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Biological Effect of Orally Active Platelet-Activating Factor Receptor Antagonist SM-10661

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SUMMARY

SM-10661 [(±)-(*cis*)-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one HCl] displayed marked *in vitro* inhibition of rabbit platelet aggregation induced by 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkyl-PAF), 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (C16-PAF), and 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, with IC_{50}, values of 5.50, 5.94, and 3.68 μM, respectively. It also inhibited alkyl-PAF-induced aggregation of human platelets with an IC_{50} of 3.00 μM, but it did not inhibit platelet aggregation induced by ADP, collagen, arachidonic acid, the thromboxane A_2 agonist U46619, or the Ca ionophore A23187, at concentrations up to 400 μM. Furthermore, SM-10661 antagonized [^{3}H]-C16-PAF binding to rabbit platelets competitively, with an IC_{50} of 1.0 μM. SM-10661 protected against alkyl-PAF-induced lethality in mice with an ID_{50} of 6.0 mg/kg intravenously or 24 mg/kg orally. In guinea pig, SM-10661 inhibited the alkyl-PAF (0.1 μg/kg)-induced increase in bronchial pressure, with an ID_{50} of 0.7 mg/kg intravenously or 15 mg/kg orally. Bronchial hyperreactivity to bombesin after the infusion of alkyl-PAF was also inhibited dose-dependently by the infusion of SM-10661, with an ID_{50} of 25 mg/kg. In addition, SM-10661 inhibited alkyl-PAF (0.01 μg/kg)-induced hypotension in rats, with an ID_{50} of 0.36 mg/kg intravenously or 33 mg/kg orally. SM-10661, when given orally, showed rapid absorption and good duration of pharmacological activity in rats and rabbits.

PAF (1-*O*-alkyl-2-acetyl-*sn*-glycerophosphoryl choline) is a phospholipid mediator that shows powerful platelet-stimulating properties (1). It also has several pharmacological actions such as induction of neutrophil aggregation, bronchoconstriction, and hypotension, increases vascular permeability (2, 3), and is believed to be involved in anaphylaxis, shock, inflammation, and allergic responses. The development of specific PAF antagonists is, therefore, essential for further elucidation of the pathophysiological significance of this mediator. Recently, several PAF antagonists have been reported, which include natural products (4–8) and synthetic compounds (9–14).

We obtained evidence of the potency and selectivity of a newly developed PAF antagonist, SM-10661 [(±)-(*cis*)-3,5-dimethyl-2-(3-pyridyl)-thiazolidin-4-one HCl] (15). Here we describe *in vitro* properties and *in vitro* activities of SM-10661 in mice, rats, guinea pigs, and rabbits.

Experimental Procedures

Materials

SM-10661 (Fig. 1) was synthesized by Sumitomo Pharmaceuticals Co., Ltd.. Structure and purity (>97%) were determined by ^{1}H and ^{13}C NMR, microanalysis, high pressure liquid chromatography, and thin layer chromatography. Alkyl-PAF, C16-PAF, and C18-PAF were purchased from Funakoshi Chemicals (Tokyo, Japan), BSA, ADP sodium salt, and arachidonic acid sodium salt from Sigma Chemicals Co. (St. Louis, MO), Ca ionophore A-23187 from Calbiochem Co. (La Jolla, CA), U-46619 from Cayman Chemical (Denver, CO), ACS II from Amersham Japan, and 1-*O*-(hexadecyl-1′,2′-^{3}H(N))-2-acetyl-*sn*-glyceryl-3-phosphorylcholine from NEN Research Products (Boston, MA). PAF was dissolved in phosphate-buffered saline containing 0.25% BSA. SM-10661 was dissolved in saline for the purpose of *in vitro* studies or intravenous administration to animals. When it was administrated orally to animals, it was dissolved in water.

In Vitro Determinations

Platelet aggregation. The effect of SM-10661 on platelet aggregation was determined according to the method of Born (16). Blood was collected from unanesthetized rabbits or humans into a 30-ml plastic syringe containing 3.8% sodium citrate as anticoagulant, in the volume proportion 9:1 (blood to citrate). An aliquot of human or rabbit platelet-rich plasma (0.2 ml at approximately 2.5×10^8 platelets/μl) was incubated in a cuvette at 37° and stirred at 900 rpm in an aggregometer. Varying concentrations of SM-10661 were added to the cuvette 2 min before 0.02 μM concentrations of the three kinds of PAF, 22 μM ADP, 10 μg/ml collagen, 19 μM A-23187, 310 μM arachidonic acid, or 2.9 μM U46619. Aggregation was allowed to proceed for 5 min and was monitored by turbidity changes in light transmission and quantified by measurement of the peak height from chart tracings. An

ABBREVIATIONS: PAF, platelet-activating factor; alkyl-PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; C16-PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; C18-PAF, 1-*O*-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; BSA, bovine serum albumin; MAP, mean arterial pressure; EGTA, ethylene glycol bis(β-amino ethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

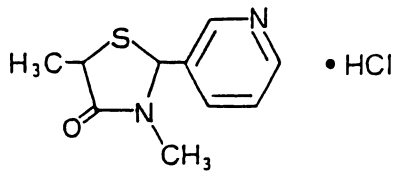

Fig. 1. Structure of SM-10661, (±)-(*cis*)-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one HCl.

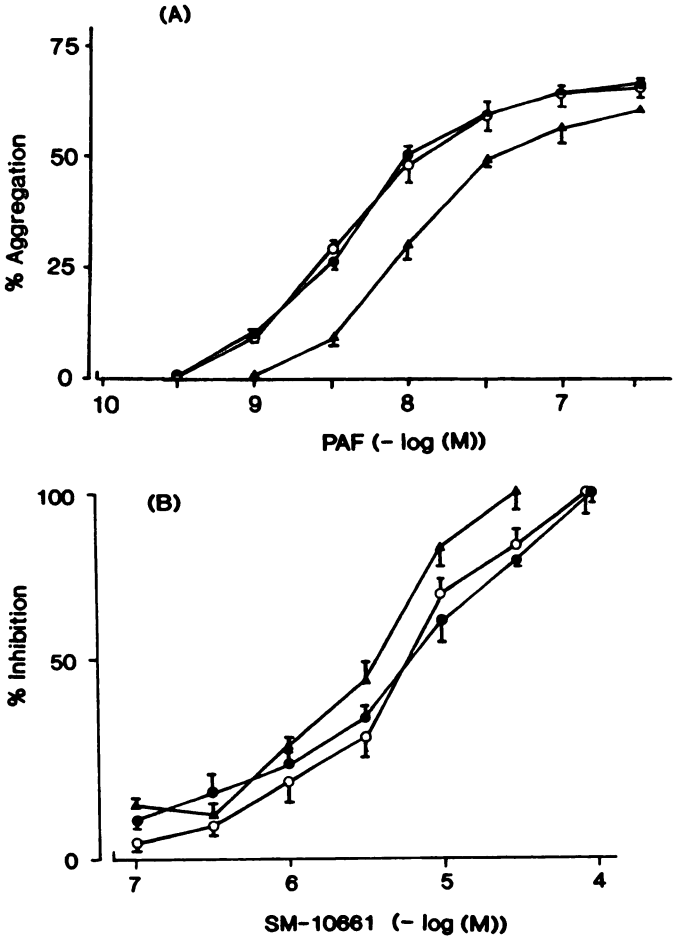

Fig. 2. A, Rabbit platelet aggregation induced by alkyl-PAF (○), C16-PAF (●), and C18-PAF (▲). B, Dose-related inhibition by SM-10661 of alkyl-PAF- (○), C16-PAF- (●), or C18-PAF- (▲) induced platelet aggregation. The aggregation assay was performed as described in Experimental Procedures. Each value represents the mean ± standard error of three determinations.

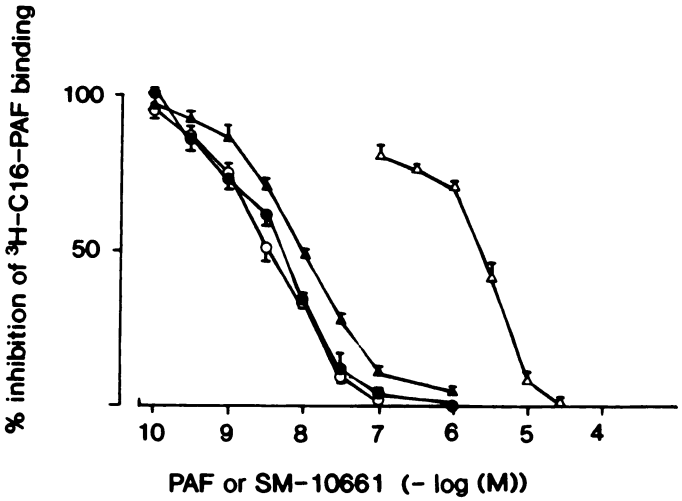

Fig. 3. Inhibitory effects of alkyl-PAF (○), C16-PAF (●), C18-PAF (▲), and SM-10661 (△) on [^{3}H]C16-PAF binding to rabbit washed platelets. The binding assay was performed as described in Experimental Procedures. Each value represents the mean ± standard error of three determinations.

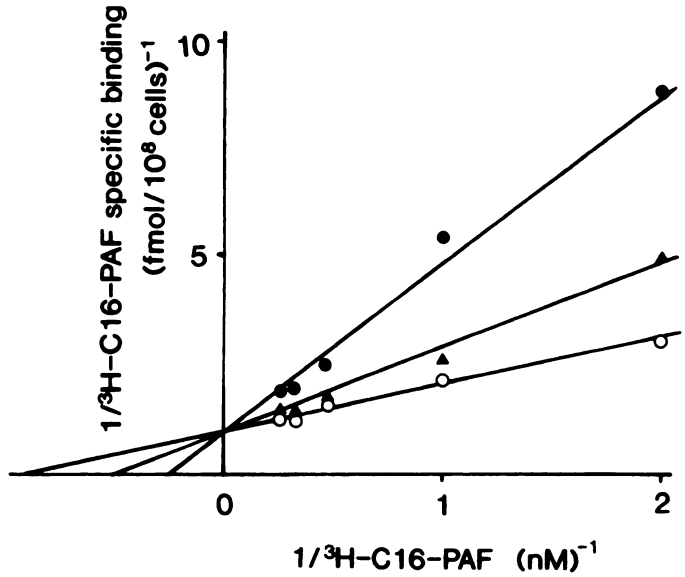

Fig. 4. Lineweaver-Burk plot of competitive inhibition of [^{3}H]C16-PAF binding by SM-10661. Rabbit platelets were incubated with increasing concentrations of [^{3}H]C16-PAF (from 0.50 to 4.00 nM) in the absence (○) or presence of 1.0 μM (▲) or 3.0 μM (●) SM-10661. Each point is the mean of three determinations.

IC_{50} value was generated from regression analysis of the dose-response curve.

[^{3}H]C16-PAF binding to platelets. The [^{3}H]C16-PAF binding assay was carried out according to the method of Homma *et al.* (17). Briefly, washed rabbit platelets were prepared and incubated with a specific radioligand, at a final concentration of 1.0 nM, in Tyrode's buffer containing 0.1% BSA and 0.1 mM EGTA, for 10 min at 22°. Free ligand was separated by suction on a Whatman GF/C filter, and the radioactivity was counted in ACS II. Specific binding was calculated as the difference in radioactivity between total binding and nonspecific binding determined in the presence of excess (1000-fold) unlabeled C16-PAF.

In Vivo Determinations

Animals were housed for at least 7 days in our animal quarters before the experiments. They were kept at a constant ambient temperature of 20° and were fed a standard diet (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum*. Experiments were carried out during the same time interval (10:00 a.m. to 4:00 p.m.) to minimize diurnal variations.

Mouse lethality. Alkyl-PAF (60 μg/2.0 ml/kg) was administered intravenously to mice (ICR, male, 23–28 g; Charles River, Japan Inc.). SM-10661 was given to mice intravenously 2 min or orally 60 min before administration of alkyl-PAF. The percentage of mortality was recorded up to 24 hr.

Rat hypotension. Alkyl-PAF-induced hypotension was carried out according to the method of Handley *et al.* (18). Male Wistar rats (KBL, Japan Inc.) weighing 200 to 250 g were anesthetized with urethane (1.2 g/kg, 5 ml/kg, i.p.). Indwelling catheters were inserted in the left carotid artery and right jugular vein. A catheter, placed in the left carotid artery was connected to a pressure transducer (Physiological Pressure, Transducer 1280; Hewlett Packard). MAP was measured continuously with the pressure transducer connected to an amplifier (Hewlett Packard) and a recorder (Yokogawa Electric). SM-10661 was given intra-

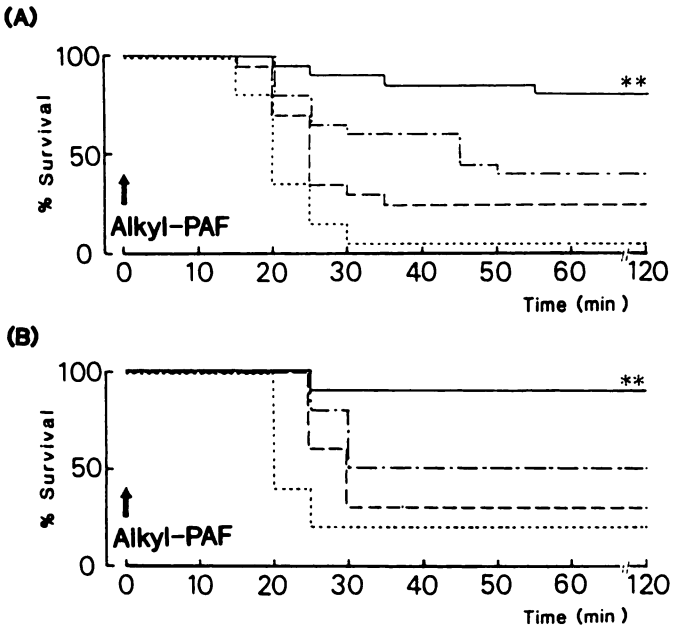

Fig. 5. Effect of SM-10661 on alkyl-PAF-induced lethality in mice. A, SM-10661 (– · –, 10 mg/kg; – – –, 5 mg/kg; · · · ·, 2 mg/kg) or saline (——) was administered to mice intravenously. Two minutes later, alkyl-PAF (60 μg/kg) was administered intravenously. B, SM-10661 (– – –, 50 mg/kg; – – –, 20 mg/kg; · · · ·, 10 mg/kg) or saline (——) was administered to mice orally. Sixty minutes later, alkyl-PAF was administered intravenously. Each group consisted of 10 to 20 mice. Statistical significance, **, $p < 0.01$.

venously 2 min or orally 60 min before intravenous administration of alkyl-PAF (0.01 μg/kg). Inhibition was calculated from the lowest levels of the MAP of rats given alkyl-PAF alone (control).

Guinea pig increase in bronchial pressure. Alkyl-PAF-induced increases in bronchial pressure were determined according to the method of Konzett and Rossler (19). Male Hartley guinea pigs (Japan SLC, Inc.) weighing 400 to 500 g were anesthetized with pentobarbital (30 mg/kg intraperitoneally) and phenobarbital (120 mg/kg intraperitoneally). Animals were ventilated (60 strokes/min, 5–7 ml/kg) with air supplemented with O_2 (50%, v/v), through an intratracheal cannula. Indwelling catheters were inserted in the left carotid artery and right jugular vein. As an index of pulmonary function, respiratory flow was measured with a pneumotachograph, a bronchospasm transducer (Ugo Basile), and a recorder (Ugo Basile). MAP was measured continuously as described above in Rat hypotension. SM-10661 was given intravenously after the animal was paralyzed with gallamine (10 mg/kg, intramuscularly), followed by 0.08 μg/kg intravenous administration of alkyl-PAF. For oral administration, SM-10661 was given 1 hr before administration of alkyl-PAF.

Guinea pig bronchial hyperreactivity. Alkyl-PAF-induced bronchial hyperreactivity was determined (20–22). Male Hartley guinea pigs (Japan SLC, Inc.) weighing 400 to 500 g were anesthetized with pentobarbital (30 mg/kg intraperitoneally) and phenobarbital (120 mg/kg intraperitoneally). Animals were ventilated (60 strokes/min, 5–7 ml/kg) with air supplemented with O_2 (50%, v/v). Pulmonary inflation pressure was measured as described in Guinea pig increase in bronchial pressure. Alkyl-PAF (3 μg/kg) was infused into the right jugular vein for 1 hr. Bronchial reactivity was assessed by intravenous administration of bombesin (300 ng/kg). SM-10661 was administered by intravenous bolus and infusion.

***Ex vivo* rabbit platelet aggregation.** SM-10661 was given orally or intravenously to rabbits. Aliquots of platelet-rich plasma were collected at varying times and alkyl-PAF-induced platelet aggregation (10^{-10} to 10^{-5} M) was monitored. Pharmacological activity of SM-10661 in blood was indicated by the alkyl-PAF concentration required to induce half maximal aggregation (EC_{50}).

Statistical Analysis

The values in the text and figures represent the mean ± standard error. The inhibitory effects and the reversing effects of SM-10661 on alkyl-PAF-induced hypotension, increases in bronchial pressure, and bronchial hyperreactivity were evaluated with analysis of variance, and individual differences between the vehicle-treated group and SM-10661-treated groups were tested by Student's t test. The χ^2 test was used for the statistical analysis of the protecting effects of drugs on alkyl-PAF-induced death in mice. p values of less than 0.05 were considered significant.

Results

Effect of SM-10661 on PAF-induced aggregation of rabbit and human platelets. Three kinds of PAF-induced aggregation of platelets are indicated in Fig. 2A. The EC_{50} values for alkyl-PAF, C16-PAF, and C18-PAF were 5.37, 5.70, and 15.2 nM, respectively. SM-10661 at final concentrations of 0.1 to 10.0 μM inhibited the three kinds of PAF-induced platelet aggregation in a concentration-dependent manner, and the IC_{50} values of SM-10661 for alkyl-PAF-, C16-PAF-, and C18-PAF-induced aggregation of rabbit platelets were 5.50, 5.94, and 3.68 μM, respectively (Fig. 2B). The IC_{50} value for alkyl-PAF-induced aggregation of human platelets was 3.00 μM. SM-10661

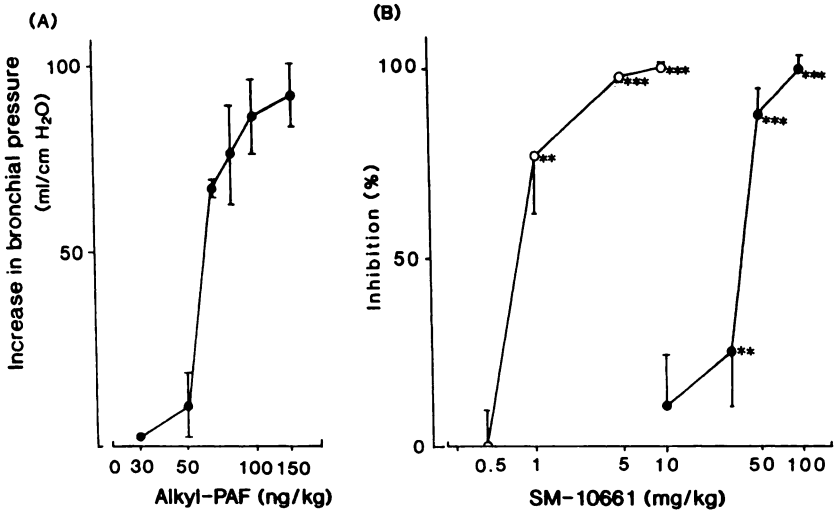

Fig. 6. A, Effect of intravenously administered alkyl-PAF on the increase in bronchial pressure in guinea pigs. B, Inhibition by SM-10661 of the alkyl-PAF-induced increase in bronchial pressure in guinea pigs. SM-10661 was given intravenously 2 min (○) or orally 60 min (●) before administration of alkyl-PAF. Each value represents the mean ± standard error of five to eight animals/dose. Statistical significance, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

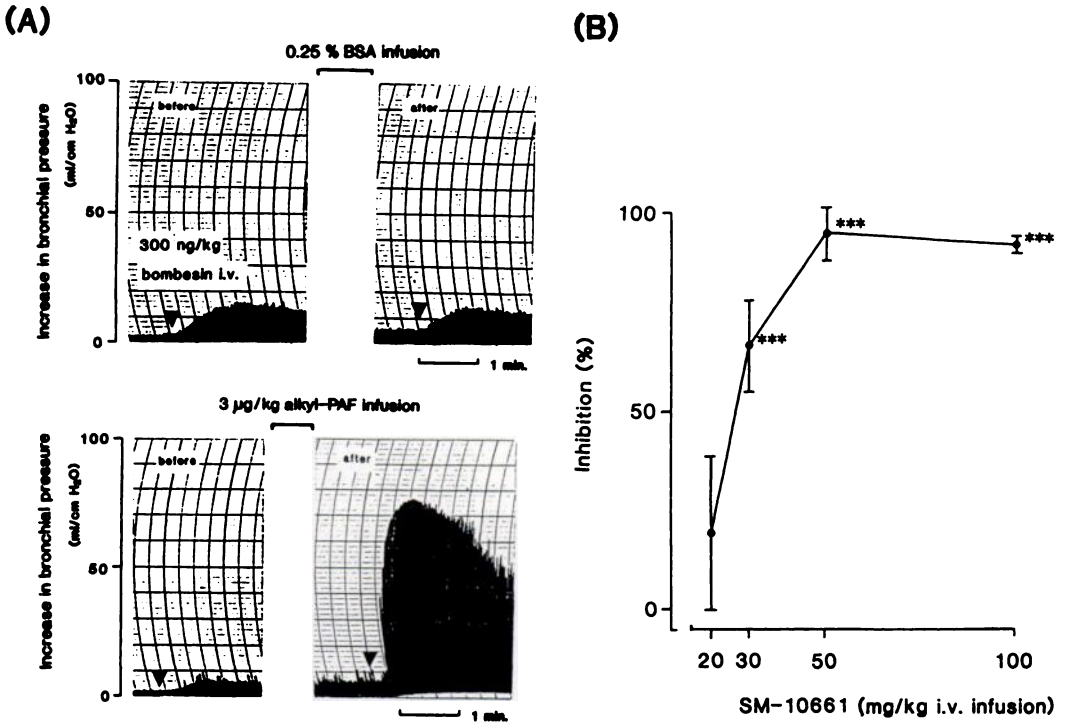

Fig. 7. A, Bronchial hyperreactivity following intravenous administration of bombesin (300 ng/kg) in guinea pigs 1 hr after infusion of 0.25% BSA (control) (*upper*) or alkyl-PAF (3 μg/kg) (*lower*). B, Inhibition by SM-10661 of alkyl-PAF-induced bronchial hyperreactivity. SM-10661 was infused for 1 hr together with alkyl-PAF. Each value represents the mean ± standard error of four to seven animals. Statistical significance, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

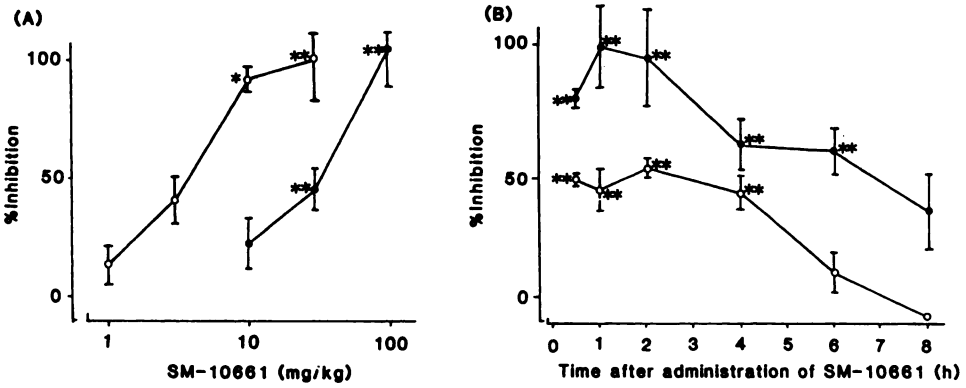

Fig. 8. Inhibition by SM-10661 of alkyl-PAF-induced hypotension in rats. A, SM-10661 was administered orally 60 min (●) or intravenously 2 min (○) before anesthesia, followed by intravenous administration of alkyl-PAF (10 ng/kg). B, SM-10661 was administered orally to rats at 100 (●) or 30 mg/kg (○) and alkyl-PAF was given to rats at various times. Each value represents the mean ± standard error of three rats. Statistical significance, *, $p < 0.05$; **, $p < 0.01$.

at a high concentration of 400 μM showed little inhibitory effect on the aggregation of rabbit and human platelets induced by ADP, collagen, arachidonic acid, the thromboxane A_2 agonist U46619, or the Ca ionophore A23187.

Effect of SM-10661 on [^{3}H]C16-PAF binding to washed rabbit platelets. First, inhibitory effects of the three kinds of PAF on [^{3}H]C16-PAF (1.0 nM) binding to washed rabbit platelets were determined (Fig. 3A). [^{3}H]C16-PAF binding was inhibited in a concentration-dependent manner, and the IC_{50} values for alkyl-PAF, C16-PAF, and C18-PAF were 3.43, 4.02, and 9.73 nM, respectively. SM-10661 competed with [^{3}H]C16-PAF with an IC_{50} value of 1.0 μM (Fig. 3B).

Rabbit platelets were incubated with increasing concentrations of [^{3}H]C16-PAF (0.5 to 4.0 nM) in the absence and in the presence of two concentrations of SM-10661 (1.0 and 3.0 μM) (Fig. 4). The K_d values, calculated from the analysis of the data by a Lineweaver-Burk plot, were 0.93 nM in the absence of SM-10661 and 1.96 and 4.02 nM in the presence of 1.0 and 3.0 μM SM-10661, respectively. Scatchard analysis confirmed that the B_{max} was not modified at these concentrations of SM-10661. These data indicate that SM-10661 inhibited [^{3}H]C16-PAF specific binding in a competitive manner.

Effect of SM-10661 on alkyl-PAF-induced death in mice. Injection of alkyl-PAF (60 μg/kg) to mice resulted in sudden death; lethality occurred mostly between 30 min and 1 hr, with additional mortality seen up to 24 hr. Animals exhibited hypothermia, labored breathing, comatose behavior, and prostration.

SM-10661, when administered orally or intravenously, protected the mice from alkyl-PAF-induced death dose dependently; the ID_{50} for inhibition was 6 mg/kg intravenously and 24 mg/kg orally (Fig. 5).

Effect of SM-10661 on alkyl-PAF-induced increases in bronchial pressure of guinea pigs. Administration of alkyl-PAF to guinea pigs produced a dose-dependent increase in bronchial pressure (Fig. 6A). When SM-10661 was given orally 1 hr before administration of alkyl-PAF (0.08 μg/kg), it inhibited the alkyl-PAF-induced increase in bronchial pressure with an ID_{50} of 15 mg/kg. SM-10661 given intravenously 2 min before the administration of alkyl-PAF inhibited the increase in bronchial pressure with an ID_{50} of 0.7 mg/kg (Fig. 6B).

Effect of SM-10661 on alkyl-PAF-induced bronchial hyperreactivity of guinea pigs. Infusion of alkyl-PAF (3 μg/kg) to guinea pigs induced an increase in the reactivity of

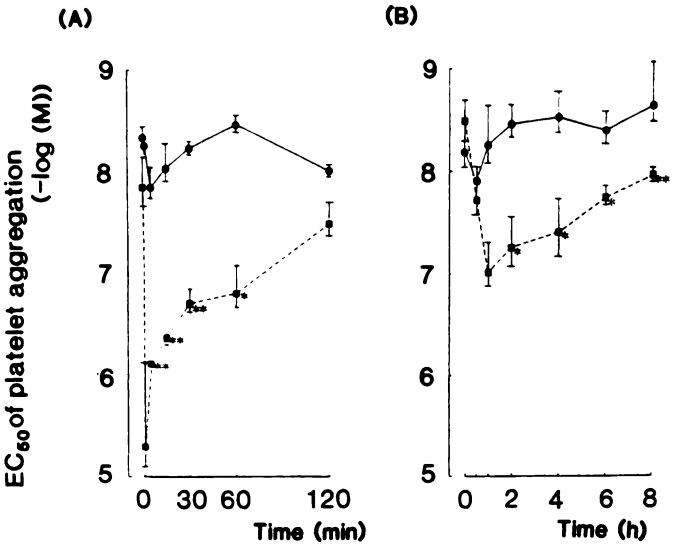

Fig. 9. Duration of action of the inhibitory effect of SM-10661 against alkyl-PAF-induced aggregation in rabbit citrated platelet-rich plasma *ex vivo*. A, SM-10661 (30 mg/kg) (■) or saline (●) was administered intravenously. B, SM-10661 (100 mg/kg) (■) or saline (●) was administered orally. From treated rabbits, platelet-rich plasma samples were then collected at varying times. Half-maximal concentrations (EC_{50}) for alkyl-PAF-induced platelet aggregation were monitored. Each value represents the mean ± standard error of three rabbits. Statistical significance, *, $p < 0.05$; **, $p < 0.01$.

the airways to intravenously administered bombesin (300 ng/kg). In contrast, infusion of 0.25% BSA did not induce any significant change in airway hyperreactivity (Fig. 7A).

Infusion of SM-10661 together with alkyl-PAF to guinea pigs inhibited alkyl-PAF-induced bronchial hyperreactivity to bombesin dose dependently, with an ID_{50} of 25 mg/kg (Fig. 7B).

Effect of SM-10661 on alkyl-PAF-induced hypotension of rats. Alkyl-PAF produced dose-dependent hypotension in the rat. The administration of alkyl-PAF (0.01 μg/kg intravenously caused an immediate decrease in MAP (21.6 ± 1.2%, $n = 27$), which returned to baseline at 3 min. SM-10661 showed dose-dependent inhibition of alkyl-PAF-induced hypotension, when given intravenously (ID_{50} of 3 mg/kg) or orally (ID_{50} of 10 mg/kg), as shown in Fig. 8A. SM-10661 given at 30 or 100 mg/kg orally showed rapid absorption and extended the duration of activity, in terms of inhibition of alkyl-PAF-induced hypotension (Fig. 8B).

Duration of action of SM-10661 against *ex vivo* alkyl-PAF-induced platelet aggregatory responses in rabbits. After oral or intravenous application of SM-10661 to rabbits, SM-10661 levels in plasma were quantified as an indicator of pharmacological activity, using *ex vivo* alkyl-PAF-induced platelet aggregation (EC_{50}) (Fig. 9). When SM-10661 (30 mg/kg) was administered intravenously, its effect was observed for about 2 hr (Fig. 9A). On the other hand, when SM-10661 (100 mg/kg) was administered orally, its protective effect continued for about 8 hr (Fig. 9B).

Discussion

The results of the present studies demonstrate that SM-10661 is an inhibitor of PAF *in vitro* and *in vivo*. PAF is not a single molecular entity but is a family of structurally related acetylated phospholipids, mainly two alkyl chain homologs C16-PAF and C18-PAF (23). Alkyl-PAF, which is used generally, is prepared from fresh beef heart and is reported to be composed of mainly these two species (23). We checked the activity of alkyl-PAF, compared with C16-PAF and C18-PAF. In *in vitro* studies, its potency was almost identical to that of C16-PAF and was greater than that of C18-PAF.

SM-10661 inhibited all three kinds of PAF-induced aggregation of rabbit platelets and [^{3}H]C16-PAF binding to rabbit washed platelets in a dose-dependent manner. Its inhibitory effect on alkyl-PAF-induced aggregation was almost identical to the effect on the C16-PAF-induced response and was weaker than the effect on the C18-PAF-induced response. *In vitro*, the anti-PAF activity of SM-10661 was specific, because SM-10661 had little or no effect on the responses to other aggregating agents. A Lineweaver-Burk plot revealed that SM-10661 interacted with PAF receptors in a competitive manner.

SM-10661, given either orally or intravenously, blocked several alkyl-PAF-induced responses in mice, rats, and guinea pigs. Systemic hypotensive (24, 25) and lethal effects of PAF (26–29) have been observed in a variety of species. The ability of SM-10661 to inhibit alkyl-PAF-induced hypotension or lethality strongly supports the hypothesis that these responses are mediated through specific receptors(26, 30–31).

PAF, given either intravenously or by aerosol, induces an increase in bronchial pressure in many animal species (32–37). The alkyl-PAF-induced increase in bronchial pressure was dose-dependently inhibited by intravenously or orally administered SM-10661. The present observations are also consistent with findings from other laboratories indicating that the PAF antagonists Ginkgolide B (ID_{50} of 3 mg/kg intravenously) (8), WEB-2086 (ID_{50} of 2.76 mg/kg orally, 0.37 mg/kg intravenously) (13), CV-398 8 (ID_{50} of 0.73 mg/kg intravenously) (9), CV-6209 (ID_{50} of 0.009 mg/kg intravenously) (8), and SDZ 64-412 (ID_{50} of 13 mg/kg orally, 0.23 mg/kg intravenously) (14) blocked PAF-induced responses.

Asthma is characterized not only by intermittent reversible episodes of bronchoconstriction but also by bronchial hyperreactivity and inflammation. Bronchial hyperreactivity is an increased bronchoconstrictor response to a wide variety of inhaled spasmogens, such as methacholine or histamine, or noxious stimuli (38). A good correlation between the degree of bronchial hyperreactivity, the presence of asthmatic symptoms, and the need for antiasthmatic medication has been reported (39). PAF has a wide range of biological actions that mimic several important features of asthmatic airways along with bronchoconstriction, such as induction of eosinophil chemotaxis, an increase in airway microvascular permeability, and sustained bronchial hyperreactivity (40–42). SM-10661 inhibited alkyl-PAF-induced bronchial hyperreactivity dose dependently. SM-10661 did not inhibit histamine- or leukotriene D_4-induced increases in bronchial pressure at 100 mg/kg intravenously, and it did not inhibit antigen-induced release of histamine or leukotriene D_4 from minced lungs of sensitized guinea pig (43). Thus, this PAF antagonist might be useful for understanding of the mechanism of bronchial hyperreactivity in asthma.

SM-10661, when given orally, shows rapid absorption and good duration of activity in rats and rabbits. The oral doses were, in general, 5 to 10 times higher than the intravenous dosages. This indicates a fairy good enteral absorption. In acute toxicity studies, SM-10661 showed very high median lethal doses in mice. The LD_{50} values were 1300 mg/kg orally and 460

mg/kg intravenously. Furthermore, SM-10661 is a highly water-soluble compound, whereas most PAF antagonists that have been reported so far are only slightly soluble in water (4–14). These properties may facilitate its usefulness in investigating the role of PAF in disease. The effectiveness of SM-10661 in these animal models may indicate its potential usefulness in pathophysiological diseases states, such as inflammation, pulmonary dysfunction, shock, and anaphylaxis.

At present, the use of PAF antagonists in humans is extremely limited. So far, the PAF antagonist BN-52063 has been studied in skin responses to allergens in atopic individuals. It inhibited early wheal and late skin responses, which may indicate an effect of the PAF antagonist against cell infiltration in the skin, particularly by eosinophils (44). It was also reported to be an effective treatment for adult systemic mastocytosis (45). Another PAF antagonist, WEB-2086, was reported to be effective in the treatment of a patient with idiopathic thrombocytopenic purpura (46). However, there have been no reports of the effects of PAF antagonists on induced bronchial hyperreactivity in humans. So the precise contribution of PAF to bronchial hyperreactivity in human asthmatic patients awaits further investigation.

Acknowledgments

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References

1. Benveniste, J., P. M. Henson, and C. G. Cochrane. Leukocyte-dependent histamine release from rabbit platelets: the role of IgE, basophil, and a platelet-activating factor. *J. Exp. Med.* **136**:1356–1377 (1972).
2. Pinckard, R. N., L. M. McManus, H. Halonen, and D. J. Hahahan. Biological activities of acetyl glyceryl ether phosphorylcholine in experimental animals and in man, in *Role of Chemical Mediators in the Pathophysiology of Acute Illness and Injury* R. McConn, ed.). Raven Press, New York, 81–92.
3. Vargaftig, B. B., M. Chignard, J. Benveniste, J. Lefort, and F. Wal. Background and present status of research on platelet-activating factor (PAF-acether). *Ann. N. Y. Acad. Sci.* **370**:119–137 (1981).
4. Hwang, S. B., C. L. Li, M. H. Lam, and T. Y. Shen. Characterization of cutaneous vascular permeability induced by platelet activating factor in guinea pigs and rats and its inhibition by a platelet-activating factor receptor antagonist. *Lab. Invest.* **52**:617–630 (1985).
5. Pan, J. X., O. D. Hensens, D. L. Zink, M. N. Chang, and S. B. Hwang. Ligands with platelet activating factor antagonist activity from *Magnolia biondii*. *Phytochemstry* **26**:1377–1379 (1987).
6. Braquet, P. BN52021 and related compounds: a new series of highly specific PAF-acether receptor antagonist. *Prostaglandins* **30**:687 (1985).
7. Okamoto, M., K. Yoshida, I. Uchida, M. M. Kohsaka, and H. Aoki. Studies of platelet activating factor (PAF) antagonists from microbial products. 1. Bisdethiobis(methylthio)gliotoxin and its derivatives. *Chem. Pharm. Bull.* **34**:340–344 (1986).
8. Terashita, Z., Y. Imura, M. Takatani, S. Tsushima, and K. Nishikawa. CV-6209, a highly potent antagonist of platelet activating factor *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.* **242**:263–268 (1987).
9. Terashita, Z., S. Tsushima, Y. Yoshioka, H. Nomura, Y. Inada, and K. Nishikawa. CV-3988: a specific antagonist of platelet activating factor (PAF). *Life Sci.* **32**:1975–1982 (1983).
10. Hwang, S. B., M. H. Lam, T. Biftu, T. R. Beattie, and T. Y. Shen. *Trans*-2,5-bis(3,4,5-trimethoxyphenyl)tetrahydrofuran: an orally active specific and competitive receptor antagonist of platelet activating factor. *J. Biol. Chem.* **260**:15639–15645 (1985).
11. Toyofuku, T., K. Kubo, T. Kobayashi, and S. Kusama. Effects of ONO-6240, a PAF antagonist, on endotoxin shock in unanesthetized sheep. *Prostaglandins* **31**:271–281 (1986).
12. Tokumura, A., H. Homma, and D. J. Hanahan. Structual analogs of alkylacetyl glycerophosphocholine: inhibitory behavior on platelet activation. *J. Biol. Chem.* **260**:12710–12714 (1985).
13. Casals-Stenzel, J., G. Muacevic, and K. H. Weber. Pharmacological actions of WEB2086, a new specific antagonist of platelet activating factor. *J. Pharmacol. Exp. Ther.* **241**:974–981 (1987).
14. Handley, D. A., R. G. Van Valen, M. K. Melden, W. J. Houlihan, and R. N. Saunders. Biological effects of the orally active platelet activating factor receptor antagonist SDZ 64-412. *J. Pharmacol. Exp. Ther.* **247**:617–623 (1988).
15. Morooka, S., Y. Komuro, R. Ando, and M. Uchida. *In vitro* and *in vivo* effects of SM-10661, a platelet activating factor (PAF) antagonist. *Prostaglandins* **35**:840 (1988).
16. Born, G. V. R. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (Lond.)* **194**:927–929 (1962).
17. Homma, H., A. Tokumura, and D. J. Hanahan. Binding and internalization of platelet-activiting factor 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine in washed platelets. *J. Biol. Chem.* **262**:10582–10587 (1987).
18. Handley, D. A., J. C. Tomesch, and R. N. Saunders. Inhibition of PAF-induced responses in the rat, guinea pig, dog and primate by the receptor antagonist SRI 63-441. *Thromb. Haemostasis* **56**:40–44 (1986).
19. Konzett, H., and R. Rossler. Versuchsanordnung zu untersuchungen an der bronchial muskulatur. *Arch. Exp. Pathol. Pharmakol.* **195**:71–75 (1940).
20. Mazzoni, L., J. Morley, C. P. Page, and S. Sanjar. Induction of airway hyperreactivity by platelet activating factor in the guinea pig. *J. Physiol. (Lond.)* **365**:107 (1985).
21. Barnes, P. J., B. M. Grandordy, C. P. Page, K. J. Rhoden, and D. N. Robertson. The effect of platelet activating factor on pulmonary β-adrenoreceptors. *Br. J. Pharmacol.* **90**:709–715 (1987).
22. Coyle, A. J., S. C. Urwin, C. P. Page, C. Touvay, B. Villain, and P. Braquet. The effect of the selective PAF antagonist BN 52021 on PAF- and antigen-induced bronchial hyperreactivity and eosinophil accumulation. *Eur. J. Pharmacol.* **148**:51–58 (1988).
23. Pinckard, P. N. Platelet activating factor (PAF): molecular heterogeneity and (patho)biology, in *Platelet-Activating Factor and Diseases* (K. Saito and D. J. Hanahan, eds.). International Medical Publishers, Japan, 37–50 (1989).
24. Blank, M. L., F. Snyder, L. W. Byers, B. Brooks, and E. E. Muirhead. Antihypertensive activity of an allyl ether analogue of phosphorylcholine. *Biochem. Biophys. Res. Commun.* **90**:1194–1200 (1979).
25. Sanchez-Crespo, M., F. Alonso, P. Inarrea, V. Alvarez, and J. Egido. Vascular actions of synthetic PAF-acether (a synthetic platelet activating factor) in the rat: evidence for a platelet independent mechanism. Immunopharmacology **4**:173–185 (1982).
26. Terashita, Z., Y. Imura, A. Shino, and K. Nishikawa. A lethal role of platelet activating factor in anaphylactic shock in mice. *J. Pharmacol. Exp. Ther.* **243**:378–383 (1987).
27. Criscuoli, M., and A. Subissi. Paf-acether induced death in mice: involvement of arachidonate metabolites and β-adrenoceptors. *Br. J. Pharmacol.* **90**:203–209 (1987).
28. Lefer, A. M., H. F. Muller, and J. B. Smith. Pathophysiological mechanisms of sudden death induced by platelet activating factor. *Br. J. Pharmacol.* **83**:125–130 (1984).
29. Myers, A., E. Ramey, and P. Ramwell. Glucocorticoid protection against PAF-acether toxicity in mice. *Br. J. Pharmacol.* **79**:595–598 (1983).
30. Doebber, T. W., M. S. Wu, J. C. Robbins, B. M. Choy, M. N. Chang, and T. Y. Shen. Platelet-activating factor (PAF) involvement in endotoxin-induced hypotension in rats: studies with PAF-receptor antagonist kadsurenone. *Biochem. Biophys. Res. Commun.* **127**:799–808 (1985).
31. Sanchez-Crespo, M., S. Frenandes-Gallard, M. L. Nieto, J. Barnes, and P. Braquet. Inhibition of the vascular actions of immunoglobulin G aggregates by BN-52021, a highly specific antagonist of PAF acether. *Immunopharmacology* **10**:69–75 (1985).
32. Chung, K. F., H. Aizawa, G. D. Leikauf, I. F. Ueki, T. W. Evans, and J. A. Nadel. Airway hyperresponsiveness induced by platelet activating factor: role of thromboxane generation. *J. Pharmacol. Exp. Ther.* **236**:580–584 (1985).
33. Cuss, F. M., C. M. S. Dixon, and P. J. Barnes. Effects of inhaled platelet activating factor on pulmonary function and bronchial responsiveness in man. *Lancet* **2**:189–192 (1986).
34. Denjean, A., B. Arnoux, R. Masse, A. Lockhart, and J. Benveniste. Acute effects of intratracheal administration of platelet activiting factor in baboons. *J. Appl. Physiol.* **55**:799–804 (1983).
35. Gateau, O., B. Arnoux, H. Deriaz, P. Viars, and J. Benveniste. Acute effects of intratracheal administration of Paf-acether (platelet-activating factor) in humans. *Am. Rev. Respir. Dis.* **3**:129 (1984).
36. Halonen, M., J. D. Palmer, I. C. Lohman, L. M. McManus, and R. N. Pinckard. Respiratory and circulatory alterations induced by acetyl glyceryl ether phosphorylcholine, a mediator of IgE anaphylaxis in the rabbit. *Am. Rev. Respir. Dis.* **122**:915–924 (1980).
37. Vargaftig, B. B., J. Lefort, M. Chignard, and J. Benveniste. Platelet-activating factor induces a platelet-dependent bronchoconstriction unrelated to the formation of prostaglandin derivatives. *Eur. J. Pharmacol.* **65**:185–192 (1980).
38. Boushey, H. A., M. J. Holtzman, J. R. Sheller, and J. A. Nadel. Bronchial hyperreactivity. *Am. Rev. Respir. Dis.* **121**:389–413 (1980).
39. Juniper, E. F., P. A. Frith, and F. E. Hargreave. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. *Thorax* **36**:575–579 (1981).
40. Morley, J. Platelet-activating factor and asthma. *Agents Actions* **19**:100–108 (1986).
41. Pretolani, M., P. Ferrer-Lopez, and B. B. Vargaftig. From anti-asthma drugs to PAF-acether antagonism and back. *Biochem. Pharmacol.* **28**:1373–1384 (1989).
42. Chung, K. F., and P. J. Barnes. PAF antagonists: their potential therapeutic role in asthma. *Drugs* **35**:93–103 (1988).
43. Uchida, M., N. Imanishi, T. Sugasawa, and S. Morooka. Effect of a selective

PAF antagonist, SM-10661, on asthmatic model. 1. Effect on passive anaphylactic bronchoconstriction in guinea pigs. *Lipids*, in press.
44. Roberts, N. M., C. P. Page, K. F. Chung, and P. J. Barnes. Effect of a PAF antagonist, BN-52063, on antigen-induced acute and late-onset cutaneous responses in atopic subjects. *J. Allergy Clin. Immunol.* **82**:236–241 (1988).
45. Guinot, C. S., L. Berdah, J. Duchier, and R. J. Revilland. Treatment of adult systemic mastocytosis with a PAF-acether antagonist, BN-52063. *Lancet* **9**:114 (1988).
46. Lohman, H. F., W. S. Adamus, and C. J. Meade. Idiopathic thrombocytopenia treated with PAF-acether antagonist WEB-2086. *Lancet* **12**:1147 (1988).

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