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Effect of a platelet-activating factor (PAF) antagonist, SR 27417A, on PAF-induced gas exchange abnormalities in mild asthma

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ABSTRACT: Inhaled platelet-activating factor (PAF), both in normals and in asthmatic patients, provokes transient systemic effects, neutropenia, bronchoconstriction and arterial oxygenation abnormalities similar to those shown in spontaneous exacerbations of asthma.

To investigate the efficacy of a new PAF-receptor antagonist, SR 27417A, on all these changes after PAF challenge, 12 nonsmoking patients (four females and eight males) (mean±SEM) age 24±1 yrs with mild asthma (forced expiratory volume in one second (FEV_1) 93±3% predicted) were studied in a double-blind, placebo-controlled, cross-over fashion 2 weeks apart. PAF aerosol challenge (18 μg) was carried out 3 h after oral administration of either SR 27417A (20 mg) or placebo. Respiratory system resistance (R_{rs}) and arterial blood gases and neutrophil cell counts were measured at baseline, before compound/placebo administration, and at 5, 15 and 45 min after PAF.

Compared to vehicle, SR 27417A brought about moderate attenuation of PAF-induced neutropenia at 5 min (by 140%; p<0.025), and rebound neutrophilia at 15 and 45 min (p<0.025), increases of R_{rs} (by 90–65%) (p<0.01) and of alveolar-arterial pressure difference for oxygen ($P_{A\text{-}a,O_2}$) at 5 min (by 68%) and 15 min (by 63%), and decreases of arterial oxygen tension (P_{a,O_2}) at 5 min (by 57%; p<0.025, each). Furthermore, systemic effects and platelet aggregation tests (p<0.001) were abolished after the administration of the compound.

We conclude that SR 27417A is effective in inhibiting systemic, cellular and pulmonary effects after platelet-activating factor challenge in patients with mild bronchial asthma.

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Platelet-activating factor (PAF) [1] is a potent ether-linked phospholipid mediator of inflammation which is considered to have a potential role in the pathogenesis of bronchial asthma. Recently, we have shown that PAF (dosage range 24–12 μg) disturbed pulmonary gas exchange in normal individuals [2–4] and in patients with mild asthma [5, 6] in an identical manner to the entire spectrum of ventilation-perfusion (V'_A/Q') mismatching shown in patients with bronchial asthma [7, 8]. We suggested that the V'_A/Q' defects could be preferentially related to an augmented bronchial vascular permeability due to post-capillary venoconstriction induced by PAF, thereby supporting the notion that PAF may play a key role, as a putative potent mediator of inflammation in human airways [5, 8]. However, negative results have been obtained over the last few years, in stable patients with mild to moderate asthma with different PAF antagonists, and these question the role of PAF in the pathobiology of asthma.

The compound SR 27417A (N-(2-dimethylaminoethyl)-N-(pyridinylmethyl)(4-(2,4,6 triisopropylphenyl) thiazol-2-yl)) amine difumarate is a novel class of anti-PAF medication that has been shown to be a potent, long-lasting and selective second generation PAF antagonist [9, 10]. The present study was undertaken to assess the efficacy of this new PAF receptor antagonist, SR 27471A, in attenuating or preventing the PAF-induced systemic, neutropenic, lung mechanical and pulmonary gas exchange effects observed in patients with mild asthma [5, 6]. To our knowledge, the efficacy of this class of anti-asthma compounds on pulmonary gas exchange abnormalities provoked by PAF has not been investigated previously.

Methods

Study population

Twelve patients with mild asthma (table 1) were recruited from our Outpatient Department for the study, which was approved by the Ethical Research Committee of the Hospital Clinic. All subjects gave informed written consent after the purpose, risks and potential benefits of the study were explained to them. The inclusion criteria were: no respiratory infection or exacerbation of asthma within the preceding 6 weeks; forced expiratory volume in one second (FEV_1) ≥80% predicted and positive methacholine bronchial challenge; maintenance therapy with aerosol short-acting beta-adrenergics and/or inhaled corticosteroids, but no previous treatment with oral corticosteroids; and absence of any systemic or cardiopulmonary disease

Table 1. – Anthropometric and baseline function data on SR 27417A and placebo studies

Subjects n	12	
Age yrs	24±1	
Sex F/M	4/8	
Height cm	170±3	
Weight kg	69±3	
FEV_1 L	3.8±0.2	
FEV_1 %	93±3	
FEV_1/FVC %	76±2	
PD_{20} µmol	0.3±0.1	
	SR 27417A	Placebo
Neutrophils $\times 10^9 \cdot L^{-1}$	2.9±0.2	3.1±0.3
V'_E $L \cdot min^{-1}$	8.2±1.3	7.9±0.6
f_R breaths·min^{-1}	14±1	14±2
R_{rs} $cmH_2O \cdot L^{-1} \cdot s$	3.6±0.4	3.2±0.3
P_{sys} mmHg	89±3	88±2
f_C beats·min^{-1}	72±2	70±2
P_{a,O_2} mmHg	99±4	98±3
P_{a,CO_2} mmHg	38±1	38±1
pH	7.42±0.01	7.42±0.01
$P_{A\text{-}a,O_2}$ mmHg	5.8±1.8	6.2±2.3
V'_{O_2} $mL \cdot min^{-1}$	256±12	245±11
V'_{CO_2} $mL \cdot min^{-1}$	218±22	215±17

Values are absolute number, or mean±SEM. For arterial blood gases, n=10. F: female; M: male; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; PD_{20}: provocative dose of methacholine causing FEV_1 to fall 20% from baseline; V'_E: minute ventilation; f_R: respiratory frequency; R_{rs}: resistance of the respiratory system; P_{sys}: mean systemic arterial pressure; f_C: cardiac frequency; P_{a,O_2}: arterial oxygen tension; P_{a,CO_2}: arterial carbon dioxide tension; $P_{A\text{-}a,O_2}$: alveolar-arterial pressure difference for oxygen; V'_{O_2}: oxygen consumption; V'_{CO_2}: carbon dioxide production. 1 mmHg= 0.133 kPa.

other than asthma. All subjects were nonsmokers and all but one were atopic as judged by the presence of a positive response to skin tests to one or more common aeroallergens.

Measurements

Blood samples were collected anaerobically through a catheter inserted into the radial artery. Arterial oxygen and carbon dioxide tensions (P_{a,O_2} and P_{a,CO_2}, respectively) and pH were analysed using standard electrodes (IL 1302; Instrumentation Laboratories, Milano, Italy). Haemoglobin concentration was measured by a Co-oximeter (IL 482; Instrumentation Laboratories, Milano, Italy). Oxygen uptake (V'_{O_2}) and CO_2 production (V'_{CO_2}) were calculated from mixed expired O_2 and CO_2 (CPX System; Medical Graphics, St Paul, MN, USA). Minute ventilation (V'_E) and respiratory frequency (f_R) were measured using a calibrated Wright spirometer (Respirometer MK8; BOC-Medical, Essex, UK). The alveolar-arterial pressure difference for oxygen ($P_{A\text{-}a,O_2}$) was calculated according to the alveolar gas equation using the measured respiratory exchange ratio (RER).

Total white cell counts in arterial blood were measured with a Technicon H.1™ System (Technicon, Tarytown, NY, USA). For measurements of platelet aggregation, arterial blood (9 mL) was collected and centrifuged at 150 ×g for 20 min at room temperature to obtain the platelet-rich plasma (PRP). Aggregation was induced by addition to the PRP of the stated concentration of PAF or adenosine diphosphate (ADP). The maximal aggregation of the PRP was recorded over a period of ~5 min (Hitachi/Aggrecorder PA3210, Kyoto, Japan) and measurements were expressed as percentages of the maximal aggregation. Inhibition of PAF-induced aggregation in post-treatment samples was calculated as a percentage of the maximal aggregation to PAF in PRP obtained from blood samples withdrawn before intake of SR 27417A/vehicle.

Total resistance of the respiratory system (R_{rs}) was measured by the forced oscillation technique and its analysis restricted to 8 Hz, as reported in detail elsewhere [11, 12]. A three-lead electrocardiogram, cardiac frequency (f_C) and systemic pressure (P_{sys}) and arterial oxygen saturation (S_{a,O_2}) through a pulsioximeter (HP M1166A; Hewlett-Packard, Boblingen, Germany) were continuously recorded throughout the whole study (HP 7830A Monitor and HP 7754B Recorder; Hewlett-Packard, Waltham, MA, USA).

Study design

A randomized double-blind, placebo-controlled, crossover design was used. All patients were challenged on two occasions, 2 weeks apart, with inhaled PAF after the administration of either 20 mg of oral SR 27417A or placebo (lactose) with patients breathing room air and seated in an armchair. Asthma medication was withheld for 12 h before arrival in the laboratory. After the establishment of adequate steady-state conditions, a set of duplicate measurements of arterial blood respiratory gases and white blood cell counts and of ventilatory, haemodynamic and R_{rs} was carried out (baseline). Maintenance of steady-state conditions after PAF challenge was demonstrated by stability (±5%) of both ventilatory and haemodynamic variables, and by the close agreement between duplicate measurements of mixed expired and arterial O_2 and CO_2 (within ±5%). These conditions were met in all patients throughout the study period. Likewise, a test of platelet aggregation was carried out before and 3 h after either SR 27417A or vehicle.

Three hours after compound/placebo administration, the patient was challenged with PAF (C_{16}) (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) (18 µg) (Novabiochem AG, Laufelfingen, Switzerland). The preparation of the PAF solution and details of the PAF challenge have been reported in full elsewhere [2–6]. Duplicate measurements were taken then at 5, 15 and 45 min following PAF inhalation, as described previously [2–6]. All sets of measurements consisted of the following steps in sequence: ventilatory recordings; respiratory gas and circulating white blood cell samplings; and haemodynamic and R_{rs} measurements.

Statistical analysis

The results are expressed as mean±SEM and 95% confidence interval. All the analyses were performed with version 6.0.1. of the Statistical Package for the Social Sciences (SPSS), (SPSS Inc., Chicago, IL, USA). Both the effects of PAF challenge and pretreatment with SR 27417A or placebo on white cell counts and arterial and mixed expired blood gases, ventilatory and haemodynamic variables and R_{rs} were assessed by a two-way repeated analysis of variance (ANOVA). Whenever an interaction between

the effects of PAF challenge and those of treatment was found, mean differences between SR 27417A and placebo at each time point were analysed using paired t-test corrected for multiple comparisons. Likewise, paired t-test was used to assess differences in platelet aggregation before and after drug/placebo administration. Pearson's correlations were used when appropriate to assess relationships between variables. Significance was set at pð0.05 in all instances.

Results

Baseline data before PAF

Mean anthropometric and functional measurements and baseline values are presented in table 1. All were similar to those reported in our previous investigations [5, 6] with no differences between SR 27417A and placebo studies. Platelet aggregation tests at 40 nM PAF (from 69±8% to 6±1%) and 80 nM PAF (from 79±7% to 6±1%) were significantly abolished (both p<0.001) 3 h after oral administration of the compound, but not after vehicle (from 60±7% to 63±7% and from 73±7% to 76±7%, respectively).

Effects of PAF after placebo (table 2 and fig. 1)

All but three patients noticed facial flushing, three coughed and two felt shortness of breath immediately after PAF challenge. Compared with pretreatment with SR 27417A, circulating peripheral blood neutrophils fell in all but three patients at 5 min (p<0.025) after PAF inhalation, followed by a rebound neutrophilia in 11 of them at 15 and 45 min (p<0.025). Total R_{rs} increased in all but two patients at 5 min, an increase which persisted 15 and 45 min after PAF challenge (p<0.01). As for arterial blood gas abnormalities (n=10), P_{a,O_2} decreased at 5 min in all patients (p<0.025), a finding probably explained by the development of V'_A/Q' mismatch provoked by inhaled PAF as shown previously [2–6]; similarly, the increases in $P_{A\text{-}a,O_2}$ were significant at 5 and 15 min after PAF challenge (p<0.025 each). By contrast, ventilatory and haemodynamic variables and the other gas exchange indices, including arterial pH, did not change.

Effects of SR 27471A on PAF challenge (table 2 and fig. 1)

Compared with vehicle, pretreatment with SR 27471A effectively abolished PAF-induced systemic effects: cough and dyspnoea were prevented in all patients, while facial flushing was only observed in one. In addition, the PAF-induced decrease of peripheral neutrophil counts at 5 min (by 140%) and the subsequent rebound neutrophilia observed at 15 and 45 min were completely offset in all but one patient. Equally important, PAF-induced lung function abnormalities were also blocked to a moderate to profound extent. Thus, pretreatment with SR 27471A prevented the increase in R_{rs} in half of the patients over the whole period of study (by 90–65%), while the P_{a,O_2} reductions at 5 min (by 57%) in all but three patients and the increases of $P_{A\text{-}a,O_2}$ at 5 min (by 68%) and 15 min (by 63%) in all but five patients were attenuated when compared to vehicle pretreatment.

There were no significant correlations between the changes in the different lung function variables and cellular abnormalities after PAF.

Table 2. – Effects of SR 27417A/placebo on platelet activating factor (PAF) challenge (n=12)

	Difference from baseline			
	5 min	15 min	45 min	p-value†
Neutrophils $\times 10^9$ cells·L^{-1}				
Placebo	-1.5 (-2.6– -0.4)	1.5 (0.5–2.4)	2.1 (0.8–3.3)	0.01
SR 27417A	0.6+ (0.2–1.0)	0.7 (0.2–1.1)	0.6+ (0.2–1.0)	
V'_E L·min^{-1}				
Placebo	0.7 (-0.2–1.7)	0.7 (-0.1–1.5)	0.1 (-9.0–1.1)	NS
SR 27417A	-0.6 (-3.0–1.7)	-0.4 (-2.6–1.7)	-0.5 (-2.9–1.9)	
f_R breaths·min^{-1}				
Placebo	0.6 (-0.7–1.8)	0.3 (-1.1–1.8)	-0.4 (-1.8–1.0)	NS
SR 27417A	0 (-1.1–1.1)	-0.2 (-1.3–0.9)	0.5 (-1.1–2.1)	
R_{rs} cmH_2O·L^{-1}·s				
Placebo	2.0 (0.8–3.1)	1.7 (0.4–3.0)	1.0 (0.1–1.9)	0.006‡
SR 27417A	0.7 (0.2–1.3)	0.6 (0.1–1.2)	0.1 (-0.4–0.6)	
P_{a,O_2} mmHg				
Placebo	-27.1 (-36.3– -17.9)	-17.1 (-27.9–-7.2)	-6.8 (-14.3– -0.7)	0.004
SR 27417A	-11.7+ (-21.4– -1.9)	-9.2 (-16.4– -2.0)	-5.6 (-12.8–1.6)	
P_{a,CO_2} mmHg				
Placebo	-0.4 (-1.6–0.7)	-0.6 (-2.5–1.3)	-0.9 (-2.9–1.0)	NS
SR 27417A	-0.6 (-2.1–1.0)	0.3 (-0.9–1.4)	-0.3 (-1.5–1.0)	
$P_{A\text{-}a,O_2}$ mmHg				
Placebo	29.3 (19.6–38.9)	15.8 (7.7–23.9)	6.5 (2.3–10.8)	0.004
SR 27417A	9.4 (0.5–18.3)	5.8+ (-0.7–12.3)	3.6 (-1.7–8.9)	

For arterial blood gases, n=10. Values are means, and 95% confidence intervals in parentheses. +: p<0.025 for comparison with placebo; †: significance of the interaction between the effects of PAF challenge and pretreatment with SR 27417A/placebo; ‡: significance of the effect of treatment (SR 27417A *versus* placebo) calculated by repeated measures analysis of variance. For definitions of abbreviations, see table 1.

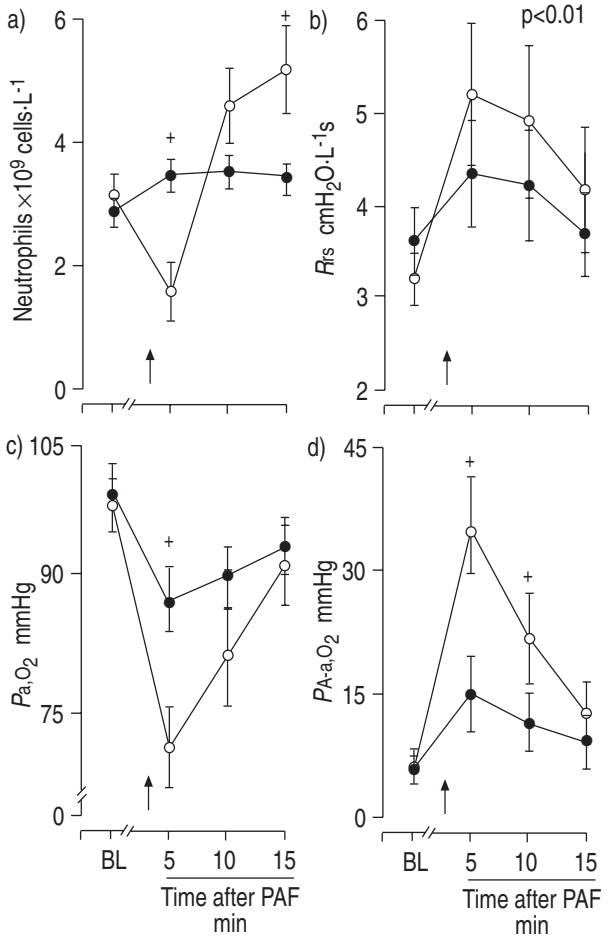

Fig. 1. – Mean (±SEM) time courses of: a) circulating neutrophils (n=12); b) resistance of the respiratory system (R_{rs}) (n=12); c) arterial oxygen tension (P_{a,O_2}); and d) alveolar-arterial pressure difference for oxygen ($P_{A\text{-}a,O_2}$) (n=10), after inhaled platelet activating factor (PAF) (arrow) with pretreatment with vehicle (lactose) (○) and PAF receptor antagonist (SR 27417A) (●) at baseline (BL) and at 5, 15 and 45 min. +: p<0.025. The p-value for R_{rs} denotes the effect of treatment after PAF challenge. 1 mmHg=0.133 kPa.

Discussion

Dosing with SR 27417A inhibited all PAF-induced systemic effects and abnormal neutrophil kinetics while effectively minimizing both bronchoconstriction and gas exchange disturbances. By contrast, PAF challenge after vehicle induced all previously reported systemic, neutropenic and lung function abnormalities both in healthy subjects [2–4] and in patients with asthma [5, 6]. Accordingly, SR 27417A is an effective inhibitor of PAF-induced effects on human airways and the dose administered (20 mg) was sufficient, as shown by the significant inhibition of platelet aggregation after SR 27417A. In comparison, in the same model of human asthma after PAF challenge, using identical dosage [6], inhaled salbutamol, but not ipratropium bromide, was efficacious in completely antagonizing all PAF-induced systemic, white cell count and lung function abnormalities. This suggests an anti-oedema effect of short-acting beta-adrenergic agonists that probably attenuates the post-capillary venoconstriction of the bronchial vasculature [13, 14]. This is the first study to demonstrate inhibition of gas exchange abnormalities with a PAF antagonist.

The data after PAF in patients pretreated with vehicle complement and extend our previous investigations, both in healthy individuals [2–4] and in patients with asthma [5, 6]. The effects induced by 18 μg PAF at 5 min on cellular and lung function end-point variables after pretreatment with vehicle were quantitatively more profound than those obtained after a lower dose (12 μg) of PAF in a similar subset of asthmatic patients [5]. Compared to the challenge with 12 μg [5], we observed an approximately twofold increase in the fall of circulating neutrophils (-49% *versus* -29%), the decrease of P_{a,O_2} (-28% *versus* -16%) and the increase of R_{rs} (+62% *versus* +28%) while the increase of $P_{A\text{-}a,O_2}$ was even greater (+473% *versus* +112%), respectively, indicating a dose-dependent effect of PAF. We speculate that pulmonary gas exchange abnormalities and the simultaneous mild-to-moderate increases of R_{rs} caused by inhaled PAF are more related to narrowing of airway calibre secondary to increased microvascular leakage than to a primary reversible constrictor effect in airway smooth muscle [2–6, 15, 16].

SR 27417A is a new PAF receptor antagonist that fully and competitively displaces radiolabelled PAF from its high affinity binding sites on rabbit and human platelets with equilibrium dissociation constant (KD) values of 57± 0.02 pM and 50±0.08 pM, respectively. On human polymorphonuclear leucocytes SR 27417A inhibits the specific binding of ^{3}H-PAF to its high affinity receptors (50% inhibitory concentration (IC50), 0.17±0.002 nM) and displays the same inhibitory pattern already reported for platelets [9]. In animal studies, SR 27417A protects actively sensitized mice from anaphylactic death when given intravenously 5 min before ovalbumin rechallenge (50% protective dose (PD50), 45 μg·kg^{-1}) or from endotoxin-induced death (PD50 100 μg·kg^{-1}). A long-lasting protection against endotoxin-induced shock was also found using the oral route in mice at the single dose of 1.0 mg·kg^{-1} [10]. Clinical data in healthy volunteers indicate that, at the 2.5 mg oral dose, maximum inhibition of PAF-induced platelet aggregation is achieved and lasts over 24 h. Similarly, at the same dose, PAF-induced bronchoconstriction (dosage 36 μg) is also abolished (unpublished data, Report 656.6.033, DARP, Sanofi Recherche, Paris Gentilly, France). Moreover, SR 27417A (10 mg *p.o.*) significantly protected asthmatic patients against allergen late phase reaction [17].

Although many PAF-receptor antagonists have been tested in animal and human models of allergy and asthma, there have been no data regarding their effects on pulmonary gas exchange disturbances provoked by PAF. In comparison to our findings, BN 52063 showed an inhibitory effect on weal-and-flare response to intradermal injection of PAF and *in vitro* PAF-induced platelet aggregation [18]. However, one of the most investigated PAF-receptor antagonists, WEB 2086, did not alter allergen challenge responses [19, 20], or show differences when compared to placebo, in reducing glucocorticosteroid demands in atopic asthmatics [21]. Another PAF-receptor antagonist, UK 74505, did not alter early or late responses to allergen inhalation or airway hyperresponsiveness to histamine in sensitized patients [22]. Also, its (+)-enantiomer (modipafant) did not show beneficial effects in patients with moderately severe asthma, as assessed by diurnal variations of peak expiratory flow (PEF) or FEV1 [23]. On the other hand, Y-24180, a potent PAF-receptor antagonist, slightly improved

airway hyperresponsiveness in asthmatic patients but had no effect on lung function [24]. More recently, SR 27417A minimally attenuated the late allergen-induced asthmatic response without effects on early response, airway responsiveness or lung function tests [25].

In conclusion, platelet activating factor antagonists remain the best tool available to assess whether platelet activating factor plays an important role in the pathobiology of human asthma, a hypothesis always invoked, but never definitively proven. On the basis of most of the previous pharmacological studies, it is unlikely that platelet activating factor is a conspicuous mediator in asthma or airway hyperreactivity in humans. However, it might be surmised that many studies with platelet activating factor antagonists have failed to antagonize platelet activating factor rather than demonstrating that platelet activating factor is not a cardinal mediator in bronchial asthma. Furthermore, the fact that several of these studies show conflicting data suggests that, in different challenges, either there are different concentrations of platelet activating factor released or perhaps expression of distinct platelet activating factor receptors. The presence of varying receptor subtypes is supported by pharmacological studies with platelet activating factor antagonists that have demonstrated enormous differences in potency in different cell types in the same species [26]. Because lung tissue harbours specific receptors for a wide variety of inflammatory mediators, a single mediator antagonist alone may not display an effective overall antimediator therapeutic response in natural forms of asthma. In this regard, a study with SR 27417A in the setting of patients with spontaneous acute severe asthma would be of great interest to further assess any potential beneficial effects on pulmonary gas exchange abnormalities shown in the current laboratory-induced model of platelet activating factor.

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