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Triflavin potentiates the antiplatelet activity of platelet activating factor receptor antagonist on activated neutrophil-induced platelet aggregation

Lin-Wen Lee [a], Hui-Chin Peng [b], Wun-Chang Ko [c], Wei-Chun Hung [c,d], Ching-Hua Su [a], Chien-Huang Lin [c], Tur-Fu Huang [b], Mao-Hsiung Yen [d], Joen-Rong Sheu [c,*]

[a] *Department of Microbiology and Immunology, Taipei Medical College, Taipei, Taiwan*
[b] *Institude of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan*
[c] *Graduate Institute of Medical Sciences and Department of Pharmacology, Taipei Medical College, Taipei, Taiwan*
[d] *Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan*

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Abstract

In this study, specific platelet activating factor (PAF) receptor antagonist ginkgolide B (BN52021) was tested for its antiplatelet activity in zymosan activated polymorphonuclear neutrophil-induced platelet aggregation. Triflavin was also tested for its antiplatelet activity compared with PAF receptor antagonist. Triflavin, an Arg–Gly–Asp-containing disintegrin purified from venom peptide inhibited platelet aggregation by interfering with the interaction of fibrinogen with the glycoprotein IIb/IIIa complex. Furthermore, we also report an efficient high resolution method for quantitative analysis of PAF using high-performance capillary electrophoresis (HPCE). The supernatant of polymorphonuclear neutrophils after their activation by opsonized zymosan induces the aggregation of washed rabbit platelets. In rabbit platelets, BN52021 (100–1000 μM) only partially inhibited activated polymorphonuclear neutrophil-induced platelet aggregation, and its maximal inhibition was estimated to be about 79%. Triflavin also partially inhibited platelet aggregation about 82% induced by activated polymorphonuclear neutrophils. Furthermore, after treatment with a combination of triflavin (0.26 μM) with various concentrations of BN52021 (4–1000 μM), the inhibitory effect of platelet aggregation was almost completely. This inhibition was greater than that produced by the individual drugs alone. These results indicate that a combination of glycoprotein IIb/IIIa complex and PAF receptor antagonist could completely inhibit activated polymorphonuclear neutrophil-induced platelet aggregation. In addition, the amount of PAF released from zymosan (6 mg/ml)-activated polymorphonuclear neutrophils was accurately calculated about 11.8 ± 1.5 ng/10^6 cells, and did not further increase even at a high concentration of zymosan (10 mg/ml). These results suggest that PAF play a major role in the interaction between platelets and polymorphonuclear neutrophils. This interaction may be important in the pathogenesis of thrombosis and inflammatory diseases. Our present findings support the hypothesis that combination therapy with glycoprotein IIb/IIIa complex antagonists and PAF receptor antagonists may represent a new approach to the treatment of ischemic disorders.

Keywords: PAF (platelet activating factor); Disintegrin; Triflavin; BN52021; Polymorphonuclear neutrophil; Platelet aggregation

1. Introduction

Thrombosis and inflammation are multicellular processes involving biochemical interactions between platelets and polymorphonuclear neutrophils (Marcus, 1990). Morphologic studies have long demonstrated the presence of leukocytes, mostly polymorphonuclear neutrophils, in histology sections of platelet-rich arterial thrombus (Henry, 1965). Aggregation platelets are known to cause strong activation of polymorphonuclear neutrophils either by released soluble mediators or simply by intercellular contact (Ruf et al., 1992). As a result, stimulated polymorphonuclear neutrophils release arachidonic acid derivatives, oxygen-derived free radicals and proteolytic enzymes. The overall impact of these polymorphonuclear neutrophil-derived substances on the dynamic process of thrombosis is still unresolved and subject to speculation.

Among the many products derived from activated polymorphonuclear neutrophils, platelet activating factor (PAF) is a phospholipid, which has been early recognized as a

* Corresponding author. Graduate Institute of Medical Sciences, Taipei Medical College, No. 250, Wu-Shing Street, Taipei 110, Taiwan. Tel.: +886-2-27390450; Fax: +886-2-27390450; E-mail: sheujr@tmc.edu.tw

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potent platelet agonist (O'flaherty et al., 1983). PAF is produced by platelets, monocytes, polymorphonuclear neutrophils and endothelial cells (Koltai et al., 1991). Its biological activities are multiple, including an effect on smooth muscle cell proliferation, a chemotactic and agonist activity on monocytes and neutrophils, and a direct toxicity on endothelial cells (Koltai et al., 1991). Specific PAF receptors have been identified on cell surfaces. Many PAF receptor antagonists such as ginkgolides or synthetic molecules, have been reported to compete with these receptors (Koltai et al., 1991). Some of them can be used safely in human being and may be effective in the therapeutic management of ischemic disorders.

Recently, many trigramin-like antiplatelet peptides (or disintegrins) have been reported (Gould et al., 1990). These peptides all contain Arg–Gly–Asp (RGD), are rich in cysteine, and bind with high affinity to integrins on the cell surface. A family of cell surface adhesion receptors termed 'integrins' has been described (Gould et al., 1990). The integrins comprise a superfamily of transmembrane receptors that participate in cell–cell and cell–substrata interactions. Integrin receptors are membrane-spanning heterodimers consisting of noncovalently associated α and β subunits (Kunicki, 1989). Trigramin, an RGD-containing peptide purified from the venom of *Trimeresurus gramineus*, is a specific fibrinogen receptor antagonist with a high binding affinity (K_d, 20 nM) for the activated platelet fibrinogen receptor (glycoprotein IIb/IIIa complex; $\alpha_{IIb}\beta_3$ integrin) (Huang et al., 1987). Triflavin is a trigramin-like antiplatelet peptide purified from *Trimeresurus flavoviridis* snake venom that is more potent than trigramin (Huang et al., 1991a,b; Sheu et al., 1997a). Its primary structure consists of 70 amino acid residues including 12 cysteines with an RGD sequence at positions 49–51 (Huang et al., 1991c). We previously reported that triflavin inhibits platelet aggregation by interfering with the interaction of fibrinogen with the glycoprotein IIb/IIIa complex (Huang et al., 1991c; Sheu et al., 1992). Binding of fibrinogen to the glycoprotein IIb/IIIa complex appears to be the final common pathway for platelet aggregation. Furthermore, we also demonstrated that triflavin has a more powerful influence on anti-thrombotic effect and anti-angiogenic activity in vivo and in vitro (Sheu et al., 1994, 1995, 1997b).

To resolve the pharmacological interventions directed against the interaction of polymorphonuclear neutrophils and platelets may represent a novel therapeutic approach. A major issue confronting current approaches to the pathogenesis and treatment of thrombosis is whether therapy directed against a single cell type or thrombus component will be satisfactory on thrombotic therapy? This study was designed to address this question by determining if Arg–Gly–Asp-containing disintegrin could markedly potentiate the effect of PAF receptor antagonist in activated polymorphonuclear neutrophil-induced platelet aggregation. On the other hand, the amount of polymorphonuclear neutrophil-derived PAF was also determined by high-performance capillary electrophoresis (HPCE) in this study.

2. Materials and methods

2.1. Purification of triflavin

Triflavin was purified from *T. flavoviridis* snake venom as previously described (Huang et al., 1991a). The procedure consisted of Fractogel TSK HW-50 gel filtration, CM-Sephadex C-50 column chromatography, gel filtration on Sephadex G-75 and G-50 columns. The last step of purification was accomplished on high-performance liquid chromatography (HPLC) reverse-phase C-18 column. The purified triflavin showed a single band, and its molecular mass was estimated to be 7500 Da on sodium dodecyl sulfate-polyacrylamide (SDS-PAGE; 20% gel).

2.2. Preparation of rabbit platelet suspensions

Blood was collected from healthy rabbits, and was mixed with 100 mM EDTA (1:15, v/v). EDTA–blood was immediately centrifuged at $120 \times g$ for 10 min at 25°C, and the supernatant (platelet-rich plasma) was retained. The platelet-rich plasma was followed by centrifugation at $500 \times g$ for 10 min at 25°C. The platelet was suspended in Tyrode's solution, pH 7.3 [containing (mM): NaCl (11.9), KCl (2.7), $MgCl_2$ (2.1), NaH_2PO_4 (0.4), $NaHCO_3$ (11.9) and glucose (11.1)]. Apyrase (1 U/ml) and prostaglandin E_1 (0.5 μM) were then added, and the mixture incubated for 10 min. The washing procedure was repeated. The washed platelets were finally suspended in Tyrode's solution containing bovine serum albumin (3.5 mg/ml) and adjusted to about 4.5×10^8 platelets/ml. The final concentration of Ca^{2+} in the Tyrode's solution was 1 mM.

2.3. Preparation of rabbit polymorphonuclear neutrophils

Blood anticoagulated with sodium heparin (10 U/ml) was collected in plastic tubes from healthy rabbits. Polymorphonuclear neutrophils were isolated using dextran sedimentation and Hypaque/Ficoll gradients as previously described (Gresele et al., 1986). Contaminating erythrocytes were removed by hypotonic lysis and polymorphonuclear neutrophils were washed twice with phosphate-buffered saline (PBS) (NaCl 131 mM, NaH_2PO_4 5 mM, KH_2PO_4 1.5 mM, pH 7.2). The washed polymorphonuclear neutrophils were finally resuspended in Tyrode's solution containing bovine serum albumin (3.5 mg/ml), and adjusted to various concentrations (1×10^6–1×10^8 cells/ml). Viability of the polymorphonuclear neutrophils was more than 95% ($n = 10$) by trypan blue dye exclusion test and the percentage of polymorphonuclear neutrophils in cell preparation was about 95–98%.

2.4. Activation of polymorphonuclear neutrophils

A total of 300 μl aliquots of various concentrations of polymorphonuclear neutrophil suspensions was activated by opsonized zymosan (6 mg/ml) at 37°C for 30 min. The suspension was then centrifuged at high speed ($1000 \times g$, 6 min, 25°C) in order to obtain a cell-free supernatant. The cell-free supernatant was stored at −80°C for subsequent assay. Complete dose–response curves were carried out on PMN preparations from each blood donor in order to minimize variability due to the different individual response of polymorphonuclear neutrophils to stimuli.

2.5. Platelet aggregation

The turbidimetric method (Born and Cross, 1963) with a Lumi-Aggregometer (Payton, Canada) was used to measure platelet aggregation. Platelet suspensions (0.4 ml) were prewarmed at 37°C for 2 min (stirring at 1200 rpm) in a silicone-treated glass cuvette. Triflavin and/or ginkgolide B (BN52021) were added 3 min before the addition of platelet-aggregation inducers (i.e., collagen or supernatant of activated polymorphonuclear neutrophils). The reaction was allowed to proceed for at least 6 min and the extent of aggregation was expressed as a percentage of the control.

2.6. HPCE analysis of PAF

HPCE was performed with a P/ACE 500 capillary electrophoresis system (Beckman) and a 5.7 cm × 75 μm i.d. Fused-silica capillary tubes. Detection was carried out at a position 530 nm from the injection end of the capillary by on-column measurements of ultraviolet (UV) absorption at 214 nm. Beckman system Gold software was used for data processing. The separations were performed at 25°C and 25 kV for 12 min.

The separation buffer was a solution of 0.1 M sodium phosphate and 0.1 M sodium borate adjusted to pH 9.0 with 1.0 M HCl. Prior to use, the buffer was filtered through a 0.2 μm membrane filter. All samples were introduced from the positive end of the capillary by vacuum for 3 s. When running several successive samples, the capillary was washed with 1.0 M NaOH for 4 min, followed by buffer for 10 min after each analysis. Identification of the PAF peak was made on using an authentic PAF and defined by relative migration time values. All separations were repeated at least 4 times to ensure the reproducibility.

2.7. Drugs

Collagen (bovine tendon type I), prostaglandin E_1, bovine serum albumin, heparin, sodium phosphate, sodium borate, EDTA and PAF were purchased from Sigma (St. Louis, MO, USA). Dextran T 500, Hypaque-Ficoll were obtained from Pharmacia (Uppsala, Sweden). Ginkgolide B (BN52021) was obtained from Biomol. Res. Lab. (Plymouth Meeting, PA, USA). *T. flavoviridis* venom was purchased from Latoxan (Rosans, France). ReoPro™ was obtained from Eli Lilly (Indianapolis, IN, USA).

3. Results

3.1. Effect of the supernatant of activated polymorphonuclear neutrophils on the aggregation of washed rabbit platelets

The cell-free supernatant of zymosan (6 mg/ml)-activated polymorphonuclear neutrophils dose-dependently induced platelet aggregation in washed rabbit platelet suspensions (5×10^7 cells/ml) (Figs. 1 and 2). In this study, 5 μl of supernatant significantly induced platelet aggregation. A maximal aggregation was reached at 30 μl of supernatant as compared with collagen (5 μg/ml). The EC_{50} value for inducing platelet aggregation was estimated to be about 12.5 μl of supernatant from activated polymorphonuclear neutrophils (5×10^7 cells/ml).

3.2. Effect of triflavin and PAF receptor antagonist BN52021 on platelet aggregation induced by the supernatant of activated polymorphonuclear neutrophils

In our previous studies (Huang et al., 1991a,b), triflavin completely inhibited agonist-induced platelet aggregation such as collagen, ADP, thrombin, epinephrine and prostaglandin endoperoxide analogue compound, 9,11-dideoxy-9α, 11α-methanoepoxy $PGF_{2\alpha}$ (U46619), not only in washed platelet suspensions but also in platelet-rich plasma and whole blood. We concluded that triflavin inhibits platelet aggregation by interfering with fibrinogen binding to its specific receptor associated with the glycoprotein IIb/IIIa complex on the platelet surface membrane (Sheu et al., 1992). In this study, triflavin (0.13–4.08 μM) inhibited platelet aggregation in a dose-dependent manner stimulated by the supernatant (30 μl) of activated polymorphonuclear neutrophils (5×10^7 cells/ml) in washed rabbit platelet suspensions (Fig. 3). The IC_{50} was estimated to be about 1.0 μM. However, triflavin only partially inhibited platelet aggregation in this reaction, and its maximal inhibition was about 82%. The inhibitory activity of triflavin did not further increase even at a higher concentration (20 μM) or prolonged the incubation time (30 min) (data not shown). It is unlikely that triflavin can completely inhibit platelet aggregation induced by various agonists (i.e., ADP, collagen, thrombin and U46619) as described previously (Huang et al., 1991a). ReoPro™ (Abciximab) is the Fab fragment of the chimeric human-murine monoclonal antibody 7E3. It binds to the glycoprotein IIb/IIIa complex of platelets and inhibits platelet aggregation (Coller and Scudder, 1985). We found that

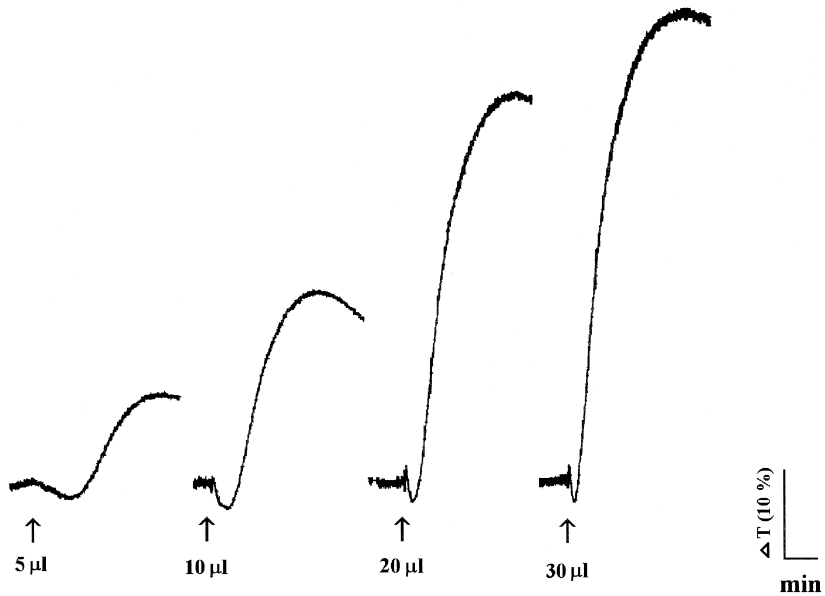

Fig. 1. Typical patterns of the supernatant of zymosan-activated polymorphonuclear neutrophils on platelet aggregation of washed rabbit platelet suspensions. Polymorphonuclear neutrophil suspensions (5×10^7 cells/ml) were activated by opsonized zymosan (6 mg/ml) at 37°C for 30 min followed by centrifugation. The various volumes of cell-free supernatants were then added into the platelet suspensions (4.5×10^8 cells/ml) to trigger platelet aggregation. The pattern is a representative example of four similar experiments.

ReoPro™ (200 μg/ml) showed a maximal inhibition about 40% in this reaction, and its inhibitory activity did not increase at a higher concentration (500 μg/ml) (data not shown). These results indicate that glycoprotein IIb/IIIa antagonists did not completely inhibit platelet aggregation induced by the supernatant of zymosan-activated polymorphonuclear neutrophils. Moreover, Fig. 3 also reveals the inhibitory effect of PAF receptor antagonist on activated polymorphonuclear neutrophil-induced platelet aggregation. In this experiment, washed platelets were preincubated with dimethyl sulfoxide (DMSO) (0.5%) or PAF receptor antagonist BN52021, a potent member of the ginkgolide family of PAF receptor antagonists (Braquet et al., 1991). BN52021 partially inhibited platelet aggregation induced by the supernatant of activated polymorphonuclear neutrophils at concentrations varying from 100 μM to 1 mM, and its maximal inhibition was about 79%. The inhibitory activity of BN52021 did not further increase

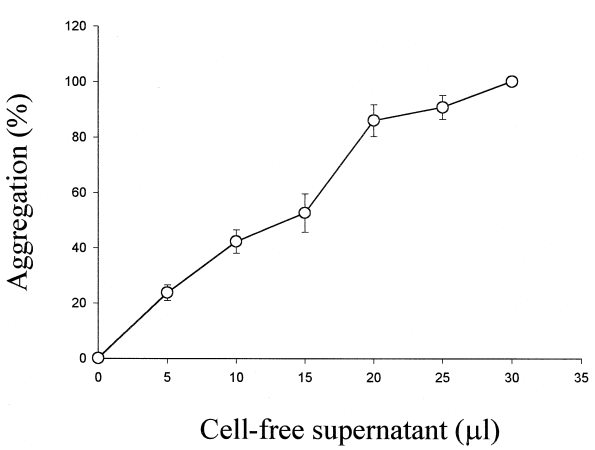

Fig. 2. Dose–response curve of the supernatant of zymosan-activated polymorphonuclear neutrophils on platelet aggregation in washed rabbit platelet suspensions. For detailed, see Fig. 1 legend. Data are presented as percentage of the collagen (5 μg/ml)-induced platelet aggregation (means ± S.E.M., $n = 4$).

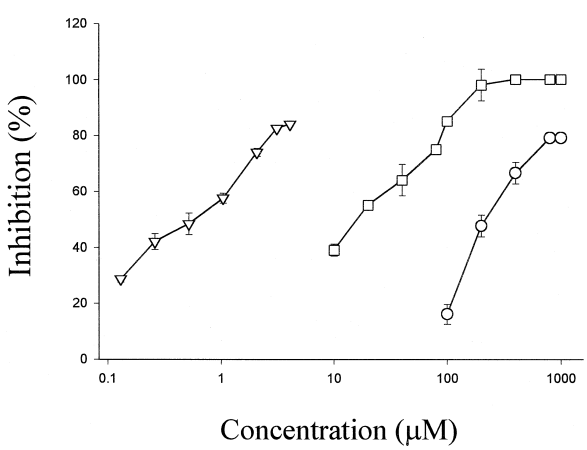

Fig. 3. Dose–inhibition curves of triflavin and/or ginkgolide B (BN52021) at inhibition the supernatant of zymosan-activated polymorphonuclear neutrophil-induced platelet aggregation. Platelets (4.5×10^8 cells/ml) were preincubated with various concentrations of triflavin (0.13–4.08 μM; ▽) or BN52021 (100–1000 μM; ○), or a combination of fixed concentration of triflavin (0.26 μM) with various concentrations of BN52021 (10–1000 μM; □) at 37°C for 3 min, then 30 μl aliquots of the cell-free supernatant of zymosan-activated polymorphonuclear neutrophils (5×10^7 cells/ml) were added to trigger platelet aggregation. Data are presented as percentage of the inhibition as compared with control (30 μl aliquots of the supernatant of activated polymorphonuclear neutrophils without triflavin or BN52021), and expressed by means ± S.E.M. ($n = 5$).

even a prolonged the incubation time (30 min) (data not shown). The IC_{50} of platelet aggregation was estimated to be about 443.6 μM. This result implies that the supernatant of zymosan-activated polymorphonuclear neutrophils may be involved in a PAF-independent pathway. When triflavin (0.26 μM) was combined with various concentrations of BN52021 (4–1000 μM), the inhibitory effect of platelet aggregation was almost completely as in the presence of BN52021 (200 μM), and was greater than that produced by the individual drugs alone (Figs. 3 and 4A). The IC_{50} (μM) value of BN52021 in the presence of a fixed concentration of triflavin (0.26 μM) was estimated to be about 6.2 μM. On a molar basis, the inhibitory activity of BN52021 in the presence of triflavin (0.26 μM) was 70-fold more potent than BN52021 alone, as compared with IC_{50} values. According to the work of Berenbaum (1990) described previously, let the concentrations of triflavin and BN52021, that each produces 50% inhibitory effect (IC_{50}) when they were used alone, be called A_e and B_e, and let A_c and B_c be their concentrations when used in combination. Then, $A_c/A_e + B_c/B_e = 0.26/1.0 + 6.2/443.6 = 0.27$. The sum is < 1, it thus appears that the combination is synergistic. Moreover, the antiplatelet effect of a combination of triflavin with BN52021 also revealed a synergistic effect in pure PAF (80 ng/ml)-induced platelet aggregation (Fig. 4B), however, the synergistic effect was not observed in non-PAF (collagen, 5 μg/ml)-induced platelet aggregation (Fig. 4C). These results suggest that a combination of glycoprotein IIb/IIIa antagonists and PAF receptor antagonists may completely inhibit activated polymorphonuclear neutrophil-induced thrombosis in vivo.

3.3. Quantification of PAF in zymosan-activated polymorphonuclear neutrophils

Accurate quantification of PAF was extremely important to this study. In general, PAF was quantified after extraction and purification by thin layer chromatography (TLC) and HPLC as previously reported (Bussolati et al., 1997). However, these methods are experimentally rather complicated, relatively expensive and time-consuming, and proper identification of PAF in a mixture can be difficult. This paper is the first report a high resolution, fast, simple and efficient method for analysis of PAF using HPCE. This technique has a higher peak capacity, resolution and improved reproducibility compared with HPLC. Identification of the peak, corresponding to the PAF compound, was

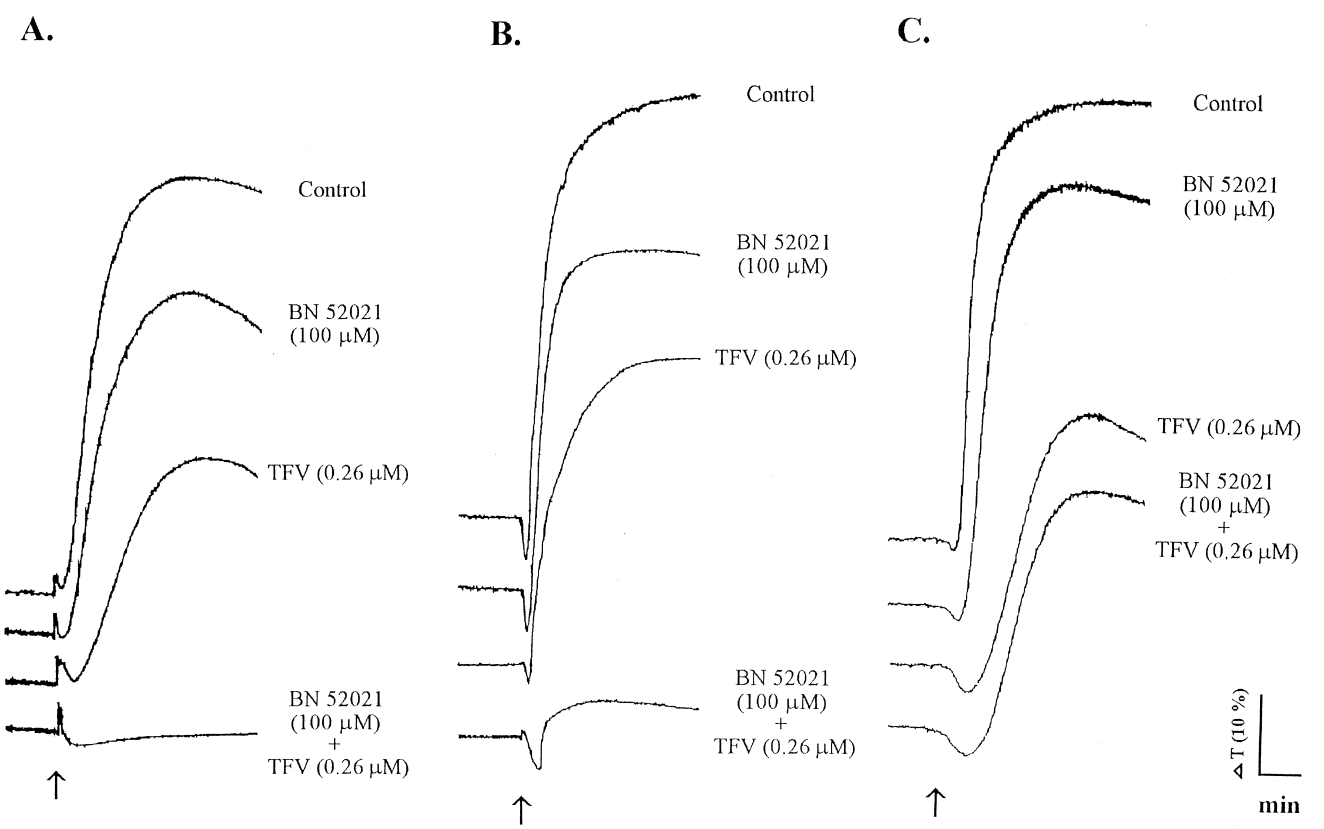

Fig. 4. Typical patterns of triflavin potentiates the inhibitory effect of BN52021 on washed rabbit platelet suspensions induced by the supernatant of zymosan-activated polymorphonuclear neutrophils, pure PAF, and collagen. Platelets (4.5×10^8 cells/ml) were preincubated with triflavin (0.26 μM) or BN52021 (100 μM) or a combination of triflavin (0.26 μM) with BN52021 (100 μM) at 37°C for 3 min, then (A) 30 μl of the cell-free supernatant of zymosan-activated polymorphonuclear neutrophils (5×10^7 cells/ml), (B) pure PAF (80 ng/ml), and (C) collagen (5 μg/ml) were added to trigger platelet aggregation. The pattern is a representative example of four similar experiments.

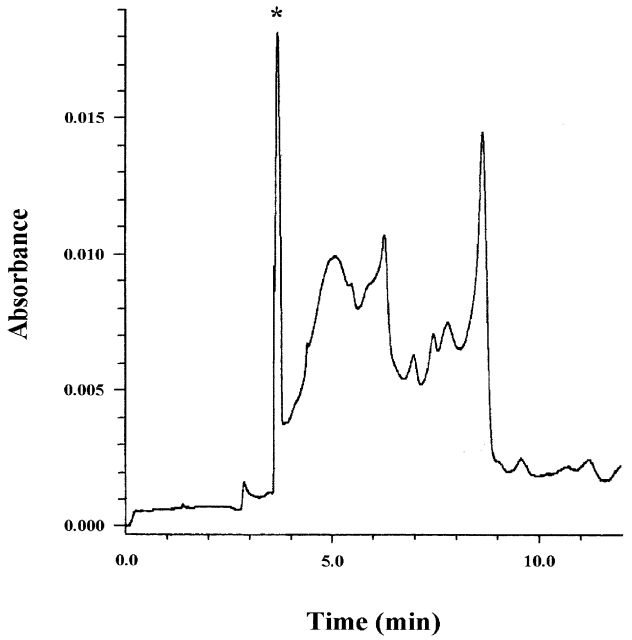

Fig. 5. The profile of quantitative analysis of PAF by using HPCE system. Buffer composition of 0.1 M sodium borate and sodium phosphate, pH adjusted to 9.0 with 1.0 M HCl; temperature 25°C; voltage 25 kV; total length of capillary 5.7 cm and detection 530 nm from injection end; ultraviolet detection at 214 nm. Vacuum injection for 3 s. One peak of PAF compound was eluted at about 3.8 min (*).

based on the use of authentic PAF and defined it by a relative migration time value. The relative migration time of the PAF peak was eluted about 3.8 min (indicated by *) (Fig. 5). Further characterization of this peak revealed it be that of PAF compound. Authentic PAF compound and samples were mixed and applied to HPCE under the same conditions. We found that the peak eluted at 3.8 min was superimposed, indicating that this peak corresponded to PAF compound.

Fig. 6 shows the regression line of cell number of polymorphonuclear neutrophils vs. total amount of PAF released from zymosan-activated polymorphonuclear neutrophils. A correlation coefficient (r) of about 0.98 was obtained from this line (Fig. 6). This result reveals that no variation in the ratio of cells associated with the release of PAF was observed. The amount of PAF released from zymosan (6 mg/ml)-activated polymorphonuclear neutrophils was calculated to be about 11.8 ± 1.5 ng/10^6 cells ($n = 10$). The PAF concentration did not further increase even at a higher concentration of zymosan (10 mg/ml) (data not shown). This indicated a high level of accuracy in the quantitative analysis of PAF concentration using HPCE.

4. Discussion

Evaluation of platelet responses in the presence of autologous polymorphonuclear neutrophils may represent an in vitro model to investigate the multicellular processes involved in thrombosis and inflammation (Marcus, 1990). Multicellular in vitro systems may help to better define the effectiveness and mode of action of new anti-thrombotic and anti-inflammatory drugs.

Activation of polymorphonuclear neutrophils participates in platelet activation and aggregation. Nguyen et al. (1995) reported that preincubation with opsonized zymosan potentiates ADP-induced aggregation in whole blood. We have thus used a model in which purified polymorphonuclear neutrophils are first activated by opsonized zymosan. A cell-free supernatant is then obtained and tested on washed platelets. The cell-free supernatant induces the aggregation of normal platelets in Tyrode's solution in a dose-dependent manner. Polymorphonuclear neutrophil–platelet interaction has been described previously (Coeffier et al., 1987) and after activation by chemotactic agents (Del Maschio et al., 1990). There is evidence that PAF is involved in this interaction. Therefore, we have thus hypothesized that PAF could play a role in this model and that platelet responses were indirectly triggered through polymorphonuclear neutrophils stimulation with zymosan. PAF is a phospholipid that is synthesized by most circulating cells particularly neutrophils, monocytes and platelets (Koltai et al., 1991). PAF receptor inhibitors are present in the plasma (Billah et al., 1986) which may explain its lack of action in plasma and whole blood.

In this study, to demonstrate the involvement of PAF in activated polymorphonuclear neutrophil-induced platelet aggregation, we used a ginkgolide compound BN52021 to compete with PAF on specific platelet membrane receptors. The results showed that BN52021 only partially

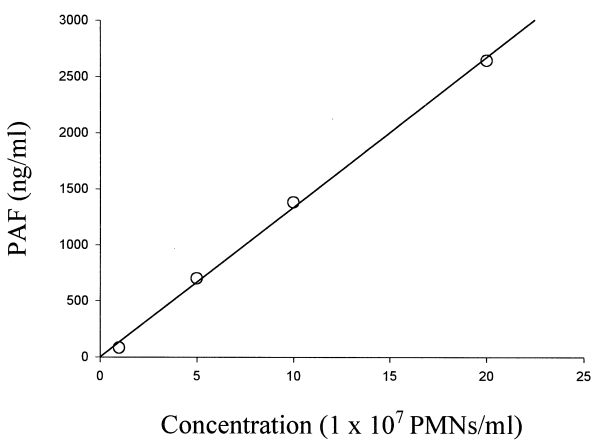

Fig. 6. The linear regression line of the amount of PAF released vs. various concentrations of polymorphonuclear neutrophils stimulated with zymosan. Various concentrations of polymorphonuclear neutrophils (1–20×10^7 cells/ml) were activated by zymosan (6 mg/ml) at 37°C for 30 min followed by centrifugation. The cell-free supernatants were applied into HPCE system to quantitative analysis of PAF. For detailed see Section 2. The line is a representative example of five similar experiments.

inhibited the activated polymorphonuclear neutrophil-induced platelet aggregation over a wide range of concentrations (100–1000 μM) (Fig. 3). This indicates that part of the PAF-independent pathway is involved in activated polymorphonuclear neutrophil-induced platelet aggregation. Indeed, it has been demonstrated that activated polymorphonuclear neutrophil-derived secretory products, such as oxygen free radical, elastase, and, more importantly, cathepsin G, may contribute to complete platelet activation (Selak et al., 1988). This may explain why BN52021 only partially inhibited platelet aggregation in this model even at a higher concentration (1 mM). However, we did not investigate the participation of cathepsin G in this experiment, the extent of cathepsin G involvement will require further study. Nevertheless, these results clearly indicate that the effect of polymorphonuclear neutrophil activation can, at least partially, be attributed to the production of PAF.

Triflavin, a specific glycoprotein IIb/IIIa complex antagonist, completely inhibited various agonist-induced platelet aggregations in our previous experiments (Huang et al., 1991a). In this study, triflavin showed a partially inhibitory effect (82%). A similar result was found using ReoPro™, a monoclonal antibody directed against glycoprotein IIb/IIIa complex. ReoPro™ was a less effective than triflavin at inhibition of activated polymorphonuclear neutrophil-induced aggregation (40%) (data not shown). These results imply that activated polymorphonuclear neutrophil-induced platelet aggregation may also be involved in a glycoprotein IIb/IIIa complex-independent mechanism. However, the exact mechanism is still unclear and requires further characterization. Therefore, in polymorphonuclear neutrophil-mediated platelet activation, different secretory products can interplay to make inhibition with triflavin or BN52021 alone more difficult. In contrast, it is noteworthy that the presence of a lower concentration of triflavin (0.26 μM) markedly potentiated the activity of BN52021 both in washed platelets (Figs. 3 and 4) and platelet-rich plasma (data not shown).

In conclusion, there were two important findings in our studies. (1) When tested alone, both glycoprotein IIb/IIIa antagonist and PAF receptor antagonist partially inhibited platelet aggregation induced by secretory products from activated polymorphonuclear neutrophils. A combination of both drugs resulted in a markedly synergistic effect. (2) A novel accurate method to quantify the amount of PAF released from polymorphonuclear neutrophils by using a HPCE system was presented. Activated polymorphonuclear neutrophils may cause damage to the vessel wall and platelet aggregation, which subsequently enhances thrombosis and inflammation. Therefore, the interaction between polymorphonuclear neutrophils and platelets may play an important role in thrombosis and inflammatory diseases. Our present findings support the hypothesis that a combination of glycoprotein IIb/IIIa complex antagonists and PAF receptor antagonists might represent a new therapeutic approach when individual drugs are not capable of completely inhibiting platelet aggregation.

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