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Estimation of anti-platelet drugs on human platelet aggregation with a novel whole blood aggregometer by a screen filtration pressure method

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1 The effects of anti-platelet drugs on human whole blood aggregation were evaluated using a novel whole blood aggregometer by a screen filtration pressure (SFP) method.

2 The SFP whole blood aggregometer was found to successfully detect whole blood aggregation induced by ADP, collagen and TRAP by measuring the SFP of blood samples. The platelet aggregation threshold index (PATI), the concentration of agonist required with an inducing pressure rate of 50%, varied time-dependently after collection of blood. High values for ADP and collagen were noted immediately after blood collection, suggesting low aggregation activity of platelets, and gradually increase thereafter.

3 Cilostazol (phosphodiesterase 3 inhibitor), dipyridamole, aspirin and tirofiban all inhibited whole blood aggregation *in vitro*. Inhibitory effects of cilostazol and dipyridamole, but not tirofiban, were markedly enhanced 6 or 7 fold by long pre-incubation (60 min), compared with short pre-incubation (2 min). Such enhancement was only observed with ADP- and not collagen-induced whole blood aggregation. A similar phenomenon was also observed for aggregation with platelet rich plasma (PRP). Cilostazol inhibition of ADP-induced platelet aggregation was more potent with PRP than whole blood ($PATI_{200} = 3.80 \pm 0.95$ μM for whole blood; 2.04 ± 0.61 μM for PRP). Inhibitory effects of dipyridamole were attenuated in PRP without erythrocytes.

4 These results demonstrate that the SFP aggregometer can sensitively detect anti-platelet aggregatory effects of various kinds of drugs. So that it is a useful tool for evaluation of anti-platelet drugs.

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Keywords: Anti-platelet drug; platelet aggregation; PDE3; screen filtration pressure; whole blood aggregation

Abbreviations: ADP, adenosine diphosphate; DMF, N,N-dimethylformamide; DMSO, dimethyl sulphoxide; PATI, platelet aggregation threshold index; PDE, phosphodiesterase; PRP, platelet rich plasma; SFP, screen filtration pressure; TRAP, thrombin receptor activating peptide

Introduction

Platelet adhesion to blood vessel walls and aggregation are crucial physiological events in thrombosis and haemostasis. Excessive platelet aggregation can cause occlusion of blood vessels and leads to ischaemia. In addition, platelet aggregates release growth factors acting on vascular smooth muscle cells such as the platelet-derived growth factor (PDGF), which induce intimal hyperplasia. These phenomena are responsible for cardiovasclular ischaemic diseases such as angina, arteriosclerosis and cardiogenic cerebral infarction. In order to treat ischaemic diseases resulting from platelet aggregation, many kinds of anti-platelet aggregatory drug have been developed, including cyclo-oxygenase and thromboxane synthase inhibitors, cyclic nucleotide phosphodiesterase (PDE) inhibitors, prostaglandin derivatives, thromboxane A_2 receptor antagonists, and GPIIb/IIIa antagonists, which have been widely used in clinical situations (Schafer, 1996).

Inhibitory effects of drugs on platelet aggregation have been generally estimated by measuring change in light transmission rate with platelet rich plasma (PRP) (Born & Cross, 1962). However, this differs from the case with whole blood. PRP contains only platelets, not erythrocytes and leukocytes, which both affect platelet aggregation by releasing substances or other interactions. In addition, PRP is prepared by centrifugation of whole blood, and it has been pointed out that this might cause platelet activation and affect the sensitivity to aggregatory stimulus. Therefore, estimation of anti-platelet aggregatory effects of drugs with PRP may not always reflect actual effects *in vivo*. From this point of view, it is necessary to estimate platelet aggregation directly in whole blood. Because there is no need for separation of platelets from other blood cells, the interval between blood sampling and starting aggregation can be reduced to a minimum, preserving the activity of labile modulators such as thromboxane A_2 and prostaglandins.

One whole blood aggregation method, featuring the impedance aggregometer, measures the aggregation of platelets in terms of increased impedance between two electrodes when platelets accumulate on them in response to aggregatory stimuli (Cardinal & Flower, 1980). This is a powerful approach for hyper cholesterolaemia and thrombocytopenia cases which for platelet aggregation is difficult to

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detect with PRP. However, it has only been used in the laboratory, and still has not been standardized for clinical diagnosis because of its low reproducibility and the inconvenience (Ingerman-Wojenski *et al*., 1982).

Another aggregation method, utilizing screen filtration pressure (SFP) has also been applied to whole blood (Swank, 1961; Swank *et al*., 1964; Dhall *et al*., 1969; Dhall & Matheson, 1969). The SFP method measures platelet aggregation in terms of increasing resistance of whole blood sample flow through a microsieve, which catches platelet aggregates. The resultant clogging formed in agonist stimulated-whole blood on screen filters, increases the resistance to blood flow. Using this approach, the effects of isometric exercise on platelet aggregability could be assessed in coronary sclerotic and cerebral arteriosclerotic patients (Sano *et al*., 1977). The effects of ethanol on micro-aggregate formation were also revealed with this method (Elmér *et al*., 1977). However, it has not been widely so far, largely due to the difficulty of measurement of SFP. This problem has been overcome by development of a novel whole blood aggregometer, which can automatically measure 'sucking pressure', that is SFP (Ozeki *et al*., 2001). The SFP aggregometer features good reproducibility and easy handling with small sample volumes, and has been shown to sensitively detect the inhibitory effect of aspirin on human platelet aggregation *ex vivo*.

In the present study, in order to evaluate whether the SFP aggregometer is suitable for examination of anti-platelet aggregatory effects of a variety of drugs, we carried out a series of pharmacological tests *in vitro* with anti-platelet drugs, cilostazol, aspirin, dipyridamole, and tirofiban and whole blood samples. Attention was also paid to the different mechanisms of anti-platelet action of these drugs.

Methods

Materials

The SFP whole blood aggregometer (WBA analyzer) was from Mebanix Co., Ltd. (Yokohama, Japan) (Ozeki *et al*., 2001). Cilostazol and tirofiban were synthesized at Otsuka Pharamaceutical Co., Ltd.. Acetylsalicylic acid (aspirin) and adenosine 5′-diphosphate (ADP) were from Sigma (St. Louis, MO, U.S.A.), arachidonic acid was from BIOMOL Research Labs. (Plymouth Meeting, PA, U.S.A.), dipyridamole, N,N-dimethylformamide (DMF), dimethyl sulphoxide (DMSO) and ethanol from Wako Pure Chemical Co. (Osaka, Japan), collagen from Nycomed Arzneimittel Gmbh. (Munchen, Germany) and thrombin receptor activating peptide (TRAP) from Sawady Technology (Tokyo, Japan). Heparin was from Shimizu Seiyaku (Shimizu, Japan). D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK) was from Calbiochem (La Jolla, CA, U.S.A.).

Sample preparation and measurement of whole blood aggregation with the SFP aggregometer

Measurement of platelet aggregation with the SFP aggregometer was carried out according to the method previously described (Ozeki *et al*., 2001). Briefly, blood was collected from human healthy volunteers into plastic syringes containing sodium citrate at a final concentration of 0.38%, heparin at a final concentration of 5 units ml^{-1}, or PPACK at a final concentration of 40 μM with 21 gauge needles using a tourniquet. PRP (3×10^8 cells ml^{-1}) was prepared as described earlier (Kimura *et al*., 1985). Two hundred μl of whole blood or PRP was necessary for one aggregation assay. Concentrations of agonist were prepared at 10 times the final concentrations. Four reaction tubes containing 200 μl aliquots of whole blood or PRP, and a stirring bar were placed in the incubation chamber. The samples were stirred

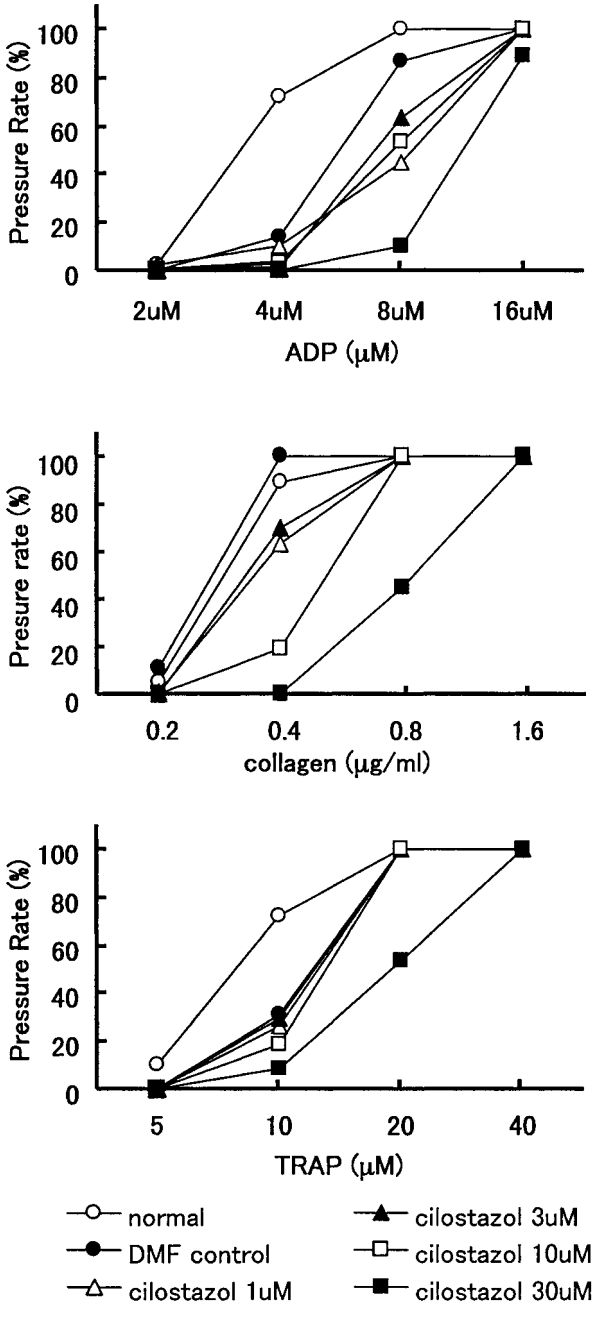

Figure 1 Inhibitory effect of cilostazol (CLZ) on human whole blood aggregation. Whole blood samples were pre-incubated with or without cilostazol, for 2 min prior to the addition of agonists. Note inhibition by 1 ~ 30 μM cilostazol of aggregation induced by ADP (upper), collagen (middle) and TRAP (lower).

at 1000 r.p.m. at 37°C. The screen microsieves are made of nickel, 3.7 mm in diameter and contain three hundred 30-μm square openings in a 1 mm diameter area. The reaction tubes were pre-incubated for 1 min at 37°C, and then 22.2 μl each of four concentrations of agonist were added. Five minutes thereafter, the filter-unit syringe with the screen microsieve was employed to suck the blood samples sequentially at a rate of 200 μl 6.4 s^{-1}. A pressure sensor was connected to the syringe. A negative pressure of -130 mmHg was established as 100%, and -6 mmHg, rather than 0 mmHg, as the 0% pressure rate, because of the viscosity of whole blood. The platelet aggregation pressure of each reaction tube was determined as the pressure rate (%).

Analysis of aggregation rates

The pressure rate was standardized using a grading curve produced by plotting data with four concentrations of agonist on the horizontal axis and plotting the pressure rates (per cent) on the longitudinal axis (Ozeki *et al*., 2001). The concentration of agonist causing a 50% increase in pressure rate was calculated and applied as the platelet aggregatory threshold index (PATI).

Evaluation of drug effects on whole blood aggregation

Cilostazol and dipyridamole were dissolved in DMF, and tirofiban in saline. Aspirin was dissolved in HEPES buffer pH7.8 and then diluted with saline. One μl of drug solution was added to 200 μl of blood 2 or 60 min before addition of aggregation agonist. The final concentration of DMF in the blood sample was adjusted to 0.25%. Whole blood was added, aggregation agonist from 60 to 90 min after blood collection for measurement. PRP samples were added to aggregation agonists from 60 to 180 min after blood

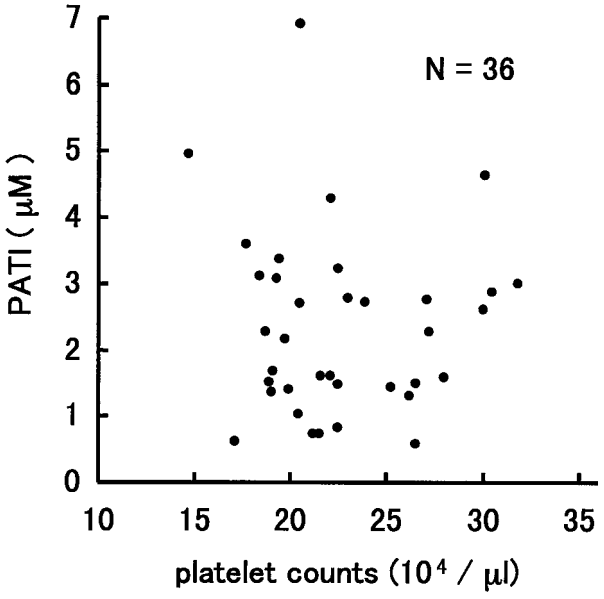

Figure 2 Relationship between platelet count and PATI value of ADP-induced whole blood aggregation. Whole blood aggregation was measured at 60 min after blood collection. Mean platelet count was $22.6 \pm 4.2 \times 10^4$ cells μl^{-1}. Mean PATI value was 2.34 ± 1.37 μM.

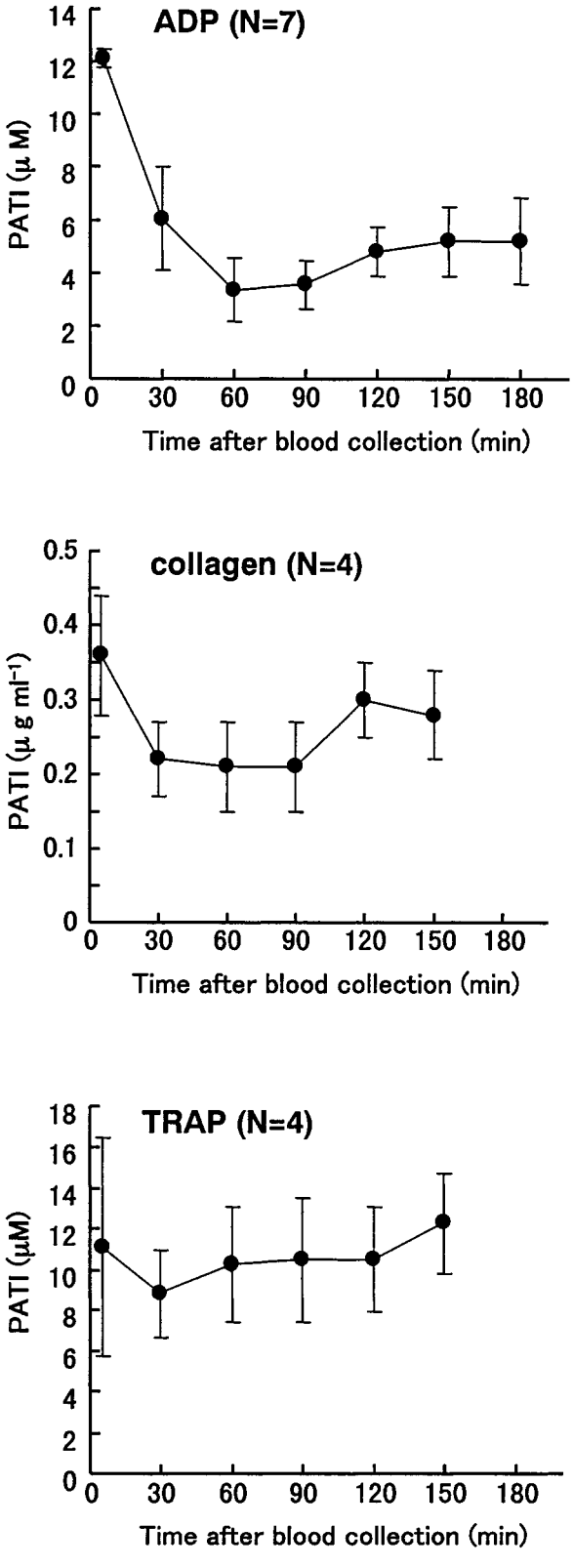

Figure 3 Time-dependent change in human whole blood aggregation after blood collection with citrate. Whole blood samples were incubated at room temperature until the measurement after blood collection. Each value is the mean$\pm$s.d. for data from the given number of determinations.

collection. Anti-aggregatory effects of drugs were evaluated by comparing PATIs of drug added-blood and control blood. As an index of the degree of anti-platelet effects, $PATI_{200}$, the concentration of the drug required to increase PATI 2 fold, was calculated with the four doubling concentrations of agonist, for example, 2, 4, 8 and 16 μM for ADP, and 0.2, 0.4, 0.8 and 1.6 μg ml^{-1} for collagen.

Statistical analysis

Data are expressed as mean$\pm$s.d. values. The statistical significance of differences between two groups was evaluated using the unpaired Student's *t*-test (two-tailed). Drug concentrations and the PATI (per cent of control) were transformed into logarithms, and $PATI_{200}$ values were calculated by log–log regression analysis. The statistical analysis system (SAS) was employed for these statistical analyses. In all cases, $P<0.05$ was considered significant.

Results

With measurement by the SFP whole blood aggregometer, ADP increased the pressure rate for whole blood dose-dependently as described previously (Ozeki *et al*., 2001) (Figure 1). Collagen and TRAP also increased the pressure rate dose-dependently. As a parameter to evaluate the degree of platelet aggregation in the SFP aggregometer, we used the platelet aggregation threshold index (PATI), which is the concentration of agonist required to increase the pressure rate to 50%, based on the results obtained with four concentrations of agonist (Imiya & Matsuo, 1993; Ozeki *et al*., 2001). For example, the control PATI values for normal blood with ADP- and collagen-induced aggregation were 3.4 μM and 0.31 μg ml^{-1}, respectively (Figure 1). Within the range from 14.7 to 31.8×10^4 μl^{-1} of platelet counts, PATI values were not dependent on platelet counts in whole blood samples (Figure 2).

The pressure rate varied time-dependently after collection of blood with a sodium citrate as a conventional anticoagulant and indicated contrary bell shape change. For ADP and collagen, the PATI values were high immediately after blood collection (Figure 3), suggesting aggregation activity of platelets to be low immediately after blood collection, with gradual increase thereafter. Stable aggregation was observed from 60 to 90 min for ADP, 30 to 90 min for collagen and 60 to 120 min for TRAP. Therefore, we examined and compared effects of drugs by adding agonists to whole blood at around 60 to 90 min after blood collection in this study.

Firstly, effects of the organic solvents, DMF, DMSO and ethanol, on whole blood aggregation were examined. These solvents suppressed the increase of the pressure rate by ADP and TRAP dose dependently (data not shown), while saline was no influence. For cilostazol and dipyridamole, 0.25% was chosen as the final concentration of DMF as a solvent based on the ease of solution preparation and the minimal effects on whole blood aggregation.

Figure 1 also shows representative results of examination of cilostazol influence on whole blood aggregation induced by ADP, collagen, and TRAP. Cilostazol suppressed the increase in pressure rate in a dose-dependent manner, so that the

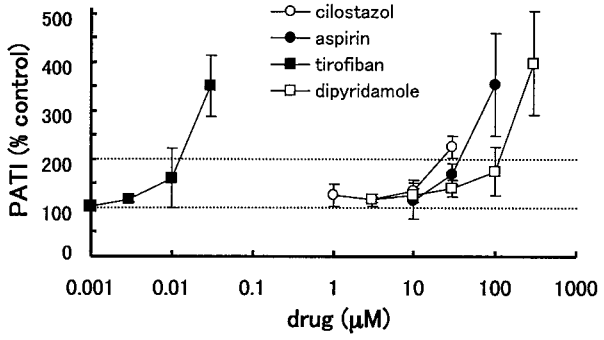

Figure 4 Effects of anti-platelet drugs on human whole blood aggregation induced by ADP. PATI values for controls (100%) were 2.14 ± 0.73 μM for saline (aspirin and tirofiban) and 4.99 ± 1.91 μM for DMF (cilostazol and dipyridamole). The lower dotted line indicates the 100% control, and the intersects on the upper dotted line are the drug-concentration for $PATI_{200}$. Each value is the mean$\pm$s.d. of data from four determinations.

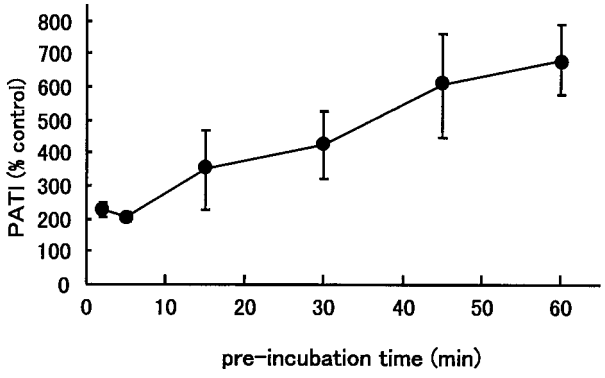

Figure 5 Effects of pre-incubation time on the inhibitory effects of cilostazol on ADP-induced whole blood aggregation. The control PATI (100%) was 4.92 ± 1.5 μM for DMF. Each value is the mean$\pm$s.d. of data from four determinations.

Table 1 Estimated $PATI_{200}$ values for whole blood aggregation with anti-platelet drugs

	$PATI_{200}$ (μM)					
	ADP		*Collagen*		*TRAP*	*Arachidonic acid*
	2 min	*60 min*	*2 min*	*60 min*	*2 min*	*2 min*
Cilostazol	28.2±11.6	3.80±0.95	25.0±15.0	17.0±1.7	>30	7.0±1.9
Dipyridamole	94.4±24.1	16.6±8.9	250.0±89.8	137.9±49.1	>300	181.8±82.3
Aspirin	36.1±11.6	ND	99.2±19.1	ND	210.4±129.4	100.3±39.9
Tirofiban	0.0115±0.0015	ND	0.0242±0.0049	ND	0.0172±0.0071	ND

ND: not determined

PATI values were elevated (see Figure 4). Aspirin, dipyridamole and tirofiban also increased PATI dose-dependently. In order to estimate the inhibitory effects of the drugs on whole blood aggregation with SFP aggregometer, we calculated the concentrations of the drugs required to increase PATI 2 fold, $PATI_{200}$ (Table 1). It means that thus for whole blood, with 28.2 μM of cilostazol, a 2 fold concentration of ADP is required to induce a 50% pressure rate. The $PATI_{200}$ values were found to be almost equal to $IC_{80\sim90}$ at submaximal concentrations of each agonist.

In order to examine the effects of pre-incubation time with cilostazol on anti-aggregation effects on ADP-treated whole blood, a 30 μM dose was applied at various period. Prolongation of the incubation period to 60 min markedly enhanced the inhibitory effects of the cilostazol on ADP-induced aggregation (Figure 5). Other anti-platelet agents were also examined, at concentrations almost equal to those for $PATI_{200}$ with short pre-incubation of 2 min, presented in Table 1. Cilostazol and dipyridamole more strongly inhibited whole blood aggregation after long pre-incubation (Figure 6), and a similar but weaker link was observed for aspirin. With tirofiban no such difference was observed between the long and short pre-incubation cases. The $PATI_{200}$ values for cilostazol and dipyridamole with ADP-induced whole blood aggregation were decreased to about one seventh or sixth, at 3.8 and 16.6 μM, respectively (Figure 7 and Table 1). In contrast, anti-aggregation effects of cilostazol and dipyridamole were only slightly enhanced by long pre-incubation in collagen-induced whole blood aggregation.

Using the SFP aggregometer, we substituted PRP for whole blood and examined the anti-platelet aggregation effect of drugs. Cilostazol inhibited platelet aggregation more potently in PRP than in whole blood (Figures 6 and 8), the $PATI_{200}$ with 60 min pre-incubation being 2.0 μM. Enhancement of anti-platelet aggregation effects was observed only with ADP, and not collagen induction. Anti-platelet effects of dipyridamole were attenuated with PRP (Figure 6). The influence of aspirin and tirofiban was not significantly changed, while effects of aspirin with long pre-incubation were enhanced.

The *ex vivo* and *in vitro* platelet aggregation assay utilizes sodium citrate as the conventional anticoagulant for the collection of whole blood to be used for the preparation of PRP. In the above experiments in this study, it was used sodium citrate as an anticoagulant in preparation of blood samples for aggregometry. However, recently, it has been reported that sodium citrate might alter the response to platelet agonists and anti-platelet aggregation effect of a part of GPIIb/IIIa antagonists such as integrilin (Phillips *et al*., 1997; Mousa *et al*., 2000; Rebello *et al*., 2000). Therefore, we examined the whole blood aggregation and drug efficacy with other types of anticoagulants, heparin or PPACK. For ADP-induced whole blood aggregation, the PATI values with heparin as an anticoagulant varied time-dependently after collection of blood and indicated contrary bell shape change (Figure 9). This aggregation pattern was similar to those with citrate. The PATI values with heparin were overall low compared with citrate. With PPACK, the overall PATI values were almost equal to with citrate. However, the PATI

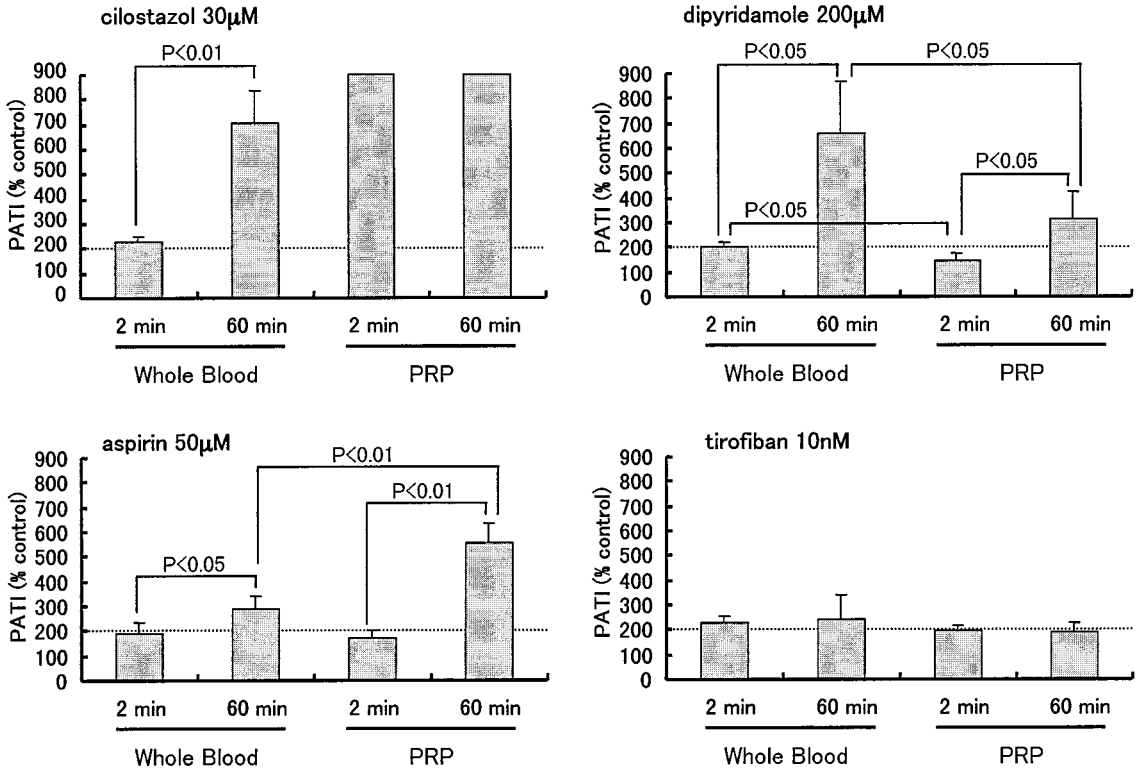

Figure 6 Effects of long pre-incubation time on inhibition by anti-platelet drugs on ADP-induced whole blood and PRP aggregation. Whole blood or PRP samples were pre-incubated with each anti-platelet drug for 2 or 60 min prior to the addition of ADP. The control PATI values (100%) for whole blood were 3.82 ± 1.59 μM for saline and 5.36 ± 1.64 μM for DMF. The respective values for PRP were 3.30 ± 2.06 μM and 3.48 ± 2.32 μM. Each value is the mean$\pm$s.d. of data from four determinations.

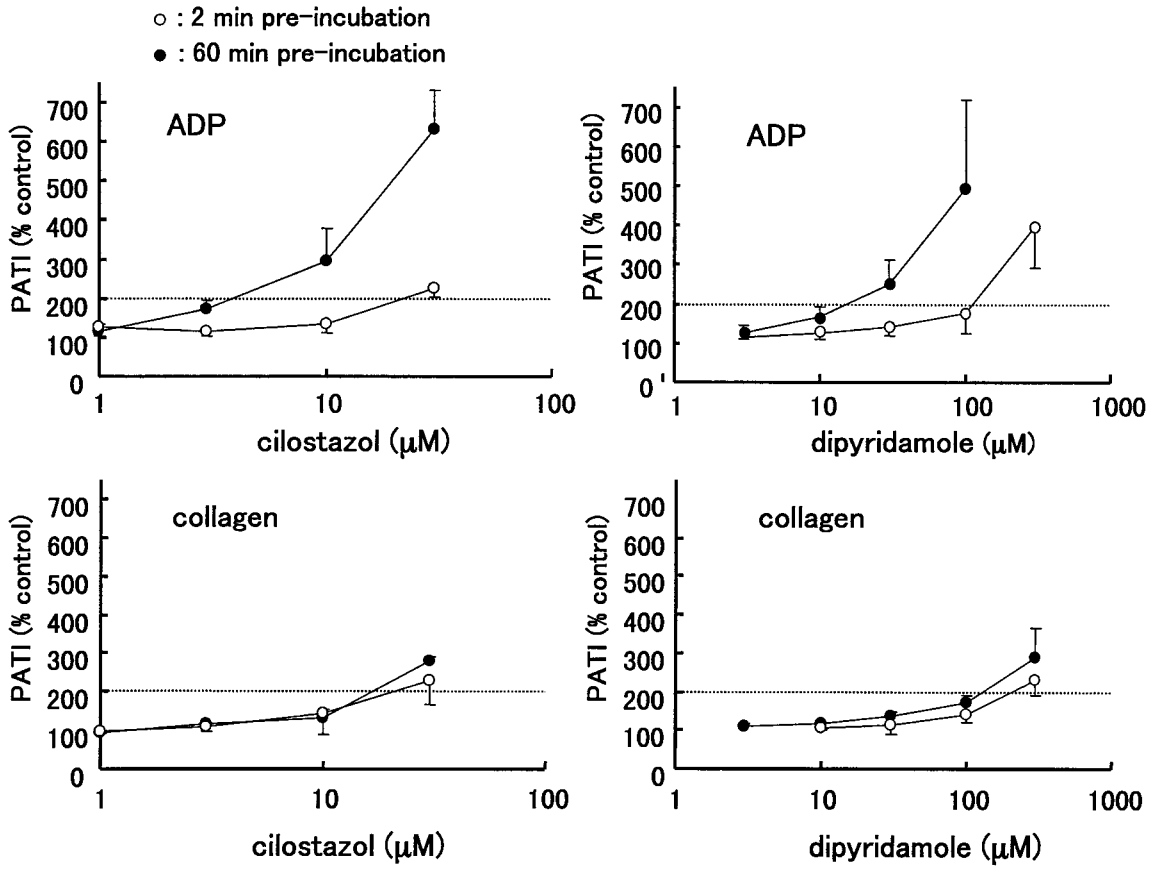

Figure 7 Effects of cilostazol and dipyridamole on ADP- and collagen-induced whole blood aggregation with long (60 min) or short pre-incubation (2 min). Intersects on the dotted lines indicate the $PATI_{200}$ drug-concentration. PATI values for DMF controls (100%) with ADP and collagen were 6.17 ± 2.70 μM and 0.81 ± 0.29 μg ml^{-1}, respectively. Each value is the mean $\pm$ s.d. of data from four determinations.

value immediately after blood collection was low, unlike with citrate and heparin. Cilostazol and tirofiban inhibited ADP-induced aggregation in whole blood collected with heparin or PPACK, similar to with citrate (Table 2). Their $PATI_{200}$ with heparin- or PPACK-anticoagulated whole blood samples were almost similar to that of citrate-anticoagulated whole blood (Table 2). $PATI_{200}$ for aspirin for inhibiting ADP-induced whole blood aggregation with PPACK-anticoagulant was significantly higher than that with citrate-anticoagulant. $PATI_{200}$ for dipyridamole with PPACK- or heparin-anticoagulant was 12–16 times lower than that in citrate-anticoagulant.

Discussion

The present study demonstrated that the SFP whole blood aggregometer is able to sensitively detect anti-platelet aggregation effects of various kinds of drugs. Platelets interact with many kinds of factors and cells in blood during activation and aggregation *in vivo*. Drugs also exert effects on other cell types and factors in addition to platelets. Therefore, it is better to evaluate the effect of drugs on platelet function in whole blood.

Cilostazol, an anti-platelet drug and vasodilator which inhibits PDE3 (Kimura *et al*., 1985; Sudo *et al*., 2000), has been used for treatment of chronic peripheral arterial occlusion in several countries, including Japan and the U.S.A. (Yasunaga & Mase, 1985; Ikeda *et al*., 1987; Dawson *et al*., 1998). The present study, the first examination of cilostazol effects on whole blood, could thereby be shown with the SFP aggregometer clear inhibition of ADP-, collagen- or arachidonic acid-induced platelet aggregation. The effective dose of cilostazol ($PATI_{200}$ in Table 1 and Figure 8) in this study was almost similar to IC_{50} estimated by measuring change in light transmission rate in PRP, 12.8 μM for ADP, 3.86 μM for collagen and 3.56 μM for arachidonic acid (Kimura *et al*., 1985), with 2 min pre-incubation. Interestingly, prolongation of the pre-incubation period markedly enhanced the inhibitory effects of cilostazol on ADP-induced aggregation (Figure 5), evident with a concentration of cilostazol attainable *in vivo* after oral administration. The data indicate that extended contact between cilostazol and blood cells is important to achieve maximal inhibition at least for ADP-induced platelet aggregation. In contrast, prolongation of the pre-incubation time did not modify significantly the efficacy of cilostazol against collagen-induced aggregation (Figure 7). Cilostazol inhibits platelet aggregation by stimulating cyclic AMP accumulation through the specific inhibition of PDE3 activity in platelets. Considering the signal pathways for ADP and collagen and the characteristics of cilostazol, it is speculated

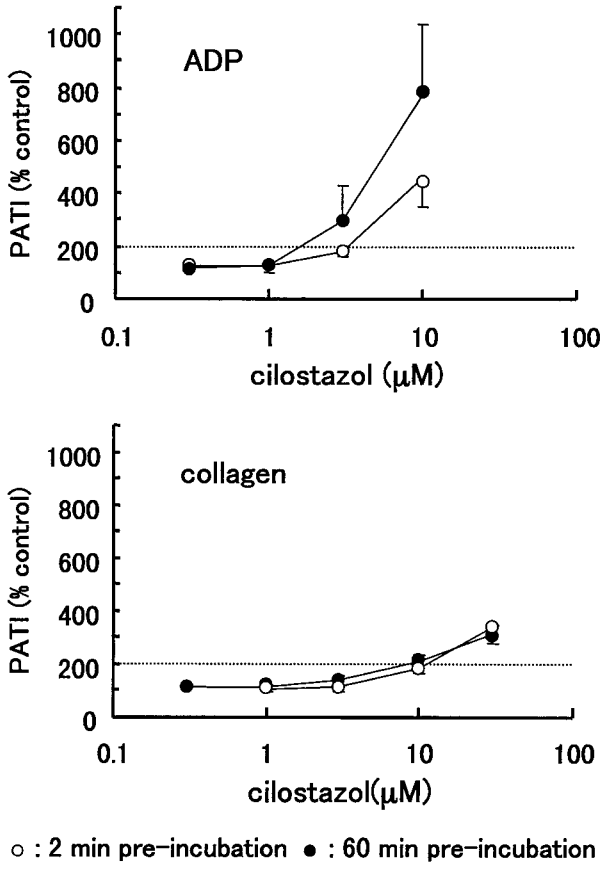

Figure 8 Effects of cilostazol on ADP- and collagen-induced PRP aggregation measured with the SFP aggregometer after long (60 min) or short pre-incubation (2 min). Intersects on the dotted line indicate the $PATI_{200}$ drug-concentration. Values for cilostazol were 2.9 μM (2 min), 2.0 μM (60 min) with ADP and 11.2 μM (2 min), 9.1 μM (60 min) with collagen. PATI values for DMF controls (100%) with ADP and collagen were 3.73 ± 1.74 μM and 1.84 ± 0.76 μg ml^{-1}, respectively. Each value is the mean $\pm$ s.d. of data from four determinations.

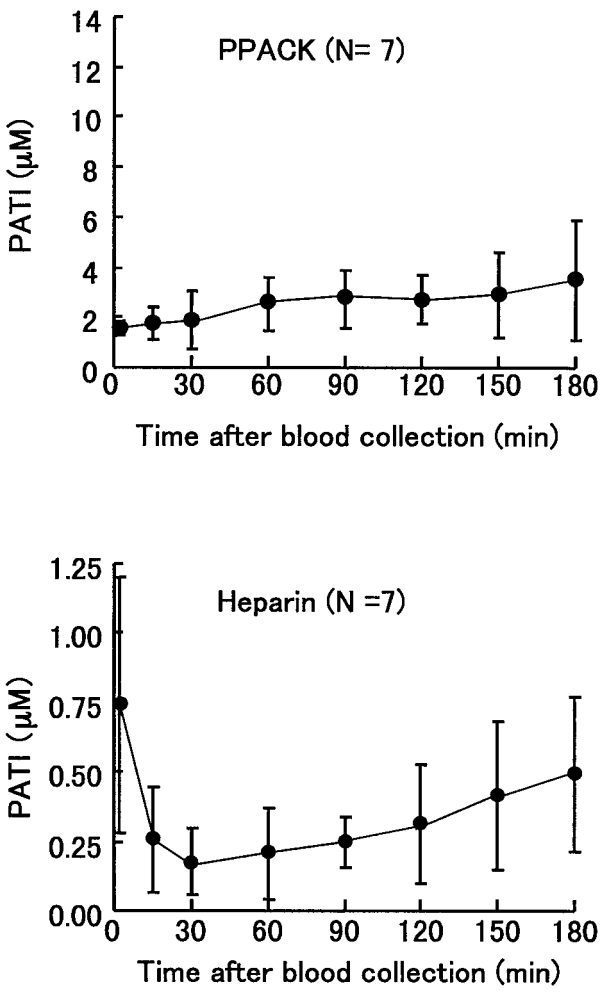

Figure 9 Time course of human ADP-induced whole blood aggregation after blood collection with PPACK or heparin. Whole blood samples were incubated at room temperature until the measurement after blood collection. Each value is the mean $\pm$ s.d. for data from the given number of determinations.

that cyclic AMP accumulation by the long pre-incubation of platelet with cilostazol might be enough to make platelet resistant to the inhibition of cyclic AMP synthesis by ADP-P_2Y_{12} receptor.

Dipyridamole is a dual inhibitor of adenosine uptake (Roos & Pfleger, 1972; Klabunde, 1983; Dawicki *et al*., 1985) and PDE5 (Sudo *et al*., 2000). In this study, potent effects of dipyridamole on platelet aggregation were observed in whole blood with the SFP aggregometer. The effects were attenuated in PRP, because erythrocytes, one of the target cell types of dipyridamole, are removed in the preparation stage. This result is consistent with previous reports with impedance whole blood aggregometry (Gresele *et al*., 1983; 1986; Dawicki *et al*., 1985). In whole blood, adenosine is continuously released, with re-uptake by erythrocytes; dipyridamole prevents this and the adenosine inhibits platelet aggregation by adenosine A_2-receptor-mediated Gs protein-linked activation of adenylate cyclase, increasing platelet cyclic AMP (Haslam & Lynham, 1972; Hourani & Cusack, 1991).

In the present study with the SFP whole blood aggregometer, tirofiban inhibited platelet aggregation at a concentration similar to that reported previously with change in light transmission rate in PRP (Peerlinck *et al*., 1993). Unlike cilostazol and dipyridamole, the effect of tirofiban was not potentiated by long-pre-incubation. This is presumably due to differences in target molecules; in the tirofiban case this is the adhesion molecule GPIIb/IIIa, involved in the final step of platelet aggregation.

Our results for aspirin are somewhat unexpected. On measurement of change in light transmission rate in PRP, it

Table 2 Effect of different anticoagulants on the potency of anti-platelet drugs in inhibiting ADP-induced whole blood aggregation

	$PATI_{200}$ (μM)		
	Citrate 2 min	*PPACK* 2 min	*Heparin* 2 min
Cilostazol	28.2 ± 11.6	16.9 ± 7.9	18.0 ± 3.8
Dipyridamole	94.4 ± 24.1	5.9 ± 3.6	8.2 ± 1.1
Aspirin	36.1 ± 11.6	>300	74.8 ± 30.4
Tirofiban	0.0115 ± 0.0015	0.0132 ± 0.0099	0.0120 ± 0.0082

was found to inhibit collagen-induced platelet aggregation, but the effect on ADP-induction was weak (Kimura *et al*., 1985). However, in this study with the SFP whole blood aggregometer, aspirin exerted potent inhibition not only on collagen-induced but also ADP-induced platelet aggregation, confirming the recent findings that orally administered aspirin potently inhibited both ADP- and collagen-induced platelet aggregation *ex vivo* in human whole blood (Kondo *et al*., 2001; Kunishima *et al*., 2001; Ohata *et al*. unpublished data; Ozeki *et al*., 2001). One reason for the sensitive detection of anti-platelet effects of aspirin might be the use of PATI, determine with four concentrations of agonist, and correlating with the minimum concentration at which irreversible aggregation is induced (Imiya & Matsuo, 1993). It is impossible to measure platelet aggregation continuously and estimate the maximum aggregation by one concentration of agonist with the SFP aggregometer, unlike change in light transmission in PRP. Therefore, it is reasonable to assume that it is better to evaluate the anti-platelet effects of drug with change of PATI using the SFP aggregometer.

It is well known that aspirin inhibits only the second aggregation of platelet induced by ADP, and not the primary aggregation. Although the latter is induced even by low concentrations of ADP, the second irreversible aggregation requires a high concentration. Therefore, the SFP aggregometer may be more sensitive for detection of anti-platelet aggregatory effects of drugs like aspirin. Inhibitory effects of aspirin on ADP-induced whole blood aggregation were slightly enhanced by long pre-incubation (Figure 5), independent of the induces, in line with earlier results (Sils *et al*., 1988).

In conclusion, the present study demonstrated that the SFP aggregometer can sensitively detect anti-platelet aggregatory effects of various kinds of drugs, and, in addition, that the results clearly reflect the pharmacological mechanisms with the agents used in the reported series of experiments. Therefore, the SFP aggregometer is a useful tool for evaluation of anti-platelet drugs.

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