Recovery of Platelet Function after Withdrawal of Cilostazol Administered Orally for a Long Period

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To clarify the recovery of platelet function after abrupt withdrawal of cilostazol, we studied platelet function and cilostazol concentration in elderly who received cilostazol, 100 mg twice a day (200 mg/day), for a long period. After interviewing the time of final cilostazol intake, platelet aggregability was determined with an aggregometer using four different concentrations of adenosine-5'-diphosphate as an inducer, which showed the grading curve (GC) type and platelet aggregatory threshold index (P ATI). Serum cilostazol concentration was also determined by high-performance liquid chromatography. The GC type and PATI showed suppressed platelet function until 15 hours after withdrawal in half of patients. Bleeding time measured by the Simplate method was prolonged within 4 hours, but recovered by 12 hours after the withdrawal. Some serum cilostazol concentrations were still high 15 hours after withdrawal, while platelets were inhibited even in patients with low serum concentration of cilostazol. In the group receiving the drug for less than 6 months, PATI correlated with serum cilostazol concentration, but platelets in the long-term administration group (more than 48 months) were suppressed at the low serum cilostazol concentration. These findings indicated that platelet function recovered within 12–16 hours after withdrawal in these patients. J Atheroscler Thromb, 2003; 10: 348–354.

Key words: Platelet aggregation, Bleeding time, Concentration, Atherothrombotic disease

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Received July 9, 2003.
Accepted for publication October 2, 2003.

Introduction

Antiplatelet therapy is common in elderly patients with a variety of atherothrombotic diseases to prevent recurrence (1–6). In practice, aspirin is used as an antiplatelet therapy, based on evidence of effectiveness and safety in prevention (1–3). It is well known that aspirin inactivates platelets permanently by blocking an arachidonic acid cascade within the cell (1). For this reason, discontinuation of antiplatelet drugs is usually needed at least 1 week before operation or biopsy to avoid bleeding complications (7). However, since it also seems to be dangerous to interrupt antiplatelet therapy in these patients, standard management of these patients has not yet been established. It might be useful to manage these patients using cilostazol, a type III phosphodiesterase inhibitor, since it inactivates platelets reversibly in a concentration-dependent manner (8, 9).

The antiplatelet effect of cilostazol on treatment and prevention in patients with intermittent claudication or stroke has also been verified in clinical trials (4, 5). Furthermore, additional effects of cilostazol on the metabolism of high-density lipoprotein cholesterol, vascular endothelial function, and bradycardiac arrhythmias have been reported (10–12). It is known that platelet function recovers completely 48 hours after withdrawal of cilostazol (8), but details concerning the period up to 48 hours remain unclear, even in patients who have received cilostazol for a long period. Thus, to clarify changes of platelet function within the 36 hours after withdrawal of cilostazol, we studied platelet function and serum concentration of cilostazol in elderly patients with a variety
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 Subjects

A total of 14 patients aged 65 or older with a variety of atherothrombotic diseases, who had been receiving 100 mg of cilostazol orally twice a day (200 mg/day) for more than 2 months, but most of whom for the reasons given below had not taken it, were enrolled in this study (Table 1). They had never received any other antiplatelet drugs. Blood samples were obtained to determine platelet aggregability and serum cilostazol concentration after the interview on the time of final cilostazol intake, and 23 of 31 samples were obtained between 12 and 36 hours after the final intake. One patient (No. 4) was admitted for biopsy of the gastric mucosa (4 consecutive samples); 11 patients had not taken cilostazol because of scheduled determination of fasting blood glucose or serum lipid (25 samples, which consisted of 2 consecutive samples and 23 repeated samples at intervals of 3 months or more); and 2 patients had forgotten to take cilostazol on the morning of their examination (2 samples). All the subjects gave fully informed consent to participate in the study after receiving an explanation of the purpose, design and procedures of this study. This study was performed in accord with the Helsinki Declaration of 1975 as revised in 1983.

 Methods

Blood was collected with a 21-gauge needle from the antecubital vein, and immediately combined with a 1/10 volume of 3.8% sodium citrate. It was then centrifuged at 180 x g for 10 min to separate the supernatant as platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was collected as the supernatant after centrifugation of the sediment at 2,000 x g for 15 min. The platelet count in the PRP was adjusted to 20 to 30 x 10^4/µl. Platelet aggregability was determined turbidimetrically according to the method of Born and Cross (13, 14) with an aggregometer PAM-8T (Mebanics Inc., Tokyo, Japan) and adenosine-5’-diphosphate (Sigma Chemicals, St. Louis, MO, USA) as inducers of aggregation.

Using an aggregometer and ADP at four different concentrations (final concentrations of 0.5, 1.0, 2.0, and 4.0 µmol/l) as an agonist, grading curve (GC) type and platelet aggregatory threshold index (PATI) were calculated on the basis of data at 5 minutes for each ADP concentration (15, 16). GC type, consisting of six grades from +3 to −2, was evaluated in a programmed manner by connecting four plotted points of ADP concentration (µmol/l) at the point corresponding to the maximum aggregatory rate on the grading curve. A GC type −2 or −1 indicated suppressed platelet aggregability, while a GC type 0 or +

Table 1. Characteristics of Subjects.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Chief diseases</th>
<th>Risk factors</th>
<th>Tx period</th>
<th>No. of exams</th>
<th>Plt. count (10^4/µl)</th>
<th>Other Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>65</td>
<td>M</td>
<td>CVD, PAD</td>
<td>+ ++ + +</td>
<td>48 m</td>
<td>2</td>
<td>19, 24</td>
<td>ACE-I, CCB, SU</td>
</tr>
<tr>
<td>2.</td>
<td>71</td>
<td>M</td>
<td>PAD</td>
<td>+ + − −</td>
<td>114 m</td>
<td>3</td>
<td>27–32</td>
<td>CCB, SU</td>
</tr>
<tr>
<td>3.</td>
<td>73</td>
<td>M</td>
<td>CVD, AA</td>
<td>+ + − +</td>
<td>24 m</td>
<td>2</td>
<td>22, 24</td>
<td>CCB</td>
</tr>
<tr>
<td>4.</td>
<td>74</td>
<td>M</td>
<td>PAD, ICst</td>
<td>− − − +</td>
<td>5 m</td>
<td>4</td>
<td>21–24</td>
<td>SU</td>
</tr>
<tr>
<td>5.</td>
<td>75</td>
<td>M</td>
<td>CVD, PAD, ICst</td>
<td>+ + + +</td>
<td>5 m</td>
<td>4</td>
<td>16–21</td>
<td>Statin</td>
</tr>
<tr>
<td>6.</td>
<td>76</td>
<td>M</td>
<td>CVD, ICst</td>
<td>+ + − +</td>
<td>36 m</td>
<td>2</td>
<td>9, 9</td>
<td>SU</td>
</tr>
<tr>
<td>7.</td>
<td>80</td>
<td>M</td>
<td>CVD, PAD, ICst</td>
<td>+ − + +</td>
<td>120 m</td>
<td>2</td>
<td>24, 26</td>
<td>ARB, alphaB, Statin</td>
</tr>
<tr>
<td>8.</td>
<td>85</td>
<td>M</td>
<td>PAD</td>
<td>− + − −</td>
<td>60 m</td>
<td>2</td>
<td>19, 19</td>
<td>betaB, SU</td>
</tr>
<tr>
<td>9.</td>
<td>88</td>
<td>M</td>
<td>ICst</td>
<td>+ − − +</td>
<td>41 m</td>
<td>2</td>
<td>22, 24</td>
<td>ARB, Diuretics</td>
</tr>
<tr>
<td>10.</td>
<td>88</td>
<td>M</td>
<td>PAD</td>
<td>+ + − −</td>
<td>21 m</td>
<td>3</td>
<td>23–24</td>
<td>CCB, SU, Statin</td>
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<td>11.</td>
<td>69</td>
<td>F</td>
<td>PAD</td>
<td>+ − − −</td>
<td>41 m</td>
<td>2</td>
<td>19, 22</td>
<td>ARB</td>
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<td>12.</td>
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<td>F</td>
<td>CVD</td>
<td>+ − + −</td>
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<td>1</td>
<td>21</td>
<td>Statin</td>
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<td>13.</td>
<td>79</td>
<td>F</td>
<td>ICst</td>
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<td>2 m</td>
<td>1</td>
<td>22</td>
<td>ACE-I, CCB, Diuretics</td>
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<td>14.</td>
<td>89</td>
<td>F</td>
<td>CVD, PAD</td>
<td>− + + −</td>
<td>36 m</td>
<td>1</td>
<td>23</td>
<td>Statin</td>
</tr>
</tbody>
</table>

1 indicated normal platelet aggregability (14). PATI, obtained from the grading curve, was defined as the ADP concentration (µmol/l) at the point corresponding to half the maximum aggregatory rate on the grading curve (14). These procedures were completed within 3 hours after blood sampling.

**Determination of bleeding time**

After venous puncture, bleeding time was determined by the Simplate method (17, 18). Bleeding time was defined as the time to reach a small fleck less than 1 mm in diameter on a filter paper every 30 seconds after incision (5 mm in width and 1 mm in depth) on the arm while adding pressure of 40 mmHg by mercury sphygmomanometer. Normal bleeding time was defined as between 2 and 9 min.

**Determination of serum cilostazol concentration**

Blood samples were simultaneously prepared for the determination of serum cilostazol concentration. Blood was centrifuged at 2000 × g for 15 min, and serum was stored at −4°C in a refrigerator. Cilostazol in the serum was measured by high-performance liquid chromatography (HPLC) at a commercial laboratory (BML Inc., Tokyo, Japan) (19). Briefly, cilostazol (OPC-13013) and the internal standard (OPC-13012) were supplied by Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan), and sample preparation was performed with 500 µl of serum samples, 50 µl of internal standard, and 25 µl of methanol on a Chem Elute (VARIAN Inc., Tokyo, Japan). After extracting the sample by evaporation with chloroform and methanol, each sample solution was injected into the HPLC system (Hitachi, Tokyo, Japan), and dissolved with a concentration gradient of acetonitrile at 50°C using a 4.6 × 250 mm column. The calibration curve was obtained in the mobile phase at a flow-rate of 1.0 ml/min. and the concentration of cilostazol was measured using 254-nm ultraviolet light with reference to that of the internal standard on the basis of the standard curve.

**Statistical analysis**

The results were divided into three groups according to the period of cilostazol administration: the short-period group (< 6 months, n = 7), the relatively long-period group (6–48 months, n = 9, middle group), and the long-period group (> 48 months, n = 15). Using StatView (SAS Institute Inc., Cary, NC, USA), the frequency of suppressed GC types among each examination period from final intake of cilostazol was studied by Fisher’s exact probability test. For pharmacodynamic and pharmacokinetic studies, polynomial regression analysis was used, since cilostazol was rapidly absorbed, and reached peak plasma levels at 3 h (22). For comparisons between platelet function test and serum cilostazol concentration, simple regression analysis was performed separately in the long- and short-period groups. A p-value of less than 0.05 was considered to indicate statistical significance.

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**Fig. 1.** Changes of ADP-induced platelet aggregability.
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Results

Characteristics of subjects
In the 14 patients with chronic atherothrombotic diseases, peripheral arterial disease, cerebral infarction, and carotid artery stenosis were seen in 9, 8, and 6, respectively (Table 1). Cilostazol was administered for periods varying from 2 months (No. 13) to 126 months (No. 7, examined twice at the period of 120 and 126 months). Most of the patients had multiple risk factors, and received a variety of medication, including antihypertensive agents, antidiabetic drugs, and statins.

Changes of platelet function
Pharmacodynamic studies on the basis of the GC type and PATI showed both non-linear correlations to time (in the polynomial regression analysis for PATI, a PATI value of over 4 was calculated as that of 4). The GC type also showed that both normal (n = 4) and suppressed (n = 4) types were seen 15 hours after withdrawal of cilostazol (Fig. 1a). After 16 h from the withdrawal of cilostazol (n = 8), there were no suppressed types. The difference in frequency of suppressed GC type between 15 and 16 hours after the withdrawal was significant. In one patient (No. 4), the GC type as well as the PATI recovered to normal between 12 and 24 hours after withdrawal (Fig. 1b).

Bleeding time, which decreased non-linearly with time, was prolonged in a half of 8 patients within 4 hours after withdrawal of cilostazol, and recovered to normal at 12 hours after withdrawal (Fig. 2).

Changes of serum cilostazol concentration
A pharmacokinetic study showed that serum cilostazol concentration decreased non-linearly with time after withdrawal of cilostazol, but a high concentration far from the polynomial regression curve was seen in 3 out of 20 samples, even 16 hours after withdrawal (Fig. 3).

Figure 2. Changes of bleeding time (Simplate method) after withdrawal of 200-mg daily intake of cilostazol.
The symbols of ●, ■, and ▲ indicate the groups of treatment period > 48 months, 48–6 months, and < 6 months, respectively. The symbols of △ and □, included in those of ▲ and ■, respectively, are mean samples, which were determined consecutively in each patient (No. 4 and No. 10 in Table 1). The dotted line indicates the upper limit of bleeding time (9 min) in normal healthy subjects. The curve indicates a polynomial regression curve.

Figure 3. Changes of serum concentration after withdrawal of 200-mg daily intake of cilostazol.
The symbols of ●, ■, and ▲ indicate the groups of treatment period > 48 months, 48–6 months, and < 6 months, respectively. The symbols of △ and □, included in those of ▲ and ■, respectively, are mean samples, which were determined consecutively in each patient (No. 4 and No. 10 in Table 1). The curve indicates a polynomial regression curve.

Relationship between platelet function and serum cilostazol concentration
Suppressed platelet function with a PATI value of more than 4.0 was seen in a wide range of serum cilostazol concentrations, and platelet function was not strongly suppressed even in patients with high serum concentrations of cilostazol (Fig. 4). However, the short-period group (A) showed suppressed platelet function correlated significantly with serum cilostazol concentration, while the long-period group (C) showed suppressed platelet function even in patients with low serum concentrations of cilostazol ($R^2$, 0.01; $p = 0.7870$). In the middle group (B), there was no consistent relationship between platelet function and serum cilostazol concentration ($R^2$, 0.397; $p = 0.1802$).

Discussion
A variety of platelet function tests have been available recently for clinical use, but there is no uniformly accepted test to evaluate platelet function (13–18). We used an aggregometer and ADP at four different concentrations, which automatically provided the GC type and PATI values (16, 17). Both parameters were useful to determine...
platelet aggregability: GC type consisted of 6 types, from GC type – 2 (severely suppressed) to that of + 3 (severely accelerated); PATI was employed as a theoretical and convenient threshold to indicate the start of platelet aggregation (15). On the other hand, bleeding time has different implications from the platelet aggregability test, and it seems to evaluate total function in vivo in the thrombosis-stasis system including platelets, coagulation factors, and fibrinolytic factors (17). Dissociation between platelet aggregability and bleeding time had been shown in previous reports (20), in which platelet adhesiveness was suppressed at cilostazol intake of 300 mg a day without causing prolongation of bleeding time.

It is well known that cilostazol inhibits not only secondary platelet aggregation but also primary platelet aggregation induced by ADP, as well as collagen, epinephrine, and arachidoic acid (8, 14, 21–23). Cilostazol is a synthetic compound, potent enough to cause a 50% reduction of aggregation induced by ADP at a concentration of 13 µmol/l in in vitro experiments using human platelets (21). The effect of the drug, examined after four weeks of treatment with cilostazol at 100 mg twice a day, demonstrated significant reduction of the aggregation rate in ADP-induced aggregation, and complete recovery 48 hours after drug withdrawal (8). However, the recovery of platelet function was unclear between 8 h to 48 h after withdrawal of cilostazol.

According to the ADP-induced platelet aggregability test (GC type), our study showed recovery of platelet function at 16 h after withdrawal of 100 mg twice a day (200 mg/day) of cilostazol given for a long-period. Bleeding time showed recovery at 12 h after withdrawal. Our results within 36 h after drug withdrawal provided additional detailed information, and there was neither a detectable drug effect nor a rebound phenomenon at 36 h after withdrawal. The bleeding time result suggested that bleeding tendency could disappear at 12 h after withdrawal of cilostazol even in patients treated with 100 mg twice a day (200 mg/day) for a long-period, although ADP-induced platelet aggregation was suppressed in half of patients till 15 h after withdrawal. Therefore, these findings indicated that platelet function recovered within 12 to 16 h after abrupt withdrawal in patients receiving 100 mg of cilostazol twice a day orally.

A previous study reported by Akiyama et al. (22) showed that plasma cilostazol levels reached a peak (765 ng/ml) at 3 h after a single oral dose of cilostazol (100 mg per body), followed by gradual decrease to approximately 400 ng/ml at 5 h after the peak. However, serum cilostazol concentration in 3 samples in our study did not decrease even at 16 h after withdrawal of cilostazol. Furthermore, elevated cilostazol of concentrations more than 1,000 ng/ml were seen in all blood samples within 6 h after withdrawal. These high serum concentrations of cilostazol might be attributable to a drug interaction in the metabolism since these patients had received several medications, including statins, antihypertensive agents, and antidiabetic drugs, in addition to cilostazol. Some of these medications could interfere with the degradation of cilostazol by the 3A4 (CYP3A4), 2C19, and 1A2 isoforms of cytochrome P450 in the liver, resulting in an increase of serum cilostazol concentrations (24). Indeed, 3 samples with high concentrations of cilostazol at 16 h or more after withdrawal were obtained from a patient (No. 10), who was taking atorvastatin for a long period. It might also be due to the accumulation of cilostazol because of long-term administration.

In our study, suppressed platelet function was found in a wide range of serum cilostazol concentrations. Platelet function was not always strongly suppressed in patients with high serum concentrations of cilostazol, while it was strongly suppressed in patients with low serum concentrations of cilostazol. The reason for this dissociation remains unclear, but one of the reasons may have been the time lag between the peaks of concentration and effect of cilostazol (8, 22). A time difference between the

![Fig. 4. Relationship between platelet aggregatory threshold index (PATI) and serum cilostazol concentration.](image)

The line in A means the regression line in the group with treatment for less than 6 months ($y = 1.003 + 0.002x; R^2 = 0.81; p = 0.0176; n = 5$; two samples were omitted because they indicated a level of more than 4 in the PATI).
peak effect and peak concentration was known to be present, although it was only 3 h (8). In addition, we speculated that the time difference might be influenced by age and smoking habit presumably due to metabolic rate and platelet function, since 4 samples showing low serum concentrations of cilostazol associated with suppressed platelet function, were obtained from the younger elderly with nosmoking habit (No. 2, No. 11). In the group receiving cilostazol for a short period of less than 6 months, platelet function inversely correlated with serum cilostazol concentration (y = 1.003 + 0.002X; R², 0.81; p = 0.0176). These findings suggest that platelet suppression in patients treated with cilostazol for a short period depends on serum cilostazol concentration. It is unclear how long the correlation persists, but the relationship between platelet function and serum cilostazol concentration was not found in the middle group.

However, in the group receiving cilostazol for a long period of more than 48 months showed suppressed platelet function even in patients with low serum concentrations of cilostazol. In a previous study by Kawamura et al. (23), the effect of cilostazol was demonstrated in patients with cerebrovascular disease at 100 and 200 mg a day for two successive weeks, and the potency became more marked as the treatment continued. This indicated that platelet function could be unexpectedly suppressed by low serum concentrations of cilostazol in patients treated for a long period.

The effect of cilostazol was greatest at 6 hours after single administration, although peak concentration preceded the peak effect by 3 h (8). Taking all these findings into consideration, a single 100-mg dose of cilostazol or short-term intake of cilostazol 100-mg twice a day (200 mg/day) could induce transient suppression of platelet function, followed by recovery within 12 h. In contrast, aspirin inhibits platelet function even after it disappears from the plasma and lasts as long as the platelets exposed to the agent remain alive (1). Therefore, patients could be treated with cilostazol instead of aspirin before operation or biopsy to prevent thrombotic events and bleeding complications during these procedures.

Study limitation
In our study, the sample size was too small to reach conclusive results and protocol was not randomized because of ethical reasons. Intentional interruption of antiplatelet therapy should not be done. Therefore, further prospective studies using cilostazol with platelet aggregability monitoring are needed in a larger number of patients receiving only antiplatelet treatment and requiring operation or biopsy.

Conclusion
Examination of changes of platelet function within 36 hours after abrupt withdrawal of cilostazol showed that platelet function recovered at 12 to 16 hours after withdrawal in patients treated with 100 mg of cilostazol twice a day (200 mg/day) for a long period. Inhibition of platelets was seen to correlate with serum cilostazol concentration in patients treated with 100 mg of cilostazol twice a day for less than 6 months, but platelet function was suppressed even at low serum cilostazol concentrations in patients treated with cilostazol for more than 48 months. These findings suggested that management with cilostazol until 12–16 hours before operation or biopsy might be valuable to prevent both thrombotic events and bleeding complications during these procedures.

Acknowledgments: The authors are indebted to Assistant Prof. Raoul Breugelmans and Prof. J. Patrick Barron of the International Medical Communications Center of Tokyo Medical University for reviewing this manuscript. All funds for this study were obtained from the authors’ department.

References


