ELSEVIER

European Journal of Pharmacology 328 (1997) 75–81

ejp

Apafant (a PAF receptor antagonist) suppresses the early and late airway responses in guinea pigs: a comparison with antiasthmatic drugs

Kozaburo Ikegami [a,*], Hiroko Hata [a], Jun-ichi Fuchigami [b], Katsuyuki Tanaka [b], Yoshiro Kohjimoto [b], Shuji Uchida [a], Kenji Tasaka [a]

[a] *Department of Pharmacology, Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd., 3-10-1 Yato, Kawanishi 666-01, Japan*
[b] *Laboratory of Pharmacokinetics and Pharmacology, Panapharm Laboratories Co., Ltd., 1285, Kurisaki-cho, Uto, Kumamoto 869-04, Japan*

Received 10 October 1996; revised 18 March 1997; accepted 21 March 1997

Abstract

We studied the effects of apafant (WEB 2086 BS), a specific and potent platelet activating factor (PAF) receptor antagonist, on the early and late airway responses in conscious and actively sensitized guinea pigs. An increase in airway resistance (Rs) was seen 1 min after the inhaled antigen challenge (early airway response), followed by another increase in Rs which peaked between 4 and 8 h after the provocation (late airway response). Oral administration of apafant as well as theophylline inhibited both early and late airway responses. Ozagrel, an inhibitor of thromboxane A_2 synthetase, salbutamol, a β_2-adrenoceptor agonist, and dexamethasone significantly inhibited either the early or the late airway response only. Disodium cromoglycate inhibited neither the early nor the late airway response. The results showed that apafant inhibited both the early and late airway responses in sensitized guinea pigs and its effect was comparable or superior to that of anti-asthmatic drugs used clinically.

Keywords: Apafant (WEB 2086 BS); Airway response, early; Airway response, late

1. Introduction

Bronchial asthma is an atopic disease characterized by bronchoconstriction, bronchial hyperreactivity and an influx of inflammatory cells, especially eosinophils, into the airway. Pepys and Hutchcroft (1975) observed that after allergen provocation, atopic asthmatic subjects could develop an early airway response which was maximal 15–30 min after provocation and then returned to the baseline within 2 h, frequently followed by a late airway response occurring 4–12 h after provocation, the incidence of which was approximately 60%. The early airway response results mostly from airway smooth muscle contraction mediated by autacoids (histamine and leukotriene C_4, etc.) released after immunoglobulin E-dependent activation of mast cells. In recent years, significant interest has been focused on the late airway response. The late airway response is accompanied by bronchial hyperresponsiveness to non-specific stimulation (Cartier et al., 1982), proceeding to serious and chronic asthma. Although the mechanisms underlying the late airway response are still poorly understood, the late airway response might be associated with an influx of inflammatory cells (e.g., eosinophils and lymphocytes) into the airway, edema in bronchial mucosa and accumulation of mucus in the lumen of the airway, suggesting that granulocytes, especially eosinophils, contribute to the functional abnormalities occurring in the airway. It is well known that there are differences between the effects of drugs on the early and late airway responses, e.g., β_2-adrenoceptor agonists inhibit only the early airway response, while steroid suppresses only the late airway response (Booij-Noord et al., 1971; Cockcroft and Murdock, 1987).

The interest in platelet activating factor (PAF) as a mediator of asthma and allergy arises because PAF has profound proinflammatory effects in the airway which closely mimic the pathophysiology of asthma. PAF is a potent stimulus to eosinophil accumulation and activation and may be involved in the eosinophilic inflammation which is characteristic of asthma and airway hyperreactivity.

The ovalbumin-sensitized guinea pig model has been

* Corresponding author. Tel.: (81-727) 93-8351; Fax: (81-727) 93-1089.

frequently used to study the pathogenesis of asthma, and it has been demonstrated that an antigen-induced airway response takes place as seen in atopic asthma with antigen provocation. This report describes the effects of apafant (WEB 2086 BS), a potent PAF receptor antagonist, on the early and late airway responses induced by inhalation of aerosolized antigen in actively sensitized conscious guinea pigs, in comparison with anti-asthmatic drugs.

2. Material and methods

2.1. Animals

Male Hartley guinea pigs (Kyudo, Kumamoto, Japan; weight range 410–756 g, age range 4–5 weeks) were used and housed in an air-conditioned room at 18–28°C with 30–70% humidity, and a 12-h dark-light cycle. The animals were given food (RC4, about 50 g/animal per day, Oriental Yeast, Tokyo, Japan) and water ad libitum.

2.2. Sensitization procedure and antigen challenge

The animals were sensitized by exposure to aerosolized ovalbumin (1%) solution for 10 min once daily for 8 consecutive days. The aerosol was generated by an ultrasonic nebulizer (NE-U12, OMRON, Tokyo, Japan). One week after the last sensitization, antigen provocation was performed with inhaled ovalbumin (2%) solution for 5 min under cover of pyrilamine maleate (10 mg/kg, i.p.), a histamine H_1 receptor antagonist, 30 min before ovalbumin (2%) challenge to protect the animals from fatal anaphylactic shock caused by released histamine. Metyrapone (10 mg/kg, i.v.), a cortisol synthesis inhibitor, was given intravenously 24 and 1 h before antigen challenge.

2.3. Measurement of airway function

Airway resistance (Rs) in the conscious guinea pig was measured with a double-flow plethysmograph, as elsewhere described (Pennock et al., 1979). Briefly, the conscious animal was positioned with its neck extending through the partition of a two-chambered, rectangular plastic box. The two chambers were joined by spring clamps and a Fearedalex collar between chambers formed a seal around the neck. Each chamber was fitted with an identical wire screen and identical flow sensors. These flow sensors were connected to an airway resistance measuring system (Pulmos-1, M.I.P.S., Osaka, Japan) to determine Rs. Rs was monitored before, and at 1 min, 2, 4, 5, 6, 7, 8 and 23–24 h after antigen challenge. The animals were removed from the plethysmograph between measurements. The effects of drugs on the early and late airway responses were evaluated as the percent change in Rs value from that before the challenge (baseline), 1 min after antigen challenge, and the AUC (area under the response curve) between 4 and 8 h after antigen challenge, respectively.

2.4. Drug treatment

Seven to 27 animals were used in the control and treatment groups. Apafant (10, 30, 100 mg/kg), ozagrel (100, 300 mg/kg), theophylline (10, 30, 100 mg/kg) and disodium cromoglycate (300 mg/kg) were administered orally 1 h before antigen challenge. To evaluate differences in effects of pre-treatment and post-treatment on the late airway response, apafant was also given orally 3 h after the antigen challenge. Dexamethasone (10 mg/kg) was administered orally 16 and 2 h before the challenge. These drugs were given in a volume of 5 ml/kg. Aerosolized salbutamol (0.2%, 0.5%) was inhaled for 10 min, 15 min before and 4 h after the challenge, with the use of an ultrasonic nebulizer. Aerosolized disodium cromoglycate (1%) was inhaled for 10 min, 15 min before the challenge. As the control, vehicles for each test drug were used.

2.5. Drugs and chemicals

Apafant (WEB 2086 BS, CAS 105219-56-5, 4-(2-chlorophenyl)-9-methyl-2-(3-morpholino-3-oxopropyl)-6*H*-thieno[3,2-*f*][1,2,4]-triazolo-[4,3-*a*][1,4]-diazepine, Lot No. 93010) was obtained from Boehringer Ingelheim (Ingelheim, Germany). Ozagrel (Dr. Karl Thomae, Biberach, Germany, Lot No. ML5828), theophylline (Sigma, St. Louis, MO, USA, Lot No. 54H0640), disodium cromoglycate (Biomol Research Lab., Plymouth Meeting, PA, USA, Lot No. P1430), salbutamol (Wako, Osaka, Japan, Lot No. TWK7612), and dexamethasone (Sigma, Lot No. 62H0202) were used. Carboxymethyl cellulose (CMC-Na, Wako, Lot No. PTG0378), ovalbumin (Grade V, Sigma, Lot No. 14H7035, 73H8025), pyrilamine maleate (Sigma, Lot No. 12H0157), and metyrapone (metopyrone, Sigma, Lot No. 94H0782, 31H2507 were also used.

Apafant was initially suspended in distilled water and then dissolved in 0.1 M HCl, and finally diluted with distilled water to the optimal concentrations. Ozagrel and theophylline were suspended in a 0.5% CMC solution. Salbutamol and dexamethasone were dissolved in a 0.9% NaCl solution. Disodium cromoglycate was dissolved in a 0.5% CMC solution and in a 0.9% NaCl solution for oral and inhalation use, respectively.

2.6. Statistical analysis

Data were normalized as percentage of Rs before saline or antigen challenge (baseline control) and given as means ± S.E.M. When data involved three or more groups, differences among groups were tested with one-way analysis of variance (ANOVA), followed by Dunnett's test. For

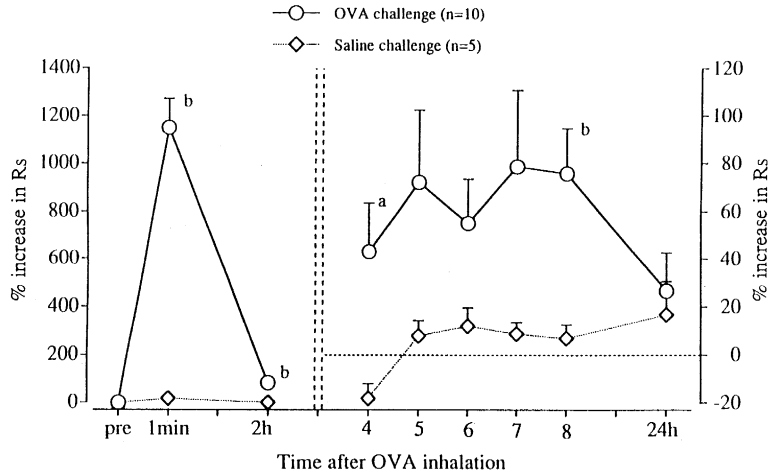

Fig. 1. Sequential changes in the airway response after saline or antigen challenge in actively sensitized guinea pigs. Rs, airway resistance; OVA, ovalbumin. Mean ± S.E.M. % increase in Rs, increase in Rs expressed as a percentage of Rs measured before antigen challenge (baseline control). Saline challenge ($n = 5$), ovalbumin challenge ($n = 10$). [a] $P < 0.05$, [b] $P < 0.01$, significantly different from saline inhalation group (Student's *t*-test).

differences between two groups, Student's *t*-test was applied. Differences were considered significant if $P < 0.05$.

3. Results

3.1. Airway resistance (Rs) after antigen challenge

The mean baseline values of Rs in the saline inhalation and ovalbumin inhalation groups were 0.59 ± 0.05 ($n = 5$) and 0.52 ± 0.01 cmH_2O/ml per s ($P = 0.210$) ($n = 10$), respectively. No significant difference was found between these two groups. On the whole, the antigen-challenged animals developed a biphasic airway response. The first phase (early phase) was a remarkable increase in Rs occurring 1 min after the challenge. The increase in Rs gradually declined and Rs returned almost to the control level 2 h after antigen challenge. The second phase (late phase) was an increase in Rs occurring 4 h or more after antigen challenge. As shown in Fig. 1, the mean Rs of the ovalbumin inhalation group increased markedly during the early phase (1150 ± 112% of baseline 1 min after antigen challenge), but the time-course curve of Rs during the late phase was without an obvious peak. From these findings, the early and late airway responses were defined as follows. Namely, the increase in Rs occurring 1 min after

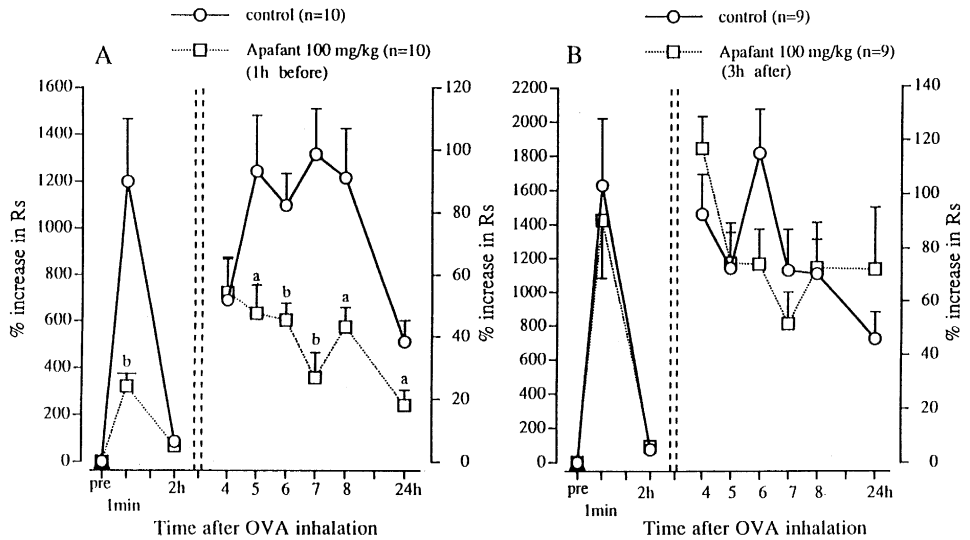

Fig. 2. Comparison of pre- and post-challenge treatment with apafant on sequential changes in airway response after antigen challenge in guinea pigs. % increase in Rs, increase in Rs as a percentage of Rs measured before antigen challenge (baseline control). For pretreatment: control ($n = 10$), apafant 100 mg/kg administered 1 h before ovalbumin challenge ($n = 10$). For post-treatment: control ($n = 9$), apafant 100 mg/kg administered 3 h after ovalbumin challenge ($n = 9$). Mean ± S.E.M. [a] $P < 0.05$, [b] $P < 0.01$, significantly different from control group (Student's *t*-test).

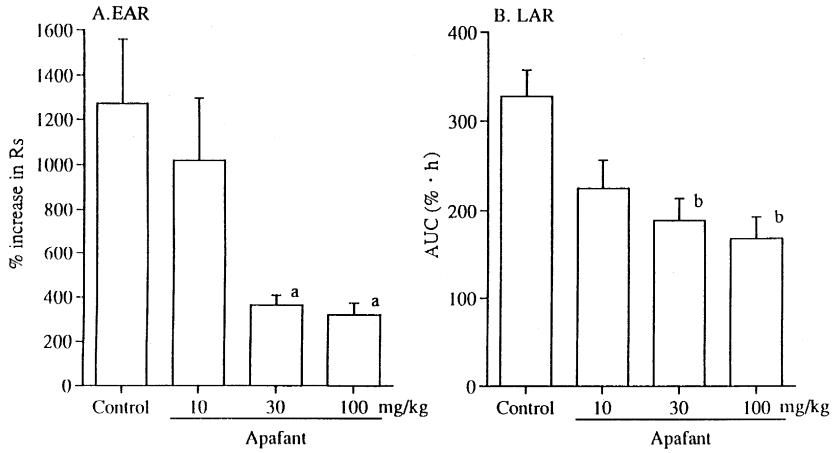

Fig. 3. Effects of apafant on the early and late airway responses in actively sensitized guinea pigs. Early airway response (EAR): percent change in Rs 1 min after antigen challenge. Late airway response (LAR): percent change in AUC (4–8 h after antigen challenge). Mean ± S.E.M. [a] $P < 0.05$, [b] $P < 0.01$, significantly different from saline inhalation group (Dunnett's multiple test).

antigen challenge was regarded as the early airway response. In contrast, to minimize the influence of a variable distribution of the peak time of Rs during the late phase among individual animals, the increase in AUC (area under the curve) for Rs between 4 and 8 h after antigen inhalation was defined as the late airway response. Both the early and late airway responses in the sensitized animals were significantly different from those of the saline inhalation animals. Rs returned almost to the baseline value within 24 h after antigen challenge.

Table 1
Effects of apafant and other anti-asthmatic drugs on antigen-induced early and late airway responses in actively sensitized guinea pigs

Drug	Dose (mg/kg)	No. of animals	EAR (1 min)		LAR (4–8 h)	
			% Change in Rs	% Inhibition	AUC (% · h)	% Inhibition
Control	–	19	1271 ± 288	–	328 ± 30	–
Apafant	10	9	1020 ± 276	20	225 ± 31	31
	30	9	365 ± 44 [a]	71	189 ± 25 [b]	42
	100	10	321 ± 55 [a]	75	168 ± 25 [b]	49
Control	–	19	1236 ± 212	–	232 ± 40	–
Ozagrel	100	10	370 ± 110 [b]	70	185 ± 54	20
	300	9	239 ± 50 [b]	81	142 ± 38	39
Control	–	27	846 ± 158	–	334 ± 51	–
Theophylline	10	9	325 ± 106	62	140 ± 46	58
	30	17	140 ± 36 [b]	83	131 ± 39 [b]	61
	100	9	309 ± 75 [a]	63	60 ± 58 [b]	82
Control	–	17	755 ± 133	–	408 ± 66	–
Salbutamol	(0.2%)	10	162 ± 22 [b]	79	319 ± 71	22
	(0.5%)	10	227 ± 41 [b]	70	341 ± 56	16
Control	–	7	864 ± 234	–	310 ± 52	–
Dexamethasone	10	8	296 ± 44	66	49 ± 25 [c]	84
Control	–	10	1448 ± 228	–	281 ± 56	–
DSCG	(1%)	10	940 ± 117	35	262 ± 54	7
Control	–	9	1364 ± 369	–	471 ± 132	–
DSCG	300	9	1212 ± 211	11	200 ± 43	58

EAR: early airway response, LAR: late airway response. Apafant, ozagrel, theophylline and DSCG (disodium cromoglycate) at 300 mg/kg were administered orally 1 h before antigen challenge. Aerosolized salbutamol was inhaled for 10 min twice (15 min before and 4 h after antigen challenge). Dexamethasone was administered orally twice (16 and 2 h before antigen challenge). The 1% solution of disodium cromoglycate was inhaled for 10 min, 15 min before antigen challenge.

[a] $P < 0.05$, [b] $P < 0.01$, significantly different from control (Dunnett's multiple test). [c] $P < 0.01$, significantly different from control (Student's *t*-test).

3.2. Effects of pre- and post-challenge treatment with apafant on the early and late airway responses

The pretreatment with apafant at 100 mg/kg, p.o. (1 h before antigen challenge) significantly reduced not only the early airway response but also the late airway response. In contrast, the post-challenge treatment with apafant at the same dose (3 h after the antigen challenge) caused no significant inhibition of the late airway response (Fig. 2). This finding meant that the test drugs could be applied 1 h before antigen challenge.

3.3. Comparison with other anti-asthmatic drugs on the early and late airway responses

Apafant at 30 mg/kg or higher, p.o., significantly inhibited both the early and late airway responses in the sensitized guinea pigs (Fig. 3). The inhibition by apafant at 30 and 100 mg/kg, p.o., was 71% and 75% for the early airway response, and 42% and 49% for the late airway response, respectively.

Ozagrel at 100 and 300 mg/kg, p.o., significantly inhibited the early airway response by 70% and 81%, respectively (Table 1). However, there were only moderate effects on the late airway response (less than 40% inhibition at the same doses), which were not significantly different from the control response. Theophylline at 10, 30 and 100 mg/kg, p.o., also inhibited the early airway response by 62%, 83% and 63%, and the late airway response by 58%, 61% and 82%, respectively. At 30 mg/kg or higher, there was significant inhibition of both the early and late airway responses. However, at 100 mg/kg, p.o., tremor and convulsions occurred in 5 out of 9 animals, and one animal died within 24 h.

Dexamethasone at 10 mg/kg was administered orally 2 and 16 h before antigen challenge. Dexamethasone also inhibited the early airway response (by 66%) and the late airway response (by 84%). However, the significant inhibition was only for the late airway response, not the early airway response. Salbutamol (0.2% and 0.5%) aerosol inhalation for 10 min, 15 min before the antigen challenge significantly inhibited the early airway response (by 79% and 70%, respectively). In contrast, even when animals additionally inhaled the same dose of salbutamol 4 h after antigen challenge, the late airway response was not significantly inhibited (22% and 16%, respectively). Inhaled disodium cromoglycate at the concentration of 1% (for 10 min, 15 min before antigen challenge) produced a moderate, non-significant inhibition of the early and late airway responses (35% and 7%, respectively). Similarly, the oral administration of disodium cromoglycate at 300 mg/kg (before antigen challenge) had little influence on the early airway response (11%). The same dose of disodium cromoglycate elicited a marked inhibition (58%) of the late airway response, which was not significantly different from that of the control.

4. Discussion

The effect of apafant on the early and late airway responses was compared with that of several anti-asthmatic drugs. In our model, the animals were actively sensitized by inhalation of an aerosolized antigen (ovalbumin), similar to the antigen delivery route in humans. The sensitized animals were challenged by ovalbumin inhalation under cover of pretreatment with pyrilamine to protect the animals from early fatal anaphylaxis, thereby making it possible to administer a sufficiently high dose of antigen into the airway. Furthermore, the measurement of pulmonary function was performed in conscious guinea pigs, taking into account the possibility that anesthesia might interact with airway responses. Metyrapone was administered to inhibit the generation of endogenous glucocorticoid.

In the present experiment, on the whole, the exposure to inhaled antigen of actively sensitized guinea pigs produced a biphasic airway response. The first phase (early phase) was an obvious increase in Rs occurring 1 min after antigen challenge. The increase in Rs in the first phase was defined as the early airway response. The second phase (late phase) was the new increase in Rs occurring approximately 4–8 h after antigen challenge; Rs returned to baseline values within 24 h. When Rs increased by more than 40% from the baseline value (before antigen challenge) at, at least, one time point during the time window between 4 and 8 h after antigen challenge, the animal was regarded as a responder (positive to the late airway response). Based upon this definition, the incidence of responders was about 90% in the present study. When we averaged Rs at every time point in the late phase, it became hard to find a clear peak in Rs due to the broad distribution of peak Rs among individual animals. Therefore, we calculated the AUC (area under the response curve) for Rs between 4 and 8 h after antigen inhalation and defined the increase in AUC as the late airway response.

Apafant (WEB 2086 BS) is a thienotriazolodiazepine compound with a specific and potent PAF receptor antagonism. Apafant at doses of 30 mg/kg or higher given orally 1 h before antigen challenge caused significant inhibition of both the early and late airway responses, in a dose-dependent manner. The early airway response is considered to be caused mostly by airway smooth muscle contraction and edema mediated by autacoids (histamine, leukotriene C_4, etc.) through immunoglobulin E-dependent activation of mast cells. In the present experiment, the effects of the test drugs were evaluated under cover of pretreatment with pyrilamine, an antihistamine, to protect animals from early fatal anaphylaxis. Therefore, the involvement of histamine in the manifestation of the early airway response in the present model was excluded. It has been reported that exposure of sensitized lung to antigen causes the release of PAF (Chignard et al., 1986) and results in bronchospasm in several species. The effects of intravenously adminis-

tered antigen are closely mimicked by intravenously administered PAF. PAF is potent in inducing microvascular leakage and plasma exudation in animal airways, and appears to act directly on postcapillary venules (Evans et al., 1987), resulting in airway edema and exaggeration of airway narrowing.

Apafant at doses of 30 mg/kg or higher given orally 1 h before allergen challenge inhibited not only the early airway response but also the late airway response in a dose-dependent fashion, suggesting that PAF plays an important role as a mediator of the late airway response. Although the mechanisms underlying the late airway response are not clear, it is considered that the late airway response might be associated with an influx of inflammatory cells (eosinophils and lymphocytes) into the airway, edema in bronchial mucosa and accumulation of mucus in the lumen of the airway. PAF attracts and activates inflammatory cells, especially eosinophils. Lellouch-Tubiana et al. (1988) reported that inhalation of PAF or antigen by guinea pigs induced eosinophil accumulation in the lungs at 6 h, and that this accumulation was prevented by treatment with apafant, suggesting that the inhibition of the late airway response by apafant might be caused by a suppression of the recruitment of eosinophils into the airway. In contrast, when given orally at 100 mg/kg 3 h after antigen challenge, apafant had no influence on the late airway response. This finding suggests that a substantial amount of PAF might be generated and released in the early stage after antigen challenge, and the released PAF may induce the migration and activation of eosinophils, leading to the development of the late airway response.

In the present study, ozagrel at 100 and 300 mg/kg given orally before antigen challenge caused a significant inhibition of the early airway response. Although the late airway response was moderately (less than 40%) reduced by ozagrel at a dose of 300 mg/kg, the inhibition was not significantly different from that of the control. Matsumoto et al. (1994) reported that the early airway response was significantly inhibited by pretreatment with CV-4151, a thromboxane A_2 synthesis inhibitor, but not by AA-2414, a thromboxane A_2 receptor antagonist, at a low dose. In contrast, the late airway response was not significantly inhibited by CV-4151 but was by AA-2414.

The inhalation of aerosolized salbutamol (0.2% or 0.5%) 15 min before antigen challenge produced a remarkable inhibition of the early airway response. However, even when inhaled again 4 h after antigen challenge, salbutamol did not significantly affect the late airway response. The failure of salbutamol to inhibit the late airway response strongly suggests that factors other than bronchocontraction may be involved in the development of the late airway response. Unlike salbutamol, the oral administration of dexamethasone at 10 mg/kg 16 and 2 h before antigen challenge significantly inhibited the late airway response (by 84%) but not the early airway response. The difference in effectiveness between salbutamol and dexamethasone on the early and late airway responses was consistent with the results in animal (Matsumoto et al., 1994) and clinical studies (Booij-Noord et al., 1971; Burge et al., 1982; Cockcroft and Murdock, 1987).

Theophylline at 30 and 100 mg/kg given orally before antigen challenge was able to significantly inhibit both the early and late airway responses. However, in the present study, the highest dose of theophylline (100 mg/kg, p.o.) induced serious side effects, such as tremor and convulsions, and caused the death of one animal.

Disodium cromoglycate is an antiallergic drug with a so-called mast cell membrane stabilizing effect. In the present study, inhaled or orally administered disodium cromoglycate caused moderate inhibition of the early and late airway responses, respectively, but no significant differences were noticed in both cases between the disodium cromoglycate-treated and control groups. Although disodium cromoglycate inhibited mast cell-mediated passive cutaneous anaphylaxis in rats, such effects are not found in guinea pigs (Mielens et al., 1974; Assem and Mongar, 1970; Assem and Richter, 1971). The oral systemic absorption of disodium cromoglycate ranges between 0.5% and 2% in both normal and asthmatic subjects (Ashton et al., 1973; Cox, 1970). Its failure to inhibit the early and late airway responses in the present study can be ascribed to species differences and to the difficult absorption of disodium cromoglycate after its oral administration.

Based upon the difference in effects between the early and late airway response, we can divide the drugs used in our experiment into three groups, e.g., significantly effective for both the early and late airway responses (theophylline), only for the early airway response (ozagrel and salbutamol) and only for the late airway response (dexamethasone). Such a spectrum is in agreement with that generally seen in the clinic, supporting that the late airway response observed in the present study was pharmacologically different from the early airway response. Furthermore, inhaled salbutamol strongly suppressed the early airway response but not the late airway response, suggesting that the late airway response did not result from the early airway response.

Modipafant (UK80,067) is a potent and specific PAF receptor antagonist but its effect was not significantly different from that of placebo on diurnal variation in peak expiratory flow, morning and evening peak expiratory flow, clinic forced expiratory volume, rescue bronchodilator usage, symptom score, or airway responsiveness in adult subjects with moderately severe asthma in a placebo-controlled parallel group study (Kuitert et al., 1995). This result suggests that PAF is not an important mediator in asthma. In contrast, Hozawa et al. (1995) reported that Y-24180, a PAF receptor antagonist, significantly ($P = 0.005$) improved the PC20-FEV_1 (forced expiratory $volume_{1\,min}$) value, compared with placebo, without a carry-over effect or a period effect by analysis of variance in a randomized, double-blind, placebo-controlled,

two-phase cross-over study, suggesting that PAF is an important mediator involved in the bronchial hyperresponsiveness of asthma in humans. Thus, the clinical effectiveness of PAF receptor antagonists remains controversial. It has been reported that 4% of Japanese adults have a deficiency of serum PAF acetylhydrolase, which is the enzyme that inactivates PAF (Miwa et al., 1988). Miwa et al. suggested that a deficiency of serum PAF acetylhydrolase might be one of the factors leading to severe respiratory symptoms in asthmatic children. Taken together, there is a likelihood that PAF receptor antagonists would be useful for the medical treatment of bronchial asthma associated with a deficiency of PAF acetylhydrolase.

In conclusion, apafant inhibited both the early and late airway responses developed after antigen challenge in sensitized guinea pigs, and its effect was comparable or superior to that of anti-asthmatic drugs used clinically.

References

Ashton, J.M., Clark, B., Jones, K.M., Moss, G.F., Neal, M.G. and Ritchie, J.T.The absorption, metabolism and excretion of disodium cromoglycate in nine animal species. Toxicol. Appl. Pharmacol.., (Vol. 26) (1973) 319.

Assem, E.S.K. and Mongar, J.L.Inhibition of allergic reactions in man and other species by cromoglycate. Int. Arch. Allergy Appl. Immunol.., (Vol. 38) (1970) 68.

Assem, E.S.K. and Richter, A.W.Comparison of in vivo and in vitro inhibition of the anaphylactic mechanism by β-adrenergic stimulants and disodium cromoglycate. Immunology., (Vol. 21) (1971) 729.

Booij-Noord, H., Orie, N.G.M. and De Vries, K.Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. J. Allergy Clin. Immunol.., (Vol. 48) (1971) 344.

Burge, P.S., Fethimiou, J., Turner-Warwick, M. and Nelmes, P.T.J.Double-blind trials of inhaled beclomethasone diproprionate and fluocortin butyl ester in allergen-induced immediate and late asthmatic reactions. Clin. Allergy., (Vol. 12) (1982) 523.

Cartier, A., Thomson, N.C., Frith, P.A., Roberts, R., Tech, M. and Hargreaue, F.E.Allergen-induced increase in bronchial responsiveness to histamine: relationship to the late asthmatic response and change in airway caliber. J. Allergy Clin. Immunol.., (Vol. 70) (1982) 170.

Chignard, M., Le Còuedic, J.P., Andersson, P. and Brange, C.Use of steroidal antiinflammatory drug provides further evidence for a potential role of PAF-acether in bronchial anaphylaxis. Int. Arch. Allergy Appl. Immunol.., (Vol. 81) (1986) 184.

Cockcroft, D.W. and Murdock, K.Y.Comparative effects of inhaled salbutamol, sodium cromoglycate, and beclomethasone diproprionate on allergen-induced early asthmatic responses, late asthmatic responses, and increased bronchial responsiveness to histamine. J. Allergy Clin. Immunol.., (Vol. 79) (1987) 734.

Cox, J.S.G.Disodium cromoglycate (Intal). Adv. Drug Res.., (Vol. 5) (1970) 115.

Evans, T.W., Chung, K.F., Rogers, D.F. and Barnes, P.J.Effect of platelet activating factor on airway vascular permeability: possible mechanism. J. Appl. Physiol.., (Vol. 63) (1987) 479.

Hozawa, S., Haruta, Y., Ishioka, S. and Yamakido, M.Effects of a PAF receptor antagonist, Y-24180, on bronchial hyperresponsiveness in patients with asthma. Am. J. Respir. Crit. Care Med.., (Vol. 152) (1995) 1198.

Kuitert, L.M., Angus, R.M., Barnes, N.C., Barnes, P.J., Bone, M.F., Chung, K.F., Fairfax, A.J., Higenbotham, T.W., O'Conner, B.J. and Piotrowska, B.Effect of a novel potent platelet-activating factor antagonist, modipafant, in clinical asthma. Am. J. Respir. Crit. Care Med.., (Vol. 151) (1995) 1331.

Lellouch-Tubiana, A., Lefort, J., Simon, M., Pfister, A. and Vargaftig, B.B.Eosinophil recruitment into guinea pig lungs after PAF-acether and allergen administration: modulation by prostacyclin, platelet depletion, and selective antagonists. Am. Rev. Respir. Dis.., (Vol. 137) (1988) 948.

Matsumoto, Y., Ashida, Y. and Tsukuda, R.Pharmacological modulation of immediate and late airway response and leukocyte infiltration in guinea pig. J. Pharmacol. Exp. Ther.., (Vol. 269) (1994) 1236.

Mielens, F.E., Ferguson, E.W. and Rosenberg, F.J.Effects of anti-anaphylactic drugs upon passive antibodies in rats. Int. Arch. Allergy Appl. Immunol.., (Vol. 47) (1974) 633.

Miwa, M., Miyake, T., Yamanaka, T., Sugatani, J., Suzuki, Y., Sakata, S., Araki, Y. and Morimoto, M.Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. J. Clin. Invest.., (Vol. 82) (1988) 1983.

Pennock, B.E., Cox, C.P., Rogers, R.M., Cain, W.A. and Wèlls, J.H.A noninvasive technique for measurement of changes in specific airway resistance. J. Appl. Physiol., Respir. Environ. Exercise Physiol.., (Vol. 42) (1979) 399.

Pepys, J. and Hutchcroft, B.J.Bronchial provocation tests in etiologic diagnosis and analysis of asthma. Am. Rev. Respir. Dis.., (Vol. 112) (1975) 829.