ORIGINAL ARTICLE

Cilostazol, a phosphodiesterase inhibitor, improves insulin sensitivity in the Otsuka Long-Evans Tokushima Fatty Rat, a model of spontaneous NIDDM

Y. Nakaya, A. Minami, S. Sakamoto, Y. Niwa, M. Ohnaka, N. Harada and T. Nakamura

Department of Nutrition, School of Medicine, University of Tokushima, Tokushima, Japan

Aim: Angiotensin converting enzyme inhibitors and α_1-adrenergic blockers improve insulin sensitivity, the mechanism of which was considered, at least in part, to be due to the increased blood flow to muscle. The present study aimed to clarify whether cilostazol, a phosphodiesterase inhibitor, improves insulin sensitivity in a model of spontaneous non-insulin dependent diabetes mellitus (NIDDM), Otsuka Long-Evans Tokushima Fatty (OLETF) rat.
Methods: OLETF rats were divided into the two groups at the age of 16 weeks: the cilostazol-supplemented group (cilostazol 40 mg/kg/day) and the normal-diet group. As a non-diabetic control, we used Long-Evans-Tokushima-Otsuka rats (non-diabetic rats). Oral glucose tolerance test and hyperinsulinemic euglycemic clamp was performed at the ages of 23 and 25 weeks, respectively. Serum levels of lipids and leptin were measured.
Results: Body weight and abdominal fat was increased in OLETF rats but cilostazol supplementation did not alter them. Insulin sensitivity, as measured by the hyperinsulinemic euglycemic clamp technique, was significantly decreased in OLETF rats (glucose infusion rate: 73.5 ± 10.0 vs. 41.5 ± 9.8 μmol/min/kg body weight, $p < 0.01$). Cilostazol supplementation improved insulin sensitivity partially but significantly 51.0 ± 5.7 μmol/min/kg body weight, $p < 0.05$) in OLETF rats at 25 weeks of age, although it did not decrease serum levels of glucose, lipids or leptin. However, this effect was not observed in non-diabetic rats.
Conclusion: Cilostazol, which is used in diabetic patients for the treatment of obstructive disease of artery, is expected to have a beneficial effect on insulin sensitivity in NIDDM.

Keywords: Otsuka Long-Evans Tokushima Fatty rat, cilostazol, insulin-sensitivity, non-insulin dependent diabetes mellitus
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Introduction

The management of concomitant conditions such as obesity, diabetes mellitus, and hyperlipidemia is advocated as one of the keys to improving the prevention of cardiovascular events [1]. Insulin sensitivity is common in the above-mentioned conditions, and non-insulin dependent diabetes mellitus (NIDDM) itself is believed to be an insulin-resistant state. It has been proposed that the management of insulin sensitivity may contribute to the prevention of development of NIDDM and cardiovascular events. Thus, in the management of NIDDM, consideration should be given to the influence of various medication on insulin sensitivity.

Correspondence:
Yutaka Nakaya, MD, Department of Nutrition, School of Medicine, Tokushima University, 3-18-15, Kuramoto-cho, Tokushima City, 770, Japan.
E-mail:
nakaya@nutr.med.tokushima-u.ac.jp

Table 1 Effect of cilostazol on body weight and abdominal fat

Group	Weight (g)	Abdominal fat	
		(g)	b.w.* (%)
LETO-Control	439±19	20.7±2.9	4.7±0.6
LETO-Cilostazol	437±14	17.3±2.6	4.0±0.5
OLETF-Control	604±38†	61.6±7.8†	10.2±0.9†
OLETF-Cilostazol	611±29†	61.9±5.8†	10.1±0.8†

*b.w., body weight. †p<0.01 LETO vs. OLETF.

It has been demonstrated that some angiotensin converting enzyme (ACE) inhibitors [2,3], α_1-blockers [4,5] and slow-acting Ca^{2+} antagonist [6] have a beneficial effect on insulin sensitivity, although other studies showed neutral effects of ACE inhibitors on metabolism [5,7]. The precise mechanism is unclear but vasodilative effect and subsequent increase in blood flow to muscle and fat tissues is considered to be an important factor for ACE inhibitors and other antihypertensive drugs.

Cilostazol is a phosphodiesterase III inhibitor and is used in diabetic patients as an antiplatelet and antithrombotic drug [8–10]. This drug also increase blood flow and is expected to improve insulin sensitivity. However, the effect of phospodiesterase inhibitor, has not been studied. Therefore, we tested in hyperinsulinemic animal models weather cilostazol can improve insulin sensitivity or not.

Materials and Methods

Animals and Experimental Design

Male OLETF rats [11] were fed standard rat chow (Oriental Yeast, Tokyo, Japan) and tap water *ad libitum* until the age of 16 weeks, when they were randomly assigned to two groups of 10 rats each: those with normal diet (OLETF-Normal) and those with a cilostazol (40 mg/kg/day) supplemented diet (OLETF-Cilostazol group). The serum concentration of cilostazol in rats was about 1.5 μg/ml, which is equivalent to those of patients treated with cilostazol. Body weight and fasting blood glucose levels were not significantly different among groups at the beginning of the experiment. A non-diabetic rat strain (Long-Evans Tokushima Otsuka; LETO) was used as the age-matched control (LETO-Normal and LETO-Cilostazol groups, respectively).

Oral glucose test was performed at 23 weeks and hyperinsulinemic euglycemic clamp studies were performed at 25 weeks after an overnight fast. After hyperinsulinemic euglycemic clamp studies, rats were anaesthetized with sodium pentobarbital (50 mg/kg) and were killed by exsanguination. Blood samples were collected from the abdominal aorta for determination of serum levels of lipids and immunoreactive insulin. We also measured the amount of abdominal fats (mesenteric, epididymal, and retroperitoneal fats).

Hyperinsulinemic Euglycemic Clamp Studies

Insulin-mediated whole-body glucose uptake was determined in 25-week-old anaesthetized rats using a euglycemic insulin clamp. After an overnight fast, rats were anaesthetized by intraperitoneal injection of pentobarbital, and catheters were inserted in the carotid artery and veins. Rats received a 1-h infusion of insulin (60 pmol/kg^{-1}/min^{-1}). Blood glucose was measured every 5 min by hexokinase methods (Tidex, Sankyo, Tokyo, Japan) throughout the study. A glucose solution (100 g/l) was initiated at time 0, the rate was adjusted to maintain the plasma concentration of glucose at ≈6.1 mmol/l. The total body glucose uptake represents the mean glucose infusion rate (GIR) during the last 20 min.

Blood Chemicals

Serum levels of triglycerides, cholesterol and HDL-cholesterol were determined by conventional enzymatic methods (Wako, Osaka, Japan). The plasma level of glucose was determined by the glucose oxidase method (Toecho Super, Kyoto Daiichi Kagaku, Kyoto, Japan). The serum level of insulin was measured by a standard radioimmunoassay technique using a commercial kit (Eiken Kagaku, Tokyo, Japan) [12], with rat insulin used as the standard (Novo, Bagsvared, Denmark). Serum level of leptin was measured by radioimuunoassey (Otsuka Assay, Tokushima, Japan) [13].

Statistical Analysis

Data in the text are expressed as the mean ± s.d., and data in figures are expressed as the mean ± s.e. Data were analysed by two-way Anova followed by the Student's *t*-test. A p-level of < 0.05 was accepted as statistically significant.

Results

Body Weight and Abdominal Fat

The body weight was significantly greater in OLETF rats than age-matched control rats (table 1). However, there

Table 2 Effect of cilostazol on serum lipids

Group	TG(mmol/l)	TC(mmol/l)	HDL-C(mmol/l)
LETO-Control	0.56±0.18	2.58±0.12	1.72±0.10
LETO-Cilostazol	0.55±0.40	2.59±0.11	1.73±0.09
OLETF-Control	1.90±0.93*	4.21±1.64*	1.91±0.17*
OLETF-Cilostazol	1.75±0.66*	3.56±0.80*	2.06±0.13*

TG, triglyceride; TC, total cholesterol; HDL-C. *$p < 0.01$ LETO vs. OLETF.

were no significant differences in body weight between those with and without cilostazol treatment. The amounts of mesenteric, epididymal, and retroperitoneal fats were significantly increased in OLETF rats compared with non-diabetic rats. However, the total fat weight in the abdominal cavity was not significantly different between those with and without cilostazol treatment.

Blood Chemical Studies

Serum levels of triglycerides were significantly higher in OLETF rats than in nondiabetic rats (table 2). Cilostazol treatment slightly decreased serum levels of triglyceride and total cholesterol, but not significantly. At 16 and 25 weeks of age, the OLETF rats had significantly higher plasma levels of glucose than in non-diabetic rats.

Serum levels of leptin was markedly increased in OLETF rats (25.9 ± 8.1 ng/ml) than in LETO rats (4.6 ± 1.4 ng/ml, $p < 0.01$) However, cilostazol did not alter leptin in OLETF (25.8 ± 6.2 ng/ml) or LEFETO rats (3.2 ± 1.1 ng/ml), respectively (figure 1).

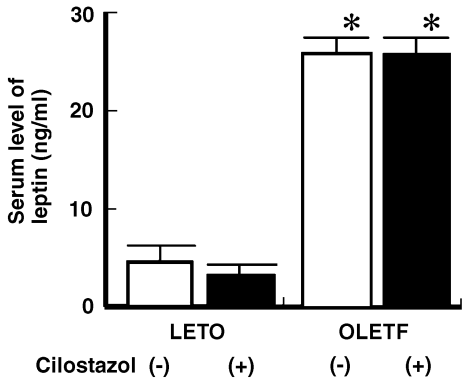

Fig. 1 Serum levels of leptin in OLETF and LETO rats and effects of cilostazol supplementation. The serum level of leptin was increased in OLETF rats, but not altered by cilostazol supplementation. *$p < 0.01$ OLETF vs. LETO.

Glucose Tolerance and In-Vivo Glucose Disposal

OGTT shows that OLETF rats showed higher blood glucose levels both before and 60 min after glucose. The fasting level of insulin was significantly higher in OLETF rats than in non-diabetic rats. Cilostazol did not alter blood levels of insulin or glucose in either OLETF or LETO rats (table 3). The GIR was decreased in OLETF rats compared with nondiabetic rats (figure 1). Cilostazol partially but significantly improved GIR in the OLETF rats, although not in the LETO rats.

Discussion

Accumulation of abdominal fats is one of the most important factors in developing insulin sensitivity in OLETF rats. In the present study, OLETF rats showed obesity, abdominal fat accumulation and impaired insulin sensitivity. Cilostazol improved significantly insulin sensitivity in OLETF rats, although it did not decrease the abdominal fat accumulation or serum levels of glucose.

Insulin resistance is generally interpreted as the physiological state under which insulin causes a reduced glucose-lowering effect. Hyperinsulinaemia is considered to be a result of insulin resistance. Male OLETF rats represent a genetic model for the spontaneous development of NIDDM. They show hyperphagia, obesity, hyperglycemia and insulin resistance at 16 weeks and most of the rats develop diabetes at 25 weeks [10,14]. Insulin resistance and impaired glucose tolerance are improved by various procedure, such as exercise training [12] and food restriction [15]. Food restriction and exercise training reduce abdominal fat accumulation and improves insulin sensitivity. There was a good correlation between fat accumulation and insulin sensitivity in OLETF rats [16]. However, in the present study, cilostazol improved insulin sensitivity without changing fat accumulation or serum lipids.

Cilostazol is now widely used as an anticoagulant but it also has a vasodilating action. Therefore,

Table 3 Oral glucose tolerance test

Group	Blood glucose (mmol/l)		Insulin (pmol/l)	
	0 min	60 min	0 min	60 min
LETO-Control	7.3±0.9	9.6±0.8	853±500	2875±1315
LETO-Cilostazol	7.4±0.7	9.4±1.0	641±467	2625±994
OLETF-Control	10.7±1.7*	17.4±7.0*	3034±840*	4215±656*
OLETF-Cilostazol	12.1±1.8*	21.6±2.1*	3211±1163*	3808±555*

$p < 0.01$ LETO vs. OLETF.

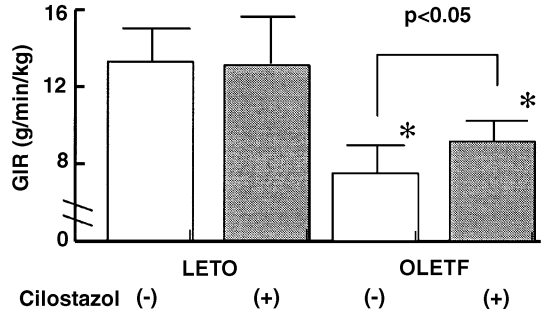

Fig. 2 Glucose infusion rate in OLETF and LETO rats and effects of cilostazol supplementation. The glucose infusion rate was decreased in OLETF rats comparered to those of LETO rats (*$p < 0.01$). Cilostazol supplementation improved glucose infusion rate in OLETF rats ($p < 0.05$) but not in LETO. *$p < 0.01$ OLETF vs. LETO.

improvement of insulin sensitivity might be due to increased blood flow. Hotta *et al.* [17] reported that cilostazol prevented the development of diabetic neuropathy by modifying vascular factors. They found, however, that cilostazol had no effects on the control of blood glucose and lipids in OLETF rats, although they did not study insulin sensitivity. In our study we found that cilostazol did not decrease glucose level but improved insulin resistance or abdominal fat accumulation.

As there have been no studies on relation among abdominal fat accumulation, triglyceride and insulin resistance during treatment of antihypertensive drugs, precise mechanism of improvement of insulin resistance by these drugs is not clear. The discrepancies between insulin resistance and blood levels of glucose and triglyceride might not be uncommon finding with other antihypertensives [18]. To improve serum lipids and glucose, additional factors must be needed. Another possibility could be that the experiement was too short to show the difference, as the improvement of insulin resistance was slight. In addition, the design was not set up to have proper placebo control groups. In the present study, those rats on cilostazol supplementation tended to gain more weight than those without cilostazol. This factor might counteract the effect of insulin sensitivity.

Muller *et al.* [19] reported that leptin impairs insulin action in adipose tissue. We also found that the serum level of leptin was markedly increased in OLETF rats and showed good correlation with insulin sensitivity. However, cilostazol did not alter the serum level of leptin. Therefore, the insulin resistance was not improved by this pathway in case of cilostazol.

Cilostazol is an inhibitor of phosphodiesterase III and increases cyclic AMP (cAMP) in platelets and also raises the vascular smooth muscle cell cAMP level causing vasodilation. It is also possible that it might have a direct effect on increased intracellular cAMP. There are several studies on the relation between the cellular cAMP levels and insulin sensitivity or its action. Parker *et al.* [20] also reported that selective inhibition of PDE III stimulated insulin secretion. The effects of type-selective inhibitors on PDE activity and insulin secretion were similar in human and rat islets. In the present study, we did not find increased insulin levels in cilostazol supplemented rats. In addition, Wesselau *et al.* [21] reported that elevating the cAMP levels impaired insulin sensitivity. These studies suggest that the improvement of insulin resistance by cilostazol is unlikely due to cAMP itself.

In our study, cilostazol improved insulin sensitivity in OLETF rats but not in LETTO, non-diabetic rats. However, the improvement in insulin sensitivity is not due to the reduction of abdominal fat accumulation. These results indicate that cilostazol seemed to have a beneficial effect on insulin sensitivity at least in patients with NIDDM.

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