Cilostazol, a selective type III phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats

Takeshi Tani a, Kenji Uehara b,*, Toshiki Sudo b, Keiko Marukawa b, Yoshinobu Yasuda a, Yukio Kimura b

a Tokushima Research Institute, Otsuka Pharmaceutical Co. Ltd., 463-10 Kagasuno Kawauchi-cho, Tokushima, 771-0192 Japan
b Thrombosis and Vascular Research Laboratory, Department of Advanced Pharmacology, Otsuka Pharmaceutical Co. Ltd., 463-10 Kagasuno, Kawauchi-cho, Tokushima, 771-0192 Japan

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Abstract

Cilostazol, a selective type III phosphodiesterase inhibitor, has antiplatelet and vasodilating effects. In this study, the effects of cilostazol on lipid metabolism and lipoprotein lipase (LPL) activity were studied in rats. Cilostazol was administered orally at doses of 30 or 100 mg/kg twice a day for 1–2 weeks to rats. Cilostazol decreased the serum triglyceride level in normolipidemic rats. The serum triglyceride level was reduced and HDL cholesterol level was increased by cilostazol in streptozotocin (STZ)-induced diabetic rats. The disappearance of exogenous triglyceride was accelerated by cilostazol in normolipidemic rats. Cilostazol increased post-heparin plasma LPL activity but had no effect on hepatic triglyceride lipase activity in STZ-induced diabetic rats. Cilostazol also increased LPL activity in the heart in STZ-induced diabetic rats. These findings suggest that an increase in LPL activity is responsible for the serum triglyceride lowering and HDL cholesterol elevating effects of cilostazol in rats. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cilostazol; Lipoprotein lipase; Triglyceride; HDL cholesterol

1. Introduction

Abnormal lipid metabolism has been extensively studied as a risk factor for atherosclerosis. Increasing evidence of an association between serum triglyceride levels and the incidence of coronary heart disease (CHD) has been obtained [1]. Chylomicrons and very low density lipoproteins, which are triglyceride-rich lipoproteins, are released from the intestine and liver into the circulation. These particles are metabolized to smaller lipoproteins, and in this process triglycerides are hydrolyzed by lipoprotein lipase (LPL). LPL activity is also responsible for the production of high density lipoproteins (HDL), anti-atherogenic lipoproteins [2,3]. Decreased LPL activity results in lipid abnormalities such as hypertriglyceridemia and hypo HDL cholesterolemia [4]. One of the major causes of hypertriglyceridemia with low HDL cholesterol level in diabetes is a decrease in LPL activity due to insulin deficiency [5].

Cyclic nucleotide phosphodiesterases (PDEs) regulate various physiological responses in many cells and tissues, including platelet aggregation [6,7], vascular relaxation [8,9], and cardiac muscle contraction [10,11]. Since PDEs exist in multiple forms in cells and tissues and each form of PDE acts in a complex manner in these responses, selective PDE inhibitors are expected to be useful for the treatment of various diseases [12,13]. Cilostazol [14,15], one such drug, can be considered the first representative of a new family of antithrombotic agents which act by selective inhibition of one of seven PDE families, the cGMP-inhibited cAMP PDEs (PDE type III) [16].

Since LPL activity has been reported to be affected by cAMP, we examined the effect of cilostazol on lipid...
metabolism and LPL activity in normolipidemic and streptozotocin (STZ)-induced diabetic rats.

2. Materials and methods

2.1. Animal experiments

Seven- to nine-week-old Wistar rats (Japan Clea or Japan SLC) were used. Diabetes was induced in fasted rats by intravenous injection of 40 or 60 mg/kg of streptozotocin (STZ) (Wako, Osaka, Japan). Cilostazol was suspended in 2% arabic gum (Wako)—tap water and administered orally at 30 or 100 mg/kg twice a day for 1–2 weeks. Control rats were administered 5 ml/kg of 2% arabic gum orally twice a day for 1–2 weeks. Rats were fasted overnight, and 2 h after final administration of the drug or arabic gum all analytical measurements were performed. Blood samples were collected from the tail vein or vena cava, and the serum and plasma separated by centrifugation were used for measurement of lipid levels, half-life of exogenous triglyceride, and LPL activity, as described below.

In this study, the following effects of cilostazol on lipid metabolism in rats were examined: (1) effect of the drug on the serum lipid levels in normolipidemic rats and STZ-induced diabetic rats, (2) effect of the drug on the disappearance of exogenous triglyceride in normolipidemic rats, (3) effect of the drug on LPL activity in post-heparin plasma in STZ-induced diabetic rats, and (4) effect of the drug on heart LPL activity in STZ-induced diabetic rats. Male and female normolipidemic rats were used in (1), while in others male rats were used.

2.2. Analytical measurements

2.2.1. Lipid and glucose levels

Serum triglyceride and cholesterol levels were assayed enzymatically using the Wako triglyceride G test (Wako) and the Wako cholesterol CII test (Wako). The HDL was separated by the phosphotungstic acid–magnesium precipitation method using an HDL-precipitation kit (Wako). Plasma glucose level was measured using the Wako glucose B test (Wako).

2.2.2. Disappearance of exogenous triglyceride

Triglyceride emulsion (20% Intralipid, Kbbi, Pharmacia AB, Uppsala, Sweden) was injected into the tail vein of rats at a dose of 1 ml/kg under ether anesthesia. Blood was collected before and 5, 10, 15 and 20 min after injection of triglyceride emulsion and the serum triglyceride level was measured. The half-life was calculated by logarithmic regression.

2.2.3. LPL activity in post-heparin plasma

For the measurement of LPL activity in post-heparin plasma, 200 U/kg of heparin (Novoheparin, Novoindustry, Bajsvaeld, Denmark) was injected into the femoral vein of rats under pentobarbital anesthesia. Ten minutes later, blood was collected with EDTA as an anticoagulant and the plasma was immediately separated by centrifugation. LPL activity in post-heparin plasma was assayed as described by Murase et al. [17]. Total lipase and LPL activity were measured using glycerol tri[1-14C]oleate (Amersham, UK), and triolein (Sigma, St Louis, MO) as substrates. Hepatic triglyceride lipase (HTGL) activity in plasma was selectively blocked by treatment with the antiserum to rat hepatic triglyceride lipase. Plasma was incubated with substrate and rat serum as a source of coenzyme apoprotein CII, in a final volume of 1 ml at 37°C for 30 min. Enzymatic reaction was stopped by the addition of 3 ml of 0.6 N H₂SO₄—isopropanol (1:4, v/v). The free fatty acid (FFA) released during incubation was extracted and the radioactivity of FFA was determined by the method of Schotz et al. [18] with minor modifications. Enzyme activity was expressed as μmol FFA hydrolyzed/ml per h. HTGL activity was calculated by subtracting LPL activity from total lipase activity.

2.2.4. Heart LPL activity

The heart was dissected after rats were sacrificed by exsanguination under ether anesthesia, and homogenized in 200 mg/ml of 50 mM ammonium–HCl buffer (pH 8.5) containing 0.5 U/ml of heparin. The homogenate was left to stand at 4°C for 1 h and centrifuged at 10 000 rev./min for 20 min. The lipolytic activity of the supernatant was assayed based on the method of post-heparin plasma LPL activity. Enzyme activity was expressed as nmol FFA hydrolyzed/min per g tissue.

2.3. Statistical analysis

Values are expressed as means ± S.D. Statistical comparisons were made between normal and diabetic groups using the unpaired Student’s t-test (two-tailed), and between control and cilostazol-treated groups using one-way analysis of variance, followed by the two-tailed Dunnett test. The level of significance was set at P < 0.05.

3. Results

3.1. Serum lipid levels in normolipidemic rats

Cilostazol decreased serum triglyceride level slightly but significantly at doses of 30 and 100 mg/kg in male normolipidemic rats (P < 0.05, each case) (Table 1).
Cilostazol had no effect on serum total cholesterol or HDL cholesterol levels in male rats. In female rats, cilostazol significantly reduced the serum triglyceride level at doses of 30 and 100 mg/kg ($P < 0.05$, each case) (Table 2). The change in triglyceride level in female rats (29% decrease) was greater than that in male rats (16% decrease). HDL cholesterol levels were increased by 20% in both the 30 and 100 mg/kg cilostazol-treated groups ($P < 0.01$), and total cholesterol levels were increased by 16 and 19% at 30 and 100 mg/kg, respectively, ($P < 0.05$ for 30 mg/kg and $P < 0.01$ for 100 mg/kg).

### 3.2. Serum lipid levels in STZ-induced diabetic rats

STZ-induced diabetic rats clearly exhibited hyperglycemia and hypertriglyceridemia compared with normal rats (Table 3). Cilostazol did not alter glucose levels but did decrease the triglyceride levels significantly at a dose of 100 mg/kg ($P < 0.05$). Cilostazol increased HDL cholesterol level significantly and in a dose-dependent fashion ($P < 0.05$ for 30 mg/kg and $P < 0.01$ for 100 mg/kg), but did not affect the increased total cholesterol level.

### 3.3. Disappearance of exogenous triglyceride in normolipidemic rats

The half-life of exogenous triglyceride was 20.6 min in control rats (Fig. 1). Cilostazol significantly decreased this half-life in a dose-dependent fashion by 30 and 37% ($P < 0.05$, each case).

### 3.4. LPL activity in post-heparin plasma in STZ-induced diabetic rats

Cilostazol significantly increased LPL activity in post-heparin plasma at a dose of 100 mg/kg ($P < 0.01$), and slightly increased LPL activity in post-heparin plasma at a dose of 30 mg/kg. Cilostazol did not significantly affect HTGL activity (Fig. 2).

### Table 1

Effect of cilostazol on serum lipid levels in male normolipidemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Serum lipids (mg/dl)</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Triglyceride</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>83 ± 11</td>
<td>78 ± 12</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>10</td>
<td>71 ± 13*</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>(30 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cilostazol</td>
<td>10</td>
<td>69 ± 10*</td>
<td>72 ± 11</td>
</tr>
<tr>
<td>(100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| * Values are expressed as means ± S.D.  
| * Significantly different from the respective values in control rats, $P < 0.05$.     |

### Table 2

Effect of cilostazol on serum lipid levels in female normolipidemic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Serum lipids (mg/dl)</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Triglyceride</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>70 ± 23</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>Cilostazol</td>
<td>10</td>
<td>49 ± 10*</td>
<td>66 ± 8*</td>
</tr>
<tr>
<td>(30 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cilostazol</td>
<td>10</td>
<td>50 ± 12*</td>
<td>68 ± 8**</td>
</tr>
<tr>
<td>(100 mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| * Values are expressed as means ± S.D.  
| * Significantly different from the respective values in control rats, $P < 0.05$.  
| ** Significantly different from the respective values in control rats, $P < 0.01$.     |

### Table 3

Effect of cilostazol on serum lipid levels in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>$n$</th>
<th>Plasma glucose (mg/dl)</th>
<th>Serum lipids (mg/dl)</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Triglyceride</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Normal rats</td>
<td>8</td>
<td>105 ± 8***</td>
<td>101 ± 15***</td>
<td>61 ± 5***</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>582 ± 37</td>
<td>380 ± 132</td>
<td>133 ± 40</td>
</tr>
<tr>
<td>Cilostazol (30 mg/kg)</td>
<td>9</td>
<td>564 ± 26</td>
<td>377 ± 102</td>
<td>133 ± 38</td>
</tr>
<tr>
<td>Cilostazol (100 mg/kg)</td>
<td>9</td>
<td>584 ± 19</td>
<td>214 ± 82*</td>
<td>120 ± 30</td>
</tr>
</tbody>
</table>
| * Values are expressed as means ± S.D.  
| * Significantly different from the respective values in diabetic control rats, $P < 0.05$.  
| ** Significantly different from the respective values in diabetic control rats, $P < 0.01$.     
| *** Significantly different from the respective values in diabetic control rats, $P < 0.001$.
3.5. Heart LPL activity in STZ-induced diabetic rats

Heart LPL activity was markedly decreased in STZ-induced diabetic rats compared with normal rats ($P < 0.001$) (Fig. 3). Cilostazol significantly increased heart LPL activity at a dose of $100 \text{ mg/kg}$ ($P < 0.01$), but at a dose of $30 \text{ mg/kg}$ had no effect on this activity.

4. Discussion

Antiplatelet agents have been shown to be effective in preventing CHD and/or cerebral stroke as well as anti-hypertensive and lipid-lowering agents [19,20]. It is important that an agent selected for prevention of vascular disease has either no or a favorable side-effect profile for other risk factors. Many clinical studies of the effects of antihypertensive agents on lipid metabolism have been carried out, and beta-blockers [21,22] and thiazide diuretics [23,24] have been shown to have adverse effects on lipid metabolism (i.e. inducing increases in triglyceride, increases in total cholesterol, and/or decreases in HDL cholesterol). However, the effects of antiplatelet agents on lipid metabolism have not been extensively studied. In this study, we showed that cilostazol significantly decreased serum triglyceride level and increased HDL cholesterol level in rats, and we also proposed a mechanism for the effects of this drug on lipid metabolism. In patients with intermittent claudication, cilostazol has been shown to improve walking distance with concomitant beneficial effects on plasma lipids (i.e. decreasing triglyceride and increasing HDL cholesterol) [25,26].
Serum triglyceride concentrations reflect the balance between triglyceride secretion into and triglyceride removal from the circulation. Maeda et al. previously studied the effect of cilostazol on triglyceride secretion from the liver using Triton WR-1339 in rats, and found that the rate of triglyceride secretion was decreased after cilostazol treatment [27]. However, plasma triglyceride levels were not significantly changed by cilostazol, and HDL cholesterol levels were not measured in that study. Therefore, it was not clear whether the alteration of plasma triglyceride level is caused by the reduced triglyceride secretion rate. In the present study, cilostazol significantly decreased serum triglyceride levels in male normolipidemic rats and concomitantly increased HDL cholesterol levels in female normolipidemic rats and male STZ-induced diabetic rats. It is known that a decrease in VLDL triglyceride itself increases HDL cholesterol by decreasing the triglyceride–cholesterol ester exchange activity of cholesterol ester transfer protein (CETP) in humans [28]. However, in rats lacking CETP [29], decrease in VLDL triglyceride secretion from the liver does not increase HDL cholesterol. Thus, a mechanism other than the effect of cilostazol on triglyceride secretion has to be considered for the serum triglyceride-decreasing and HDL cholesterol-elevating effects of the drug in rats. In this study, we focused on whether the drug enhances the triglyceride removal from the circulation. In an experiment using male normolipidemic rats, cilostazol significantly decreased the half-life of exogenous triglyceride after intravenous injection of Intralipid, suggesting that the drug increases triglyceride removal from the circulation. In addition, cilostazol significantly increased post-heparin plasma LPL activity in STZ-induced diabetic rats. Thus, the most likely mechanism by which cilostazol decreases serum triglyceride appeared to involve its effect on LPL, an enzyme playing a central role in the catabolism of triglyceride-rich lipoproteins.

Plasma HDL originates from three sources: the small intestine, liver, and LPL-mediated lipolysis of triglyceride-rich lipoproteins. Although the production of HDL by the liver and small intestine may be important in determining the plasma HDL cholesterol level, LPL also contributes to the determination of plasma HDL cholesterol levels. The increase in HDL cholesterol level induced by cilostazol might thus be the result of an increased degradation of triglyceride-rich lipoproteins by LPL.

Metabolism of cilostazol is slow in female rats, and the serum concentration of this drug is about 10-fold higher than that found in male rats [30]. Thus it appears that the effect of the drug is more pronounced in female rats. In fact, although the serum triglyceride levels were decreased by 14% with a dose of 30 mg/kg of the drug in male normolipidemic rats, the level was decreased by 30% in female normolipidemic rats. Cilostazol concomitantly increased serum HDL cholesterol levels in female rats, but affected only serum triglyceride levels in male rats. In female rats, cilostazol also increased the serum total cholesterol level, reflecting an increase in serum HDL cholesterol level, and resulting in a decrease in the ratio of non HDL cholesterol/HDL cholesterol. The same phenomenon, producing increases in both HDL cholesterol and total cholesterol levels, was observed in a study of a recently-developed compound, NO-1886, which specifically increases LPL activity [31].

Insulin upregulates LPL activity [32], and therefore in diabetes, low LPL activity results in lipid abnormalities such as hypertriglyceridemia, particularly in insulin-dependent diabetes mellitus [33]. In fact, in STZ-induced diabetic rats, which is an insulin-dependent diabetic animal model, the serum triglyceride level was markedly increased and heart LPL activity was approximately 36% of that in normolipidemic rats. On the other hand, many investigators have shown that LPL activity is also affected by cAMP [34,35]. In studies using db-cAMP, which mimics cAMP, and agents which increase cAMP level such as IBMX, forskolin and cholera toxin, an increase in LPL activity and a comparable increase in LPL mRNA were observed in rat heart cells [36] and in a neonatal mouse hepatoma cell line [37]. Since type III PDE exists in heart, liver and adipocytes [38], cilostazol may be expected to increase cAMP levels in such tissues. In this fashion, the drug affects tissue LPL activity. With regard to the effect of cilostazol, a decrease in serum triglyceride is more pronounced in male STZ-induced diabetic rats than that in male normolipidemic rats and an increase in HDL cholesterol is only observed in STZ-induced diabetic rats. The difference in the effect of cilostazol on serum lipid levels in male normolipidemic and STZ-induced diabetic rats may be related to the LPL activity and insulin level in those rats. In STZ-induced diabetic rats, tissue cAMP levels may be a dominant regulator of LPL activity because insulin, a regulator of LPL activity, is depleted in these rats. Thus the potency of cilostazol on lipid metabolism may be more clearly detected in male STZ-induced diabetic rats than in male normolipidemic rats.

LPL mainly exist in tissues such as adipose tissue, skeletal muscle and heart, and LPL activity in post-heparin plasma reflects the whole enzyme activity released in blood from those tissues. In the present study, cilostazol significantly increased LPL activity in both post-heparin plasma and heart tissue in STZ-induced diabetic rats. However, in contrast to adipose tissue and skeletal muscle, heart LPL activity accounts for only a small fraction of total LPL activity and makes a quantitatively minor contribution to the catabolism of plasma triglyceride-rich lipoproteins. In future studies, the effect of this drug on LPL in adipose tissue and...
skeletal muscle needs to be examined. It may be important to clarify the effect of other type III PDE inhibitors on LPL activity and LPL mRNA levels because the effect of cilostazol on LPL found in this study may be a common action of type III PDE inhibitors.

In summary, we found that cilostazol increased LPL activity, resulting in a decrease in serum triglyceride levels and an increase in HDL cholesterol levels. In addition to their antiplatelet and inotropic effects [39], in this study type III PDE inhibitors were shown to have other important effects. Compounds such as cilostazol may be useful to prevent vascular lesions by ameliorating both platelet hyperactivity and lipid abnormalities.

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References

in the coronary arteries of rats with experimental atherosclerosis. 