A microchip flow-chamber system for quantitative assessment of the platelet thrombus formation process

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Abstract

As the pathogenesis of arterial thrombosis often includes platelet thrombus formation (PTF), antiplatelet agents are commonly used for the prevention of thromboembolic events. Here, using a novel microchip flow-chamber system we developed to quantitatively analyze the PTF process, we evaluated the pharmacological efficacies of antiplatelet agents under different arterial shear rates. Hirudin-anticoagulated whole blood was perfused over a collagen-coated microchip at shear rates of 1000, 1500, and 2000 s⁻¹, and PTF in the absence and presence of various antiplatelet agents was observed microscopically and quantified by measuring flow-pressure changes. The onset of PTF was measured as T10 (time to reach 10 kPa), and AUC10 (area under the flow pressure curve for the first 10 min) was calculated to quantify the overall stability of the formed thrombus. Aspirin and AR-C66096 (P2Y12-antagonist) at high concentrations (50 μM and 1000 nM, respectively) prolonged T10 only modestly (AR-C66096 > aspirin), but effectively decreased AUC10, resulting in unstable PTF at all examined shear rates. With dual inhibition using both aspirin (25 μM) and ARC-66096 (250 nM), AUC10 was drastically reduced. Nearly complete suppression of AUC10 was also observed with abciximab (2 μg ml⁻¹) and beraprost (PGI2-analog; 4 nM). Although OS-1 (GPIb-α-antagonist; 100 nM) prevented complete capillary occlusion, significant amounts of microscopic thrombi were observed on the collagen surface. In contrast to abciximab and beraprost, OS-1 differentially affected PTF under higher shear conditions. Our novel analytical system is capable of distinguishing the pharmacological effects of various antiplatelet agents under physiological shear rates, suggesting that this system may aid in the determination of the appropriate type and dose of antiplatelet agent in the clinical setting.

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Introduction

The pathogenesis of atherothrombosis often involves platelet thrombus formation (PTF) at the site of vascular injury after plaque rupture. Antiplatelet agents, such as aspirin and clopidogrel, effectively inhibit the adhesion and aggregation of platelets to subendothelial connective tissues, including collagen, and are widely used in the primary and secondary prevention of cardiovascular events. However, therapeutic responses to antiplatelet therapies exhibit large individual variability, as determined by platelet function tests, and reduced sensitivity to aspirin and clopidogrel is associated with an increased risk of recurrent vascular events (Breet et al., 2010; Frelinger et al., 2009; Maree and Fitzgerald, 2007; Michelson et al., 2007; Price, 2009).

Adjusting antiplatelet therapies by increasing doses, switching to an alternative agent, or co-administering multiple agents, has been performed empirically based on clinical symptoms and recurrent cardiovascular events. More recently, several novel assay systems for platelet function have become clinically available, in addition to turbidimetric platelet aggregometry in platelet-rich plasma (Harrison, 2009; Michelson, 2009). For example, the VerifyNow™ and Multiplate™ systems measure changes in light transmission and electrical impedance, respectively, due to platelet agglutination and aggregation in response to an exogenous platelet agonist (Harrison, 2009; Michelson, 2009). Although these tests can be performed using...
anticoagulated whole blood, testing conditions are non-physiological because the sample blood is mixed in the absence of arterial flow and only a single exogenous agonist is added at a supra-physiological concentration. The choice and concentration of the platelet agonist strongly affects the observed platelet activity and the inhibitory effects of an antiplatelet agent. It is thus desirable to develop a method for evaluating platelet function under physiological conditions, such as in the presence of collagen-surface and using arterial shear flow rates, and to allow testing of multiple antiplatelet agents in a single run.

Here, we describe a new microchip flow-chamber system designed to evaluate PTF under variable flow conditions, and present preliminary data on the impact of various antiplatelet agents on PTF. We hypothesized that the PTF process mediated by platelet-collagen interaction under arterial shear rates in the microchips could be compared to whole blood aggregometry by microscopic inspection of thrombus sizes and the monitoring of flow pressure changes due to thrombus-mediated occlusion.

Materials and methods

Materials

Microchips, comprising cover- and capillary-chips, were obtained from Richell Corp. (Toyama, Japan). Type I collagen (from pig tendon) was purchased from Nitta Gelatin, Inc. (Osaka, Japan). Six microliters from Tocris Bioscience (Bristol U.K.). Abciximab, a glycoprotein (GP) IIb/IIIa antagonist, was obtained from Eli Lilly (Indianapolis, USA). A from Toray Industries, Inc. (Tokyo, Japan). Type I collagen (from pig tendon) was purchased from Richell Corp. (Toyama, Japan). Six microliters from Wako Pure Chemicals (Osaka, Japan) and was dissolved in 1% dimethylsulfoxide (DMSO) prior to use. AR-C66096 (Kunapuli, 2002), a specific antagonist of the P2Y12 receptor, was purchased from Wako Pure Chemicals (Osaka, Japan) and was dissolved in 1% dimethylsulfoxide (DMSO) prior to use. Acetylsalicylic acid was purchased from Dyna-Byte Medical (Munich, Germany). Acetylsalicylic acid was purchased from Wako Pure Chemicals (Osaka, Japan) and was dissolved in 1% dimethylsulfoxide (DMSO) prior to use. AR-C66096 (Kunapuli, 2002), a specific antagonist of the P2Y12 receptor, was purchased from Wako Pure Chemicals (Osaka, Japan) and was dissolved in 1% dimethylsulfoxide (DMSO) prior to use. Acetylsalicylic acid was purchased from Wako Pure Chemicals. De, was purchased from Toray Industries, Inc. (Tokyo, Japan). All other reagents were obtained from Wako Pure Chemicals.

Blood samples

The present study conformed to the provisions of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Kinki University (Osaka, Japan) and informed written consent was obtained from all subjects. Blood from healthy, fasting volunteers was drawn into hirudin-anticoagulant tubes. The volunteers had not taken any medications in the preceding two weeks that might have affected platelet function. Blood was used after a resting phase of 1 h from the time of collection.

Principle of the flow-chamber system to monitor PTF

An overview of the analytical process of the microchip flow-chamber system used to monitor PTF is illustrated in Fig. 1. In this system, the blood sample is placed in a reservoir that is connected to an HS210 precision pump (Uniflow, Tokyo, Japan) that impels the blood (4–60 μl min⁻¹) through the inlet port into a flow path that leads to a collagen-coated analytical path consisting of 25 capillary channels (Fig. 1A). The process of PTF in the flow chamber is continuously monitored using a video-microscope (10×) under the flow chamber and by a pressure sensor positioned between the HS210 pump and reservoir that tracks pressure changes in the flow path. In the present study, hirudin-treated whole blood was perfused at flow rates of 12, 18, and 24 μl min⁻¹, corresponding to initial shear rates of 1000, 1500, and 2000 s⁻¹, respectively, as estimated using FLUENT software (Ansys Co., Ltd., Tokyo, Japan). When blood flows through the analytical path of the microchip, platelets adhere and aggregate on the surface of the collagen-coated capillary channels. Small platelet aggregates gradually increase in size and eventually occlude the capillary, resulting in an increase of flow pressure. Thus, flow pressure patterns reflect the PTF process in the microchip.

Definitions of parameters for quantification of PTF

Two specific terms, T₁₀ and AUC₁₀, were used to quantify PTF inside the microchips. T₁₀ (time to reach 10 kPa) was defined as the onset of PTF and represents the duration (sec) for the flow pressure

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Fig. 1. Schematic diagrams of the microchip flow-chamber system designed to monitor platelet thrombus formation. A) Collagen-coated microchip. B) Analytical components of the system.
to increase to 10 kPa from baseline due to PTF-induced partial occlusions of microcapillaries. The baseline pressure was the mean flow pressure 20 to 30 s after the start of each experiment.

AUC<sub>10</sub> is the area under the flow pressure curve (less than 60 kPa) for 10 min after the start of the assay. This pressure-time integral was used to quantify the overall stability of thrombus. Lower AUC<sub>10</sub> values presumably reflect slow or reduced thrombus growth, or alternatively a rapid breakdown of PTF. Flow pressures above 60 kPa were truncated at 60 kPa for the calculation of AUC<sub>10</sub>.

**Effects of antiplatelet agents on PTF**

The changes in T<sub>10</sub> and AUC<sub>10</sub> parameters were evaluated in the presence of aspirin (25 and 50 μM), AR-C66096 (250, 500, and 1000 nM), abciximab (0.5, 1.0, and 2.0 μg ml<sup>-1</sup>, corresponding to 10.5, 21, and 42 nM, respectively), OS-1 (25, 50, and 100 nM), and beraprost (1.0, 2.0, and 4.0 nM). We speculated that antiplatelet agents that interfere with platelet adhesion would intensely affect T<sub>10</sub>, whereas agents that interfere with platelet aggregate stability would more likely affect AUC<sub>10</sub>. Antiplatelet agents were added in vitro to the whole blood sample (at ~1% of the total volume), which was incubated at 25 °C for 10 min prior to the assay. DMSO was used as the diluent for aspirin and had no effect on PTF at concentrations of up to 0.01%.

**Effects of antiplatelet agents on whole blood aggregometry**

Effects of antiplatelet agents were also evaluated using the Multiplate<sup>™</sup>-analyzer (Dynabyte Medical, Munich, Germany). Briefly, 300 μl saline and 300 μl sample blood were pipetted into a single-use cuvette. After a 3-min incubation at 37 °C, one of the following agonists was added (final concentration): ADP (6.5 μM), collagen (3.2 μg ml<sup>-1</sup>), arachidonic acid (0.5 mM), or ristocetin (0.77 mg ml<sup>-1</sup>). Platelet adhesion and aggregation were monitored for 6 min. The impedance change caused by the adhesion and aggregation was plotted against time and the area under the aggregation curve was used to measure the aggregation response, quantified in arbitrary aggregation units (U).

**Effects of aspirin intake on PTF and whole blood aggregometry**

To analyze the effects of aspirin intake on the PTF process, whole blood was first collected into hirudin-anticoagulant tubes from 5 healthy male volunteers (mean age, 35.4 ± 5.6 years) before, and 4 and 24 h after the intake of aspirin (100 mg). After a resting phase of 1 h, PTF at the three shear rates, and whole blood aggregation induced by collagen, ADP, and arachidonic acid were measured for each blood sample, as described above.

**Statistical analysis**

For the PTF analyzer system, intra-individual coefficients of variation (CV,%) were determined by conducting quadruplicate assays for individual blood samples from four different subjects. Data are shown as the mean ± SD, unless otherwise indicated. All data analyses were performed with Graphpad Prism (Graphpad Software, Inc., San Diego, USA).

**Results**

**Flow pressure patterns of PTF for untreated blood from healthy individuals**

The pressure waveforms measured by the microchip flow-chamber system characterize the onset of the PTF process (T<sub>10</sub>) and the AUC<sub>10</sub> of sustained thrombus growth up to 60 kPa. In the case of unstable thrombus growth, the pressure waveform starts to decline as platelet thrombi break apart. Here, as only the blood from healthy subjects was used, flow pressure started to increase (thrombus growth) after of lag time of approximately 2–3 min and reached a sustained peak (firm thrombi) above 60 kPa. At shear rates of 1000, 1500, and 2000 s<sup>-1</sup>, the onset times of thrombus growth (T<sub>10</sub>) in blood from healthy individuals (male, 19; female, 14; mean age, 36.5 ± 11.6 years) were 199.0 ± 38.9, 144.1 ± 25.7, and 128.6 ± 23.0 s, respectively, while the AUC<sub>10</sub> (kPa × min) values were 297.2 ± 54.2, 406.5 ± 44.5, and 438.1 ± 29.8, respectively (Fig. 2). Therefore, PTF for untreated whole blood from normal individuals was enhanced in a shear-dependent manner in this monitoring system. The intra-individual CVs at shear rates of 1000, 1500, and 2000 s<sup>-1</sup> were calculated to evaluate the reproducibility of the device. CVs for T<sub>10</sub> were 5.3% ± 2.8%, 4.5% ± 0.9%, and 4.9% ± 1.5%, respectively, and those for AUC<sub>10</sub> were 7.0% ± 3.3%, 2.8% ± 2.3%, and 2.5% ± 1.7%, respectively.

**Video microscopy of PTF**

The PTF process within the capillaries was visually inspected using the built-in light microscope. After initiating the perfusion of whole blood, platelet adhesion and aggregation were observed on the collagen surface, and many small platelet thrombi were visible within 1–3 min. These small thrombi gradually grew in size by repeated collapsing and had formed stable platelet thrombi that occluded the capillaries in the analytical field within 4–8 min (Fig. 3 and Video data 1; left).

**Effects of antiplatelet agents on PTF and whole blood aggregometry**

Fig. 4 shows examples of typical suppression patterns of PTF for whole blood samples treated with several antiplatelet agents. Aspirin (cyclooxygenase-1 inhibitor) and AR-C66096 (P2Y<sub>12</sub> antagonist) were characterized by moderate increases in T<sub>10</sub> and concentration-dependent decreases in AUC<sub>10</sub> (Fig. 4A). In contrast, abciximab (GPIIb/IIIa antagonist), OS-1 (GPIbox antagonist), and beraprost (cyclic AMP enhancer) prolonged T<sub>10</sub> in a concentration-dependent manner and completely suppressed the hike of flow pressure at high concentrations (Figs. 4B and C). The examined agents also demonstrated distinctive efficacies and sensitivities to different shear conditions for suppressing PTF and to different agonists for platelet aggregation, as described below.

The effects of various antiplatelet agents on thrombus formation revealed by the microchip-based PTF and whole blood platelet aggregation assays are shown in Figs. 4 and 5, respectively. In the following
sections, the results obtained for both assay methods are described and compared for each antiplatelet agent.

Aspirin and AR-C66096

The PTF assay of whole blood revealed that in the presence of aspirin, AUC\textsubscript{10} concentration-dependently decreased, whereas the effect on T\textsubscript{10} was relatively small (Fig. 5A). For aspirin (50 μM), T\textsubscript{10} values were prolonged by 1.5 and 1.6 fold, while AUC\textsubscript{10} values were decreased by 47.7% and 50.4% at shear rates of 1000 and 2000 s\textsuperscript{-1}, respectively. Similarly, AR-C66096 demonstrated concentration-dependent inhibitory effects on T\textsubscript{10} and AUC\textsubscript{10} values at all examined shear conditions (Fig. 5B). In the presence of 1000 nM AR-C66096, T\textsubscript{10} values were prolonged by 2.0 and 1.8 fold, while AUC\textsubscript{10} values were decreased by 77.9% and 73.5% at shear rates of 1000 and 2000 s\textsuperscript{-1}, respectively. When both aspirin (25 μM) and AR-C66096 (250 nM) were added to blood samples, T\textsubscript{10} was prolonged over 10 min at all shear rates and AUC\textsubscript{10} was reduced to nearly zero, although the individual inhibitory effects of these agents on T\textsubscript{10} and AUC\textsubscript{10} were limited, indicating synergistic antiplatelet effects (Fig. 5C). Microscopic observations demonstrated that dual inhibition significantly lowered the growth rate and firmness of platelet thrombi (Video data 1; right).

The whole blood platelet aggregation assay revealed that in the presence of 50 μM aspirin, Multiplate-AUCs were decreased by 41.3%, 13.7%, and 56.9% for collagen-, ADP-, and arachidonic acid-induced aggregation, respectively (Figs. 6A, B, and C). Similarly, in the presence of 1000 nM AR-C66096, Multiplate-AUCs were reduced by 29.6% and 74.7% for collagen- and ADP-induced aggregation, respectively. These results showed that abciximab, which interferes with both GPIIb/IIIa-fibrinogen and GPIIb/IIIa-vWF interactions, suppressed PTF on the collagen surface under flow conditions more efficiently than collagen-induced platelet aggregation under non-flow conditions.

Abciximab

Abciximab treatment of whole blood prolonged T\textsubscript{10} and decreased AUC\textsubscript{10} values in a concentration-dependent manner. Abciximab (2 μg ml\textsuperscript{-1}) nearly completely suppressed PTF inside the microchip (Video data 2; left), while T\textsubscript{10} values of all blood samples were prolonged over 10 min at all shear conditions tested (Fig. 5D). In contrast, Multiplate-AUCs were reduced by 54.9% for collagen-induced aggregation and 92.0% for ADP-induced aggregation (Figs. 6A and B). OS-1

Both T\textsubscript{10} and AUC\textsubscript{10} were concentration-dependently affected by OS-1 at all shear rates (Fig. 5E). At a concentration of 100 nM, T\textsubscript{10} variables were prolonged by 2.0-, 3.0-, and 3.3-fold, while AUC\textsubscript{10} variables were decreased by 74.8%, 84.6% and 90.1% at shear rates of 1000, 1500, and 2000 s\textsuperscript{-1}, respectively. Thus, OS-1 exerted greater inhibitory effects on PTF under higher shear conditions, which contrasted with the superior efficacy of aspirin under low shear conditions (Figs. 5A and E). Furthermore, the inhibitory effect of OS-1 on PTF markedly differed from that of abciximab. Although abciximab concentration-dependently reduced the size and number of platelet thrombi, and completely suppressed PTF at high concentration, significant amounts of platelet thrombi were observed even at the highest concentration (100 nM) of OS-1 (Video data 2; right). However, OS-1 effectively prevented the complete occlusion of the capillaries, a finding that was consistent with the result of impedance aggregometry. In the presence of 100 nM OS-1, Multiplate-AUCs were...
reduced by 78.6% for ristocetin-induced aggregation (Fig. 6D), but no inhibition of collagen-induced aggregation was observed (Fig. 6A).

Beraprost

The inhibitory effects of beraprost on PTF were also concentration-dependent and potent. At 4 nM beraprost, nearly complete suppression of PTF was achieved under all three shear conditions (Fig. 5F). In contrast, Multiplate-AUCs were reduced by only 38.0% for collagen and 58.8% for ADP with beraprost at 4 nM (Figs. 6A and B).

Effects of aspirin intake on PTF and whole blood aggregometry

The growth and stability of platelet thrombi were reduced after the intake of aspirin at all shear rates tested. AUC_{10} values of blood samples collected 24 h after the intake of aspirin were reduced by 13.1%, 20.1%, and 22.8% at shear rates of 1000, 1500, and 2000 s^{-1}, respectively, with minor prolongation of T_{10} (Fig. 7A). These findings were concordant with the characteristic antithrombotic efficacy observed in aspirin-treated blood in vitro.

In addition, Multiplate-AUCs for collagen-, ADP-, and arachidonic acid-induced aggregation were reduced by 9.7%, 5.0%, and 26.2%, respectively, 24 h after the intake of aspirin (Fig. 7B).

Discussion

In the present study, we demonstrated the utility of a novel microchip flow-chamber system to quantify the process and extent of PTF, and to monitor and quantify the effects of antiplatelet agents on PTF. The major advantages of this system include its ability to differentiate antiplatelet effects of multiple agents using a single type of collagen-coated capillary (i.e., no need for an exogenous drug-specific agonist), and its ability to adjust shear rates to mimic venous and arterial flow patterns. These properties represent significant improvements over conventional platelet function assays.

The sensitivity of the impedance aggregometry (Multiplate™ system) to different antiplatelet agents depends on the choice of agonist. We detected that the sensitivity to aspirin decreased in the order of arachidonic acid > collagen > ADP, whereas for AR-C66096 and beraprost, their inhibitory effects were more apparent with ADP compared to collagen. Abciximab potently inhibited both ADP- and collagen-induced aggregation, whereas OS-1 only exhibited concentration-dependent inhibition of ristocetin-induced aggregation, and no effects against ADP or collagen were detected. For these reasons, it is difficult for aggregometry assays to comparatively demonstrate antithrombotic efficacies among agents. Further, possible
insensitivity to antiplatelet therapy cannot be fully evaluated using these assays, because combination therapies cannot be accurately tested in an agonist-specific aggregometric assay. However, several of these limitations can be possibly overcome using the microchip flow-chamber presented here to measure PTF. As this approach uses a collagen-coated surface and adjustable shear rates, it is possible to comparatively evaluate antiplatelet efficacy for a single agent and for agents in combination (Fig. 5C).

The shear rates used in the present study ranged from 1000 to 2000 s$^{-1}$, corresponding to reported shear rates in arterioles (500–1600 s$^{-1}$) (Hanson and Sakariassen, 1998). The PFA-100 system (Siemens Diagnostics, Deerfield, IL) is another device that measures platelet function under high shear (~5000 s$^{-1}$) (Kundu et al., 1995). The assay is conducted using citrated whole blood, and its endpoint is the time for occlusion (closure time) of an aperture coated with collagen/epinephrine or collagen/ADP. Although aspirin (Mammen et al., 1998) and clopidogrel (Raman and Jilma, 2004) are known to prolong aperture closure times, elevated von Willebrand factor (vWF) levels shorten closure time (Homoncik et al., 2000). Indeed, vWF is the predominant promoter of PTF at shear rates over 1500 s$^{-1}$ (Savage et al., 1996). For these reasons, the PFA-100 system is often used as a screening test for von Willebrand disease (Fressinaud et al., 1998), but its usefulness in detecting aspirin or P2Y$_{12}$ antagonists, such as clopidogrel, remains questionable (Breet et al.). Our present data shed light on this issue, because aspirin seemed to exert better antithrombotic efficacy under lower shear (1000 s$^{-1}$), whereas OS-1 (GPIb$\alpha$ antagonist) was more effective under higher shear (2000 s$^{-1}$) (Figs. 5A and E).

Our system also permits informative data of the PTF process to be captured by videomicroscopy, which revealed that platelet adhesion...
and aggregation were triggered within 1–3 min by the collagen-coated surface under shear (Video data 1 and 2). Platelet thrombus growth was generally hindered under shear flow, and thus thrombi repeatedly fell apart and re-formed. The latter process eventually led to the complete occlusion of capillaries on the microchip. The importance of endogenous release of platelet agonists (TXA2 and ADP) was evident from the limited thrombus growth observed in aspirin- and AR-C66096-treated blood, as well as in blood samples drawn after aspirin intake.

Most previously reported flow chamber systems are based on thrombus volume and/or area covered by thrombi as measured by confocal microscopy to evaluate PTF, as these two parameters should dominantly reflect the initial PTF process on a collagen-coated surface. However, although thrombus volume and area can be quantitatively assessed using these conventional systems, assays that are able to measure the qualitative characteristics of thrombi, including stability and fragility, may provide a more comprehensive evaluation of the PTF process (Mendolicchio et al., 2011). As our developed system is able to assess both qualitative and quantitative properties of thrombi, it may provide additional benefits for evaluating the therapeutic effects of aspirin and clopidogrel.

Abciximab is a prototypical GPIIb/IIIa receptor antagonist that hinders fibrinogen and vWF from binding to this platelet receptor. Significant delays in the onset (T₁₀) of PTF underlie the importance of vWF-GPIIb/IIIa interactions after transient adhesive interaction (i.e., tethering) between vWF and GPIbrix under high shear (Savage et al., 1996). Here, the inhibition of GPIbrix using OS-1 (Benard et al., 2008) also prolonged the onset of PTF and maintained the patency of the inner lumen of capillaries (Fig. 5E and Supplemental Movie 2). Although abciximab completely suppressed PTF at > 2 μg ml⁻¹ (−42 nM), considerable platelet thrombi remained on the surface of collagen even in the presence of 100 nM OS-1 (highest concentration tested). It is thus likely that the inhibitory effect of OS-1 is more potent at shear rates well above 2000 s⁻¹, which occur in narrowed capillaries and stenotic coronary arteries (Strony et al., 1993). Taken together, these data suggest that targeted inhibition of GPIbrix-vWF interaction may be a potential approach to selectively block complete occlusion of stenosed vessels while maintaining hemostatic function (Kageyama et al., 2002).

Prostacyclin is an endogenous antiplatelet substance that is released from normal endothelial cells (Nakagawa et al., 1994). Beraprost, a prostacyclin analog (Satoh et al., 2002), and other cAMP enhancers are known to reverse ADP-induced platelet aggregation (i.e., disaggregation) (Kikura et al., 2000). Disaggregatory responses are also observed in clopidogrel-treated platelets in response to ADP (Szlam et al., 2010). In the flow-chamber system, beraprost exerted concentration-dependent prolongation of T₁₀ and almost completely suppressed PTF (−null AUC₁₀) at 4 nM. The incomplete suppression of AUC₁₀ by AR-C66096 relative to beraprost may be related to differences in the intracellular mechanisms of cAMP elevation between these agents. Beraprost directly increases cAMP by interacting with IP receptors, whereas AR-C66096 prevents the decrease of cAMP by interacting with P2Y₁₂ receptors. In the impedance aggregometry, mechanistic differences between beraprost and AR-C66096 were not detected; both agents moderately suppressed ADP-induced aggregation and only mildly inhibited collagen-induced aggregation. Collectively, these data confirm the pivotal role of ADP, which is endogenously released from collagen-activated platelets, in stabilizing platelet thrombi under shear.

A few limitations of this study warrant mention. First, the developed system does not use endothelial cells. As demonstrated in the experiment with beraprost, endothelial cells play important modulatory roles in platelet activity. Second, blood samples from healthy volunteers were used with various antiplatelet agents, and thus our results cannot be directly extrapolated to pathological thrombus formation in cardiovascular patients.

Antiplatelet therapy has become the mainstay treatment of cardiovascular disease. In addition to aspirin and clopidogrel, a number of novel antiplatelet agents are being introduced to clinical practice. Recurrent coronary stent thrombosis is often attributed to insensitivity to antiplatelet therapy, but mechanisms and alternative treatment strategies, such as switching or combining drugs remain controversial (Breet et al., 2010; Maree and Fitzgerald, 2007). Important short falls of platelet aggregometry include the inability to assess antiplatelet agents in combination and the low shear environment, although newer whole blood assays are quicker and less labor-intensive than light-transmission aggregometry. The novel automated microchip flow-chamber system described here allows the assessment of PTF in whole blood under physiological and pathological shear rates. Further evaluations of this system are warranted for improved selection and monitoring of antiplatelet agents in the clinical setting.

Supplementary materials related to this article can be found online at doi:10.1016/j.mvr.2011.11.007.

Acknowledgments

This work was supported by the Japan Science and Technology Agency (JST).

References


