

Platelet-activating factor nasal challenge induces nasal congestion and reduces nasal volume in both healthy volunteers and allergic rhinitis patients

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ABSTRACT

Background: Platelet-activating factor (PAF) is a lipid mediator produced by most inflammatory cells. Clinical and experimental findings suggest that PAF participates in allergic rhinitis (AR) pathogenesis. The aim was to assess the PAF ability to induce clinical response in nasal airway after local stimulation.

Method: Ten nonatopic healthy volunteers (HVs) and 10 AR patients out of pollen season were enrolled. PAF increasing concentrations (100, 200, and 400 nM) were instilled into both nasal cavities (0, 30, and 60 minutes, respectively). Nasal symptoms (congestion, rhinorrhea, sneezing, itching, and total 4 symptom score and nasal volume between the 2nd and 5th cm (Vol₂₋₅) using acoustic rhinometry (AcR), were assessed at -30, 0, 30, 60, 90, 120, and 240 minutes.

Result: PAF increased individual and total nasal symptom score in both HVs and seasonal AR (SAR) patients from 30 to 120 minutes (maximum score at 120', $p < 0.05$). Nasal obstruction was the most relevant and lasting nasal symptom. PAF also induced a significant reduction of Vol₂₋₅ at 90' (27%), 120' (38.7%), and 240' (36.4%). No differences in the response to PAF nasal challenge were observed between HVs and SAR subjects in either clinical symptoms or AcR.

Conclusion: This is the first description of PAF effects on human nasal mucosa using a cumulative dose schedule and evaluated by both nasal symptoms and AcR. Nasal provocation with PAF showed long-lasting effects on nasal symptoms and nasal obstruction in HVs and in patients with SAR. Nasal challenge may be a useful tool to investigate the role of PAF in AR and the potential role of anti-PAF drugs.

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Platelet-activating factor (PAF) is a potent mediator of inflammation with a relevant role in the pathogenesis of several diseases.¹ PAF is released from a variety of cells, including endothelial cells, neutrophils, macrophages, or mast cells² and most of them have PAF receptors.³ The capability of activating, inducing chemotactic responses, or proinflammatory mediators release from eosinophils, mast cells, platelets, or other circulating cells has been established in *in vitro* studies.^{2,3} PAF involvement in asthma has been long studied even though it has been associated with bronchoconstriction, bronchial hyperresponsiveness, mucus secretion, or gas exchange impairment.^{4,5} PAF receptor antagonists have not clearly proven their clinical benefits.⁵ PAF in allergic rhinitis (AR) has been less studied and its potential role as a new therapeutic target is still unknown.⁶ Some authors have found increased PAF levels in nasal lavage after nasal challenge with pollen.⁷ In addition, some studies have proposed PAF as a factor implicated in the priming phenomenon.^{1,8} In fact, some studies have shown a greater nasal response after histamine or bradykinin nasal challenge, if PAF has been previously administered.⁹

Acoustic rhinometry (AcR) is a noninvasive, rapid, and reliable method that can be performed easily with minimal patient cooperation. AcR has shown its accuracy in several nasal diseases and has shown a good direct correlation with symptoms score.^{10–12}

The aim of this study was to assess the PAF ability to induce an inflammatory response in the nasal airway. To this purpose, we evaluated nasal symptoms and nasal volume by AcR, after nasal challenge with PAF in healthy volunteers (HVs) and patients with seasonal AR (SAR).

METHODS

Study Design

This study was performed in a double-blind manner and out of pollen season. All subjects were challenged with PAF diluent (4% ethanol) at -30 minutes, before PAF administration to rule out unspecific nasal reactivity. Three consecutive increasing doses of PAF (100, 200, and 400 nM) were administered every 30 minutes (0, 30, and 60 minutes). Nasal response was objectively evaluated by AcR. Nasal symptoms (rhinorrhea, nasal congestion, nasal itching, and sneezing) were subjectively scored by visual analog scale (VAS; 0–100 mm) and Likert scale (from 0 to 3 for each symptom and from 0 to 12 for total symptom score). Both objective and subjective nasal responses were measured at 0, 30, 60, 90, 120, and 240 minutes (Fig. 1). The local Ethics Committee of our institution approved the study. J. Mullol and C. Picado contributed equally to this work.

Study Population

Twenty subjects, 10 HVs and 10 patients with SAR, were recruited. The study was performed out of pollen season. The SAR patient group had positive prick test and specific IgE to grass or plane tree pollen with compatible personal history of SAR. All subjects refrained from using any medication (antihistamines and corticosteroids) for 4 weeks before and during the study and none of them had recently suffered an episode of rhinitis. All subjects gave their informed consent before the study.

Acoustic Rhinometry

Nasal volumes were measured by AcR (Acoustic Rhinometer SER 2000; RhinoMetrics, Lynge, Denmark). All AcR measurements were repeated three times and then averaged, to ensure the reproducibility of the results. All measurements were performed in both the right and

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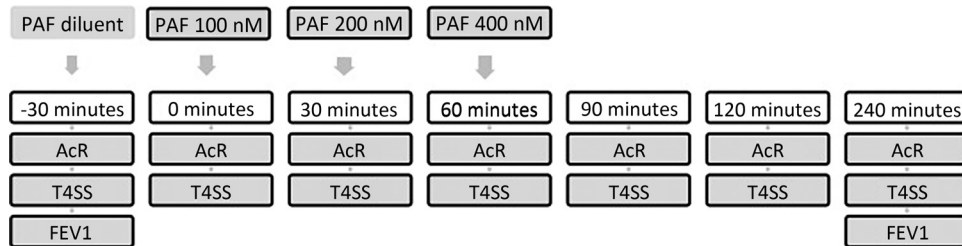


Figure 1. Chronogram.

Table 1 Patients' baseline characteristics

	All Subjects	HVs	SAR
Age, mean yr (SD)	30.2 (4.5)	30.4 (2.5)	30 (6)
Females, n (%)	11 (55%)	5 (50%)	6 (60%)
AcR Vol ₂₋₅ , mean (SD)	6.2 (1.3)	6.3 (1.7)	6.1 (0.9)
T4SS, mean (SD)	0.2 (0.4)	0.1 (0.3)	0.3 (0.4)

AcR = acoustic rhinometry; T4SS = total 4-symptoms score (Likert scale and visual analogue scale); HVs = healthy volunteers; SAR = seasonal allergic rhinitis; Vol₂₋₅ = nasal volume between the 2nd and 5th cm.

the left nasal cavities. Because the main changes in nasal geometry occur in the most vascularized segment, the nasal volume was evaluated between the 2nd and 5th cm (Vol₂₋₅) of the nasal cavity using a standardized method.¹³

Nasal Challenge

PAF (Sigma Aldrich, Madrid, Spain) was dissolved in 100% ethanol (final concentration of 4%) and diluted in 0.9% w/v NaCl solution containing 0.25% w/v serum albumin (Sigma Aldrich), with a final concentration of 10⁻² M. PAF doses were chosen according to previously reported studies.^{9,14,15} PAF (100 µL) was instilled into each nostril with an Eppendorf pipette.

Statistics

VAS and Likert scale results were expressed as mean ± SD. Nasal volume changes were expressed as the percentage change from the nasal volume value obtained with the diluent challenge. Data were compared using ANOVA test and Student's *t*-test for paired and unpaired data. The values of *p* < 0.05 were considered significant.

RESULTS

Subjects

Twenty subjects were evaluated (11 women; mean age, 30.2 years [4.5 SD]). Mean baseline AcR Vol₂₋₅ was 6.2 (1.3 SD) and mean total 4 symptoms score was 0.2 (0.4 SD). Both groups' baseline characteristics are described in Table 1.

There were no significant differences in evaluated variables between SAR and HV group.

Nasal Challenge with PAF

No increase in symptoms or in nasal volume recordings were observed after the diluent challenge in HV and SAR patients out of the pollen season. PAF was administered in progressive increasing doses (100, 200, and 400 nM) and a dose-dependent reversible increase in total nasal symptoms was observed and confirmed by symptoms score scale (Likert scale) and VAS in both groups. Nasal obstruction was the most significant and lasting symptom, and rhinorrhea, sneezing, and nasal itching increased much less after nasal PAF instillation (Table 2). Maximum symptoms score (Likert scale and VAS) were reached at 120 minutes, and a significant nasal symptom improvement (VAS and Likert) was observed at 240 minutes (Figs. 2, A and B).

A progressive decrease in nasal volume (Vol₂₋₅) after PAF challenge was observed. At minute 90 a reduction of Vol₂₋₅ of >25% compared with baseline was observed. The greatest decrease in Vol₂₋₅ was reached at 120 minutes and despite of clinical improvement, nasal obstruction (objective data from AcR) was still important at 240 minutes (Fig. 3).

Although symptom scores after PAF nasal challenge were, in general, higher in SAR patients than in HVs, no statistical differences were found between these groups.

Table 2 Effect of PAF nasal challenge on individual nasal symptom evaluated by Likert scale (0–3)

	–30 min	0 min	60 min	90 min	120 min	240 min
Itching						
HV mean (SD)	0	0	0.2 (0.4)	0.3 (0.7)	0.3 (0.7)	0
SAR mean (SD)	0	0.1 (0.3)	0.7 (0.7)	0.7 (0.7)	0.7 (0.9)	0.3 (0.7)
Rhinorrhea						
HV mean (SD)	0	0	0.4 (0.7)	0.6 (0.7)	0.8 (0.8)	0.3 (0.7)
SAR mean (SD)	0	0	0.5 (0.5)	0.9 (0.7)	1.6 (0.7)	0.5 (0.7)
Nasal Obstruction						
HV mean (SD)	0.1 (0.3)	0.1 (0.3)	0.5 (0.7)	0.9 (0.7)	1.7 (0.8)	1 (0.94)
SAR mean (SD)	0.3 (0.5)	0.3 (0.5)	0.8 (0.8)	1.1 (0.9)	1.8 (1)	1 (1)
Sneezing						
HV mean (SD)	0	0.2 (0.4)	0.3 (0.5)	0.5 (0.5)	0.3 (0.5)	0
SAR mean (SD)	0	0	0.6 (0.7)	0.5 (0.7)	0.7 (0.8)	0.1 (0.3)

Differences between 60, 90, 120, or 240 min and baseline (–30 and 0 min) were not statistically significant.

No differences were found between HVs and SAR patients at any time and for any nasal symptom.

HVs = healthy volunteers; PAF = platelet-activating factor; SAR = seasonal allergic rhinitis.

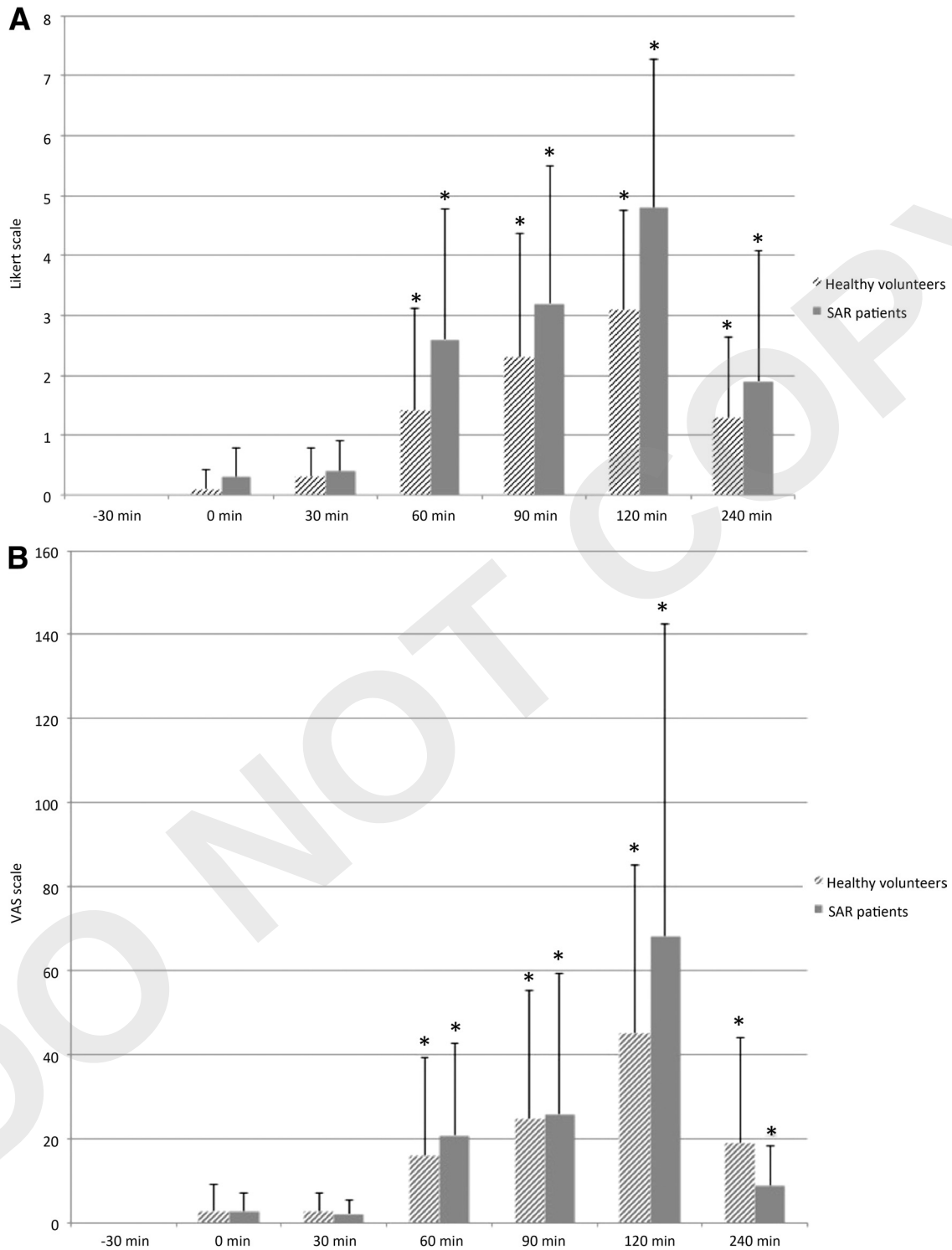


Figure 2. Effect of platelet-activating factor (PAF) nasal challenge on total nasal symptom score evaluated by (A) Likert scale (0–12) and (B) visual analog scale (VAS; 0–100 mm) in seasonal allergic rhinitis (SAR) patients and healthy volunteers (HV; * $p < 0.05$ compared with baseline at 0 minutes).

DISCUSSION

We have shown the ability of cumulative doses of PAF to induce a response in the nasal airways. We tested PAF in both HVs and SAR patients, but no differences in the responses were observed between the two groups.

Nasal response was evaluated subjectively in nasal symptoms (VAS and Likert scale), and the objective assessment of nasal challenge was made by AcR, because of its demonstrated accuracy to assess nasal cavity geometry and its close correlation with nasal symptoms, mostly with nasal obstruction.^{10,11} Previous studies have used rhinomanometry to assess nasal obstruction. In contrast to AcR,

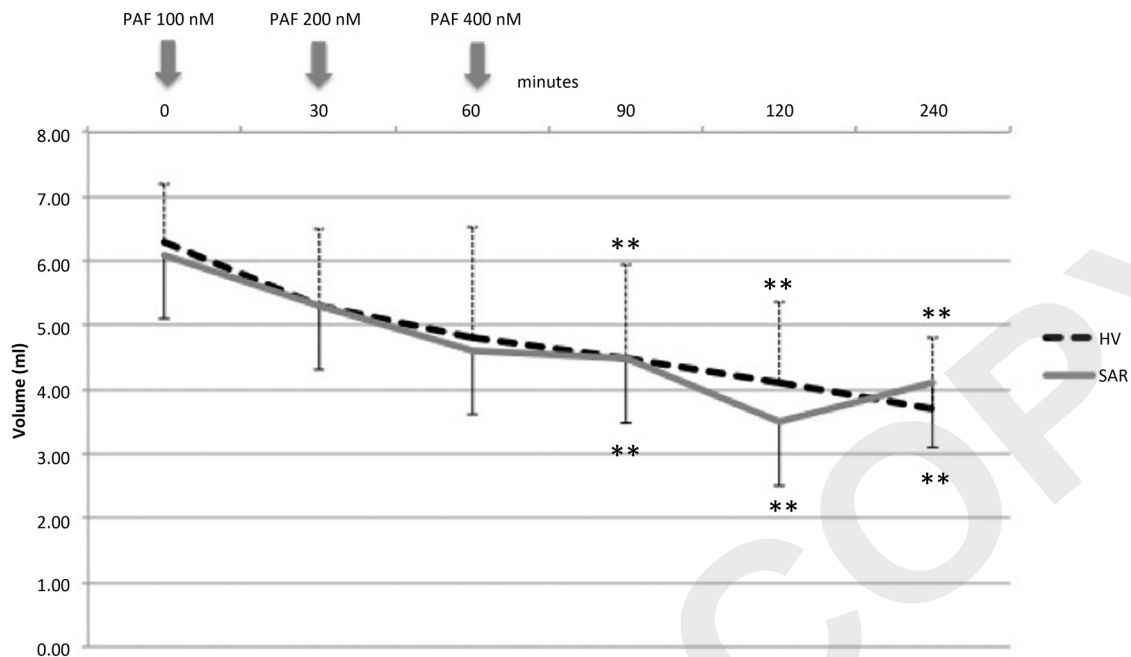


Figure 3. Nasal volume decrease evaluated by acoustic rhinometry (AcR) in healthy volunteers (HVs) and seasonal allergic rhinitis (SAR) patients.

rhinomanometry is a technique with a poor correlation with clinical symptoms.^{10,12,13}

Previous studies have examined the effects of PAF on nasal mucosa with contradictory conclusions. Klementson *et al.*¹⁴ showed a nasal response to PAF only in AR patients. In keeping with our results, Leggieri *et al.*⁹ could not find any difference in the nasal response to PAF between HVs and AR patients. Similarly, Tedeschi *et al.*¹⁵ reported that only 3 out of 10 healthy subjects did not clinically respond when they were challenged with nasal PAF.

A progressive increasing dose of PAF (100, 200, and 400 nM) with a final cumulative dose of 700 nM was used in this study, and the greatest response occurred after 60 minutes of the last dose of 400 nM. All previously published studies used a single dose of PAF (from 30 to 600 nM), and found that symptoms peaked at 30 minutes after PAF challenge.^{9,14,15} We observed an almost complete resolution of symptoms at 240 minutes after PAF instillation. These results concur with those previously reported by Tedeschi *et al.*¹⁵ In contrast with our results, another study reported improvement in symptoms, and normalization of nasal resistance (measured by rhinomanometry), 60 minutes after PAF challenge. Differences in the PAF dosage between studies (from 30 to 700 nM) most probably account for the reported discrepancies on the duration of the effects of intranasal PAF.

Nasal congestion was the predominant subjective symptom induced by PAF, and rhinorrhea was less relevant and sneezes or nasal itching were experienced only by some subjects and were usually mild. These findings probably resulted from the well-known ability of PAF to induce an increase in vascular permeability.^{9,14,15} We have used AcR for nasal blocking assessment because of its sensibility for detecting nasal volume changes even in severe obstructions, as observed in nasal polyposis, where other techniques, such as rhinomanometry, can not be used.^{12,13,16}

The role of PAF as an inflammatory mediator has been mostly studied in lung diseases. PAF levels have been found significantly higher in patients with asthma,^{17,18} and several independent lines of evidence indicate that PAF is a mediator of positive feedback mechanisms during the inflammation that occurs in asthmatic airways.¹⁹ However, specific PAF antagonists have not provided any further therapeutic option in the disease, because of minor or lacking effects.¹⁷ In contrast to asthma, some PAF antagonists such as ABT-491

have shown its efficacy in animal models of rhinitis, inhibiting the nasal response after PAF challenge.²⁰ We could be facing a paradoxical effect of PAF antagonists, without effect in asthma despite its anti-inflammatory properties but with potential therapeutic effects in rhinitis. Although there is evidence to support the concept of one airway, one disease, some differences still exist between asthma and rhinitis. For instance, antihistamines are recommended as first-line therapy for rhinitis, but they have no indication in asthma.^{21,22} However, additional studies in humans with PAF antagonists alone or combined with other mediator antagonists (*e.g.*, antihistamines) are needed to evaluate PAF relevance on rhinitis symptoms.

In conclusion, we have shown the role of PAF in nasal symptoms in a human model of PAF challenge. To our knowledge, this is the first description of PAF effects on human nasal mucosa using a cumulative dose schedule and evaluating both subjective (nasal symptoms) and objective (AcR) outcomes.

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