non-insulin dependent diabetic rats.*

Key Words : *Cilostazol; Insulin resistance; Streptozotocin-induced non-insulin dependent diabetic rat; Euglycemic hyperinsulinemic clamp technique; Glucose tolerance test*

INTRODUCTION

Insulin resistance is one of the major pathophysiologic findings in non-insulin dependent diabetes mellitus. Improvement of insulin resistance is one of the major goals in the management of non-insulin dependent diabetes mellitus. It is well known that insulin resistance accompanies increased peripheral vascular resistance. In addition, increased peripheral vascular resistance may exacerbate insulin resistance by inhibiting the access of insulin and glucose to skeletal muscle cells. These findings have recently been supported by observations that peripheral vasodilating drugs, such as angiotensin converting enzyme inhibitors and alpha-1-adreno-receptor antagonists, improve insulin resistance by increasing insulin mediated glucose disposal[1, 2].

Address reprint requests to : Bong Yun Cha, M.D., Department of Internal Medicine, Catholic University Medical College, # 505 Banpo-dong Seocho-ku, Seoul 137-040, Korea

Cilostazol (6-[4-(1-cyclohexyl-1-H-tetrazol-5-yl)butoxyl]-3,4-dihydro-2(1H) quinolinone), a novel synthetic antithrombotic drug, has been shown to have potent in vitro and in vivo inhibitory effects on platelet aggregation induced by almost all physiological aggregator substances, including adenosine diphosphate (ADP), collagen, epinephrine, platelet activating factor (PAF) and thromboxane A2 (TXA2)[3]. In addition, this drug has been shown to increase blood flow and to ameliorate the hypertriglyceridemia in insulin-resistant non-insulin dependent diabetes mellitus[4-8]. Thus, it is possible that cilostazol may improve insulin resistance by increasing peripheral blood flow. In the present study, we used non-insulin dependent diabetes model can be induced by treatment of a neonatal rat with streptozotocin (STZ). This animal model quickly developed acute diabetes characterized by overt hyperglycemia and reduced insulin stores. However, the pancreas of this model is able to regenerate within 3-4 weeks after injection of STZ and basal glucose levels are normalized. As the animals injected with STZ age, they slowly become glucose-intolerant and insulin-resistant[9]. In order to evaluate the effect of cilostazol on insulin resistance in the STZ induced non-insulin dependent diabetic rats, intraperitoneal glucose tolerance test (IPGTT) was performed and insulin dependent glucose utilization by the euglycemic hyperinsulinemic clamp study was quantified.

MATERIALS AND METHODS

1. Non-insulin Dependent Diabetes Mellitus Model

The male neonatal rats were obtained by mating the paired adult Wistar rats. Two days after birth, the male neonate siblings were separated into two groups. One group was not treated and served as the age-matched control animals and the other group was rendered diabetic by the administration of a intraperitoneal (IP) injection of 0.09 mg/g body weight of STZ as reported by Schaffer and Wilson[10]. They were allowed access to food (standard rat chow, Sam Yang Co. Seoul, Korea) and tap water ad libitum. The rats were kept in gang cages in quarters in which the temperature and humidity were maintained at 24℃ and 92%, respectively. The rats were weighed once a month.

Six months after the STZ injection, the diabetic rats were fed rat chow containing cilostazol (100 mg /kg/day) for a month. The Otsuka Pharmaceutical Company (Tokushima, Japan) provided cilostazol.

2. Glucose Tolerance Test with Measurement of Insulin and Free Fatty Acid Levels

Intraperitoneal glucose tolerance tests (IPGTT) (2 g/kg body weight) were performed under pentobarbital anesthesia (4 mg/100 g body weight IP). Control and STZ-induced non-insulin dependent diabetic rats were fasted for 16 hr after which time they were given an IP injection of 20% glucose solution. Blood samples were drawn from the eyeball capillary plexus before and 15, 60 and 120 minutes after the glucose challenge. Blood samples were immediately centrifuged at 4℃ and plasmas were stored at -20℃ until assayed. Plasma glucose concentrations were measured using a Beckman Glucose Analyzer (Beckman Instrument Co., Palo Alto, CA., USA). Plasma insulin concentrations were determined using the Incstar rat insulin RIA kit (Incstar Co., Stillwater, MN, USA). Fasting plasma-free fatty acid levels were determined by enzymatic assay using a commercial kit (Eiken Chemical Co., Tokyo, Japan).

3. Euglycemic Insulin Clamp Studies

These studies were performed according to the procedures described previously in detail[11, 12]. Rats were anesthetized with pentobarbital (4 mg/100 g body weight, IP). A carotid or the tail artery and jugular vein were cannulated for blood sampling and infusion of glucose and insulin, respectively. Two hours after the completion of the cannulation, the clamp studies were begun. Insulin (porcine monocomponent Actrapid, Novo, Copenhagen, Denmark) dissolved in 0.9% NaCl containing 0.2% bovine serum albumin (Sigma Chemical Co., St. Louis, MO., USA) was infused at a constant infusion rate (0.75 U/h/kg) with an infusion pump (Pump 22, Harvard Apparatus Co. MA., USA). The variable amounts of glucose infusions were started five minutes after the beginning of the insulin infusion. The steady state plasma insulin and plasma glucose levels were reached 45-50 minutes after the beginning of the insulin infusion. Blood samples (150 μL) were collected at 10 minute intervals and the samples that were collected at 90-120 minutes after the beginning of the insulin infusion were used to determine glucose infusion rate. Body temperature was maintained at 37-38℃ with heating lamps during the procedures.

4. Analysis of Data

Results were presented as means±SEM. Significance of differences between groups was assessed using Student's unpaired *t*-tests.

RESULTS

1. Body Weight

The body weights of STZ-induced non-insulin dependent diabetic rats were not significantly different from those of the age-matched control rats at six months of age. The body weights of cilostazol-treated diabetic rats were not significantly different from those of the age-matched control rats at seven months of age (Table 1).

Table 1. Body weight of sreptozotocin-induced non-insulin dependent diabetic and normal age-matched control rat

	Body weight (g)	
	6 months	7 months
Control	381.3±12.2 (n=39)	396.2±18.5 (n=20)
NIDDM	404.2±9.8 (n=32)	390.0±12.4 (n=25)*

Values presented as means±SEM.

*Non-insulin dependent diabetic rat with 4-weeks of cilostazol treatment. Two-day-old rats were rendered diabetic by the intraperitoneal injection of 0.09 mg/g body weight of streptozotocin.

2. IP GTT and Insulin Levels

Changes in plasma glucose and insulin levels during the IPGTT in age-matched control rats and STZ-induced non-insulin dependent diabetic rats are shown in Figure 1. During the GTT, the increases in plasma glucose of the diabetic rats were significantly greater than those of the control rats at six months of age. On the other hand, the increases in plasma insulin levels of the diabetic rats were significantly lower than those of the age-matched control rats.

When comparing the data of IPGTT of the control rats performed at the six months and the seven months of age, significant glucose intolerance was noted at the age of seven months. On the other hand, the profiles of plasma glucose and insulin levels of the diabetic rats during IPGTT, four weeks after the treatment with cilostazol, also showed the pattern of glucose intolerance. But, in comparing with those of the age-matched control rats, the degree of glucose intolerance of the seven-month-old diabetic rats treated with cilostazol was not different significantly (Figure 2).

3. Free Fatty Acid Levels

Fasting free fatty acid levels of the STZ-induced non-insulin dependent diabetic rats at the six months of age

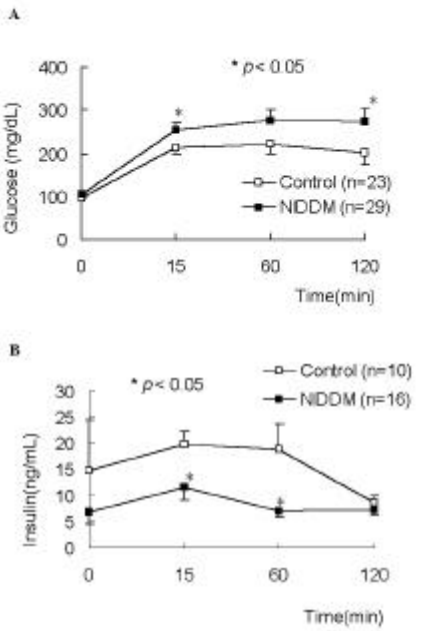

Figure 1. Plasma glucose (A) and insulin (B) concentrations in response to an intraperitoneal glucose challenge (2 g glucose/kg) in 6 month-old streptozotocin induced non-insulin dependent diabetic rats and normal control rats. Values presented as mean±SEM.

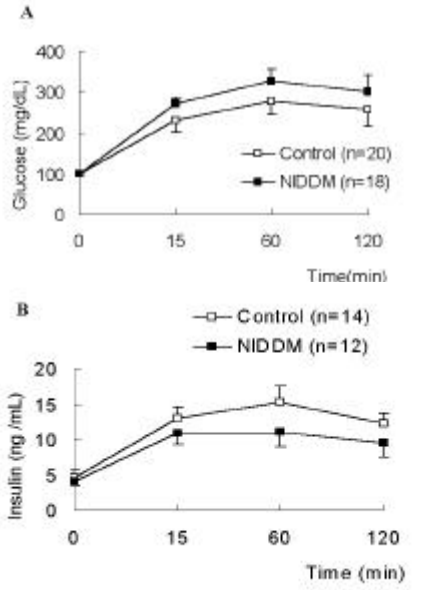

Figure 2. Plasma glucose (A) and insulin (B) concentrations in response to an intraperitoneal glucose challenge (2 g glucose/kg) in 7 month-old streptozotocin induced non-insulin dependent diabetic rats with 4 weeks of cilostazol treatment and their age-matched control rats. Values presented as mean±SEM.

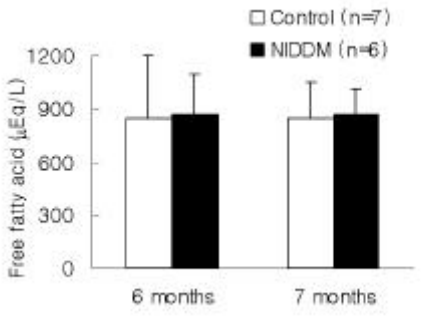

Figure 3. Fasting free fatty acid levels in normal control and streptozotocin induced non-insulin dependent diabetic rats before (6 months) and after (7 months) cilostazol treatment. Values presented as mean±SEM.

were not different from those of the control rats (874± 224 μEq/L in the diabetic rats *vs* 846±381 μEq/L in the control rats). After treatment with cilostazol for four weeks, there was also no significant difference in fasting free fatty acid levels between the diabetic and the age-matched control rats (867± 146 μEq/L in the diabetic rats *vs* 848±200 μEq/L in the control rats, Figure 3).

4. Glucose Infusion Rates (GINF) in the Euglycemic Hyperinsulinemic Clamp Study

To determine whether this insulin resistance can be improved by treatment with cilostazol, glucose clamp studies were performed in the STZ-induced non-insulin dependent diabetic rats before and after the treatment with cilostazol for four weeks. During the euglycemic hyperinsulinemic clamp studies, the GINF of the STZ-induced non-insulin dependent diabetic rats at the six months of age were significantly lower than those of the age-matched control rats (21.4± 1.1 mg/kg/min in the diabetic rats *vs* 26.5±2.2 mg/kg/min in the control rats, $p<0.05$). However, the GINF of the cilostazol-treated diabetic rats were not significantly different from those of the age-matched control rats (21.2±3.2 mg/kg/min in the diabetic rats *vs* 22.6± 1.0 mg/kg/min in the control rats, Figure 4).

In the control group, the GINF at the seven months of age tended to be lower than the GINF at the six months of age, but the difference was not significant (26.5 ±2.2 mg/kg/min at the six months of age *vs* 22.6± 1.0 mg/kg/min at the seven months of age). On the contrary, the GINF of the seven-month-old diabetic rats were not significantly different from the GINF of the six-month-old diabetic rats (21.2±3.2 mg/kg/min at the seven months of age *vs* 21.4± 1.1 mg/kg/min at the six months of age).

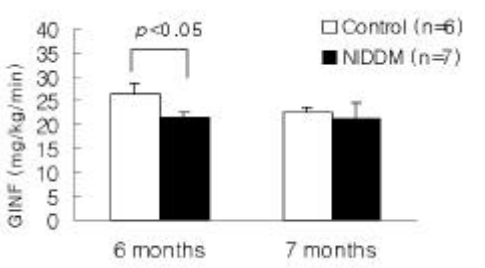

Figure 4. Effects of cilostazol on glucose infusion rate in normal age-matched control and streptozotocin induced non-insulin dependent diabetic rats before (6 months) and after 4 weeks of cilostazol treatment (7 months). Values presented as mean±SEM.

DISCUSSION

Insulin resistance in six-month old STZ-induced non-insulin dependent diabetic rats was examined using an IPGTT and a glucose utilization procedure. In the IPGTT study, significant glucose intolerance was noted in the diabetic rats. These rats were also hypo-insulinemic compared to the age-matched control rats at 15 and 60 minutes after the glucose loading. On the other hand, peripheral glucose utilization was clearly decreased in STZ-induced non-insulin dependent diabetic rats when measured by the euglycemic hyperinsulinemic clamp technique. Taken together, these results indicate that the diabetic rat model employed in the present study is similar to non-obese, insulin-deficient, non-insulin dependent diabetes mellitus in man[9, 13, 14]. A number of investigators have shown that insulin resistance develops in peripheral tissues of the neonatal animal model of non-insulin dependent diabetes mellitus[15-18]. Our data also shows that the six-month old STZ-induced non-insulin dependent diabetic rat actually had a blunted insulin secretory response to glucose challenge and decreased glucose utilization in peripheral tissue. Previously, Schaffer and Wilson[10] found that a blunted insulin response to glucose challenge in the STZ-induced non-insulin dependent diabetic rat model developed between six and fourteen months of age. They observed that the six and fourteen month-old animals were markedly glucose intolerant but the six-month animals had an enhanced insulin secretory response. They also found that the myocardium developed insulin resistance was associated with alternating periods of hyper- and then hypo-insulin secretion. Although the same protocol used by Schaffer

and Wilson[10] was used in the present study, hypoinsulinemia and insulin resistance developed earlier than they reported. At the present time, there is no explanation that can be offered to account for this discrepancy.

Our observations could not suggest where insulin resistance occurs because endogenous glucose production was not measured. However, under euglycemic hyperinsulinemic conditions, it has been reported that the major portion of an infused glucose load is used by muscle tissues[19, 20] and that the uptake of glucose by the adipose tissues accounts for only 1-2% of the infused glucose load[21, 22]. Therefore, the reason for the insulin resistance of this animal model might have originated from the muscle tissue.

When comparing between the GINF of six-month-old control rats and the GINF of seven-month-old control rats, GINF at seven months of age tend to be lower than that at six months of age, which might be the age-related deterioration of glucose tolerance, but the difference was not significant. Also, comparing between the GINF of six-month-old STZ-induced diabetic rats and the GINF of seven-month-old STZ-induced diabetic rats, which were treated with cilostazol, there was no significant difference between them. On the other hand, the GINF of STZ-induced diabetic rats at six months of age were significantly lower than those of the age-matched control rats. However, the levels of plasma glucose and insulin in the IPGTT and the GINF during the euglycemic hyperinsulinemic glucose clamp study of the cilostazol-treated seven-month-old diabetic rats were similar to those of the age-matched seven-month-old control rats. These results indicate that cilostazol may improve insulin resistance of these STZ-induced non-insulin dependent diabetic rats.

The mechanism(s) underlying the effects of cilostazol to improve insulin resistance are not clear. However, several possibilities of the mechanisms of cilostazol on improving insulin resistance can be suggested. We measured fasting plasma-free fatty acid levels, because it has been well documented that the elevated level of plasma-free fatty acids cause insulin resistance in muscle and liver[23, 24]. But the plasma-free fatty acid levels were not increased in this STZ-induced non-insulin dependent diabetic model. In addition, cilostazol treatment made no significant effect on the level of plasma-free fatty acids. Thus it seems that insulin resistance of this non-insulin dependent diabetic model is not related to plasma-free fatty acid levels.

One of the possibilities of the improvement of insulin resistance may be due to the improved insulin secretion from the pancreas. Weir et al.[14] studied insulin secretion from the isolated, perfused pancreas of the STZ-induced non-insulin dependent diabetic rat. They found that insulin secretion from the beta cells of this in vitro pancreas model was extremely insensitive to glucose exposure. However, pretreatment with phosphodiesterase inhibitor, e.g., theophylline, showed that these cells were not completely insensitive to glucose exposure. Cilostazol, which has type III phosphodiesterase inhibitory property[5], may also stimulate insulin secretion directly to the beta cells of the pancreas. With this possible effect of cilostazol, increased insulin secretion might have improved hyperglycemia, and the improvement of hyperglycemia might produce subsequent improvement of insulin resistance. However, because we did not measure insulin secretion from the pancreas, this possibility remains to be evaluated.

Another possibility to be speculated is that cilostazol might directly improve the insulin action on the muscle or adipose tissues. But, in view of many reports which showed that phosphodiesterase inhibitors impair the insulin action in insulin target tissues[25-28], it is hard to imagine that cilostazol could play a direct role on improving the insulin action in the muscle or adipose tissues. However, it can be speculated that relaxation of systemic arterioles by cilostazol might increase blood flow through muscle tissue[29], thereby improving the tissue response to glucose and insulin. This possibility is supported by several published studies reporting that cilostazol inhibits type III phosphodiesterase activity in blood vessels, leading to vasodilatation by increasing cAMP levels in the vascular smooth muscle[5].

In conclusion, these results demonstrate that these STZ-induced non-insulin dependent diabetic rats revealed significant glucose intolerance, leading to the development of insulin resistance. Four weeks cilostazol treatment of the diabetic rats improved insulin resistance. These observations raise the possibility that cilostazol may improve insulin resistance in STZ-induced non-insulin dependent diabetic rats.

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