Respiratory Medicine (2005) **99**, 519–523

ELSEVIER

respiratoryMEDICINE

Lack of systemic oxidative stress during PAF challenge in mild asthma ☆

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Received 18 October 2004

KEYWORDS
Acute asthma;
Leukotrienes;
Inflammatory mediators;
Oxidant–antioxidant imbalance

Summary To further establish the role of oxidative stress in the pathogenesis of acute bronchial asthma, we investigated the effects of platelet-activating factor (PAF) challenge on systemic oxidant–antioxidant balance in 12 asthmatic patients (age, 25 ± 3[SEM] yr; FEV_1, 95 ± 10% predicted), using a double blinded, controlled with Lyso-PAF (L-PAF), cross-over design.

Respiratory system resistance (Rrs), arterial blood gases, peripheral blood neutrophils and oxidant–antioxidant balance, including thiobarbituric acid (TBA)-malondialdehyde (MDA) adducts, protein sulphydryls and Trolox equivalent antioxidant capacity (TEAC), were assessed at baseline and 5, 15 and 45 min after PAF and L-PAF (18 μg each) bronchoprovocation. Urinary leukotriene E_4 ($uLTE_4$) elimination was measured 120 min after challenge.

Compared with baseline, as expected, PAF increased significantly Rrs and $Aa\mathit{PO}_2$ and decreased PaO_2 and peripheral blood neutrophils along with a rebound neutrophilia and increased $uLTE_4$. By contrast, markers of systemic oxidative stress remained unaltered throughout the study. Unlike PAF, L-PAF-induced changes were negligible.

We conclude that there is no systemic oxidant–antioxidant imbalance during acute bronchoconstriction induced by PAF in these patients with mild asthma.

☆Supported by Grants 99/0135 from the Fondo de Investigación Sanitaria (FIS), the Comissionat per a Universitats i Recerca de la Generalitat de Catalunya (2001 SGR00286), Red Respira-ISCIII-RTIC-03/11, and by grant-in-aids by AstraZeneca SA (Spain) and Laboratorios Esteve SA.
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Introduction

In patients with asthma platelet-activating factor (PAF) inhalation provokes neutropaenia, bronchoconstriction and airway microvascular leakage,

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doi:10.1016/j.rmed.2004.10.013

resulting in pulmonary gas exchange disturbances similar to those observed during naturally occurring acute asthma.[1,2] There is also evidence of increased neutrophil pulmonary sequestration after PAF and circulating leukocytes in asthmatic patients show an enhanced oxidative burst.[2–4] Accordingly, it has been suggested that an imbalance between oxidants and antioxidants may have a role in the pathogenesis of acute asthma.[5] We hypothesised that PAF challenge as a model of acute asthma would result in an altered systemic oxidant–antioxidant balance in the context of an abrupt bronchoprovocation resembling airway changes observed in spontaneous asthma attacks. To this end, we challenged mild asthmatics with PAF and measured the markers of oxidative stress in plasma.

Methods

Study population

Patients with mild asthma were recruited from our Outpatient Department for the study, which was approved by the Ethical Research Committee of the Hospital Clínic. 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; $FEV_1 > 70\%$ predicted and > 1.5 L; positive methacholine ($PD_{20} < 1.9\,\mu$mol) and PAF ($\geqslant 20\%$ increase of baseline resistance of respiratory system [Rrs] after PAF [8 μg]) bronchial challenges; no previous treatment with systemic steroids within the preceding 4 weeks; and, absence of any systemic or cardiopulmonary disease other than asthma. Maintenance therapy consisted of rescue inhaled medication with short-acting β_2-adrenergics in patients, inhaled glucocorticosteroids in 2, inhaled long-acting β_2-adrenergics in 1, and inhaled combined therapy in 3. All subjects were non-smokers and were atopic.

Lung function and peripheral blood cells

The resistance of Rrs, arterial PO_2, PCO_2 and pH, the alveolar-arterial PO_2 difference ($AaPO_2$), oxygen uptake ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), and total white cell counts in arterial blood were measured or calculated, as previously described.[1,6] A three-lead electrocardiogram, heart rate and systemic pressure and arterial O_2 saturation through a pulseoximeter (HP M1166A, Hewlett-Packard, Boblingen, Germany) were continuously recorded throughout the whole study. Measurements of urinary cysteinyl leukotriene E_4 ($uLTE_4$), corrected for urinary creatinine and urine volume, were carried out with a validated enzyme immunoassay (EIA), as previously described.[6,7]

Markers of oxidative stress

A 10 ml arterial blood sample was collected in two EDTA/citrate tubes, spun immediately at 1000 rpm for 5 min in order to measure plasma antioxidant capacity. The plasma was aspirated into three clean eppendorf tubes and stored at $-70\,^{\circ}$C until analysed in dry ice. Three samples at each time point were obtained.

Antioxidant capacity assay: The plasma antioxidant capacity TEAC (Trolox equivalent antioxidant capacity) was measured by the method of Miller et al.[8] TEAC was calculated by defining the concentration, in mmol L^{-1} of TEAC to a 1.0 mmol L^{-1} sample of the plasma under investigation.

Lipid peroxides assay: The level of plasma lipid peroxidation products as thiobarbituric acid (TBA)-malondialdehyde (MDA) adducts were measured spectrophotometrically by the method described by Yagi.[9] The final result was expressed as micromoles of MDA formed per litre of plasma. In one patient, this assay was not available.

Protein sulphydryl assay: Protein thiols were measured using the method of Ellman.[10] Oxidized protein sulphydryls were measured by treating plasma with 10 mM dithiothreitol (DDT), at 4 °C for 2 h, followed by dialysis overnight at 4 °C against 2×100 volumes of nitrogen-saturated PBS. Reduced protein sulphydryls were then measured with Ellman's reagent as previously described.[10]

Study design

A randomised, double-blinded, L-PAF-controlled, cross-over design was used. After completing the first visit for recruitment, patients were challenged on two separate days 1 week apart with inhaled PAF (C_{16}) (1-*O*-Hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) (18 μg) or L-PAF (C_{16}) (1-*O*-Hexadecyl-sn-glycero-3-phosphocholine) (18 μg) (Novabiochem AG, Laufelfingen, Switzerland, each), as previously described.[1,6] All asthma medication was withheld for 2 days before arrival to the laboratory. After ensuring steady-state conditions, a set of duplicate measurements of all variables were obtained (baseline). Maintenance of steady-state conditions after PAF/L-PAF challenge was demonstrated by stability ($\pm 5\%$) 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%), conditions that were met in all patients. Duplicate measurements were taken at 5, 15, and 45 min following PAF/L-PAF inhalation, as described previously.[1,6] No patient needed rescue medication with short-acting β_2-agonists during the studies. Samples for $uLTE_4$ measurement were collected at baseline and 120 min after challenge. Oral fluid intake during the period of study was not permitted.

Statistical analysis

Results are expressed as mean ± SEM and 95% confidence interval (CI). The effects of PAF/L-PAF challenge on the different primary end-point variables were assessed by a two-way analysis of variance (ANOVA) for repeated measures. When the latter was significant, post hoc comparisons within each intervention between each time point and baseline were performed using paired *Student's t-test* with *Bonferroni's* correction. Baseline data for each variable on each intervention day and $uLTE_4$ levels before and after PAF/L-PAF nebulization were also analysed using paired *Student's t-test*. Statistical significance was set at $P<0.05$ or <0.025 (for *Bonferroni's* correction).

Results

Baseline data (Before PAF/L-PAF challenge)

Twelve patients (6 females; age, 25 ± 3 yr; FEV_1, 3.2 ± 0.4 L [95 ± 10% predicted]; FEV_1/FVC, 75 ± 7%; and, PD_{20}, 0.5 ± 0.3 μmol) were enrolled in the study. Except for small differences in Rrs and products of lipid peroxidation ($P<0.02$ each), without physiological significance, all the other variables were similar between each study day (Table 1).

Effects of PAF challenge

All but three patients noticed facial flushing, one coughed and five became dyspnoeic immediately after the challenge. Compared to baseline, peripheral blood neutrophils fell at 5 min (to $3.5 \pm 0.7 \times 10^9\,L^{-1}$) ($P<0.01$) followed by a rebound neutrophilia at 15 and 45 min (to 5.1 ± 0.7 and $5.4 \pm 0.7 \times 10^9\,L^{-1}$) ($P<0.001$ each) (Table 2). In parallel, at 5 min Rrs (to 5.5 ± 0.4 cm $H_2O\,L^{-1}\,s^{-1}$) ($P<0.005$) and $AaPO_2$ (to 27.1 ± 2.8 mmHg) increased whereas PaO_2 decreased (to 76.2 ± 2.1 mmHg) ($P<0.001$ each). By 15 ($P<0.001$) and 45 min ($P<0.01$), Rrs, $AaPO_2$ and PaO_2 were still abnormal. Heart rate increased (from 70 ± 1.7 to 78 ± 2.2 min^{-1}) ($P<0.005$) at 5 min, whereas all the other ventilatory, haemodynamic and gas exchange variables, including arterial pH (all within normal limits at baseline), remained unchanged throughout the study period. By contrast, no significant changes were observed in plasma lipid peroxides measured as TBA-MDA adducts, protein sulphydryls, nor in plasma antioxidant capacity, measured as TEAC. PAF challenge increased markedly $uLTE_4$ elimination (to 2144 ± 845 pg mg^{-1}) ($P<0.02$) at 120 min.

Effects of L-PAF challenge

Except for a small increase in $AaPO_2$ at 5 and 15 min, and a mild decrease in PaO_2 at 15 min ($P<0.01$ each), compared to baseline, no other

Table 1 Baseline findings before Lyso-PAF/PAF challenge (mean ± SEM).

	Lyso-PAF	PAF	*P*-values
Neutrophils ($\times 10^9\,L^{-1}$)	4.5 ± 0.5	4.5 ± 0.6	NS
Rrs (cm $H_2O\,L^{-1}\,s^{-1}$)	4.2 ± 0.3	3.6 ± 0.3	<0.02
PaO_2, (mmHg)	92.1 ± 1.5	94.5 ± 2.0	NS
$PaCO_2$ (mmHg)	37.1 ± 0.9	37.3 ± 1.0	NS
$AaPO_2$ (mmHg)	10.5 ± 1.5	9.0 ± 2.1	NS
$ULTE_4$ (pg mg^{-1} creatinine)	680.9 ± 291.3	752.1 ± 178.9	NS
TBA-MDA (μmol L^{-1})	1.16 ± 0.06	1.42 ± 0.06	<0.02
Protein sulfhydryl (mmol L^{-1})	0.49 ± 0.02	0.49 ± 0.02	NS
TEAC (mmol)	1.41 ± 0.03	1.42 ± 0.04	NS

Definition of abbreviations: Rrs: respiratory system resistance; $AaPO_2$: alveolar-arterial PO_2 difference; $uLTE_4$: urinary leukotriene E_4; TBA-MDA: thiobarbituric acid-malondialdehyde adducts; TEAC: Trolox equivalent antioxidant capacity; NS: not significant.

Table 2 Mean differences (95% CI) from baseline after Lyso-PAF (L-PAF) and PAF challenge.

		5 min	15 min	45 min	*P*-value
Neutrophils ($\times 10^9\,L^{-1}$)	L-PAF	0.4 (−0.3/1.2)	0.6 (−0.3/1.5)	0.6 (−0.3/1.6)	<0.005
	PAF	−1.1* (−1.7/−2.7)	0.5* (0.2/0.7)	0.9* (0.5/1.3)	
Rrs (cm $H_2O\,L^{-1}\,s^{-1}$)	L-PAF	0.2 (−0.2/0.5)	0.3 (0.0/0.6)	−0.1 (−0.4/0.2)	<0.02
	PAF	1.9* (1.3/2.6)	1.5* (1.1/2.0)	0.6* (0.1/1.0)	
PaO_2 (mmHg)	L-PAF	−3.1 (−6.4/0.3)	−6.0* (−9.8/−2.3)	−1.6 (−4.4/1.3)	<0.005
	PAF	−18.3* (−22.6/−13.9)	−14.3* (−18.3/−10.4)	−5.8* (−8.7/−2.8)	
$AaPO_2$ (mmHg)	L-PAF	4.2* (0.9/7.4)	6.9* (2.8/10.9)	0.2 (−1.7/2.0)	<0.005
	PAF	18.1* (13.1/23.1)	14.3* (10.2/18.4)	4.9* (2.3/7.4)	
TBA-MDA ($\mu mol\,L^{-1}$)	L-PAF	0.08 (−0.09/0.25)	−0.04 (−0.38/0.30)	−0.01 (−0.23/0.22)	NS
	PAF	−0.20 (−0.40/0.00)	−0.30 (−0.50/−0.10)	−0.20 (−0.40/0.10)	
Protein sulfhydryl ($mmol\,L^{-1}$)	L-PAF	−0.02 (−0.04/0.00)	−0.01 (−0.05/0.02)	0.01 (−0.01/0.02)	NS
	PAF	0.01 (−0.03/0.05)	0.01 (−0.03/0.05)	0.02 (−0.03/0.06)	
TEAC (mmol)	L-PAF	−0.10 (−0.19/−0.01)	−0.05 (−0.12/0.03)	−0.07 (−0.19/0.05)	NS
	PAF	−0.03 (−0.13/0.08)	−0.06 (−0.12/0.01)	−0.02 (−0.10/0.05)	

For definition of abbreviations, see Table 1. All but TBA-MDA adducts (*n*, 11) variables correspond to 12 patients.
*Significantly different from baseline (for individual *P*-values, see Results).

systemic, cellular, lung mechanical, oxidative stress and $uLTE_4$ changes were observed after L-PAF exposure.

Discussion

Asthma is a chronic inflammatory disease of the respiratory tract of unknown aetiology. Recent evidence indicates that inflammatory abnormalities exist even in the airways of subjects with mild disease. In the current study we showed no increase in systemic markers of oxidative stress in response to PAF challenge in mild asthmatics. This was reflected by no change in total antioxidant capacity and levels of lipid peroxides in plasma after the challenge in asthmatics. In this regard, Rahman and colleagues[3] reported similar levels of lipid peroxides and proteins sulphydryls in the plasma of healthy nonsmokers and stable asthmatic patients to those observed in our study. However, these markers of systemic oxidative stress increased in spontaneous acute severe asthma. Thus, PAF inhalation as a model of acute asthma in our study did not produce increased systemic oxidative stress despite inducing consistent lung function disturbances and abnormal neutrophil kinetics akin to those shown in naturally occurring acute asthma.

There are several explanations for these negative results. Firstly, the time course of the measurements made after PAF challenge may be too short to increase systemic markers of oxidative stress. Secondly, the inflammatory response after PAF exposure may be less than that produced in spontaneous acute asthma where systemic oxidative stress occurred. Thirdly, PAF exposure may simply induce an inflammatory response but not oxidative stress. Interestingly, the administration of PAF was associated with an increase in $uLTE_4$ elimination, thereby reflecting an integrated form of endogenous whole body cysteinyl LTC_4 and LTD_4 release during the study. Leukotrienes may be involved as secondary mediators in the production of the systemic and pulmonary effects caused by PAF in asthmatic patients. Thus, PAF can increase the subsequent release of chemotactic mediator LTB_4 indicating that it may prime the constitutive lung cells to increase the inflammatory response relevant to the pathogenesis of asthma.[6]

It has been suggested that an imbalance between oxidants and antioxidants may have a role in the pathogenesis of acute asthma.[5] However, any potential increase in oxidant burden may be

balanced by an effective antioxidant defence system to prevent the development of oxidative stress.[11] A correlation between dietary antioxidant deficiency and asthma exacerbations has also been proposed.[12] In bronchial asthma the sources of the increased oxidant burden include the increased burst of reactive oxygen species (ROS) released by circulatory or airway leukocytes or by endogenous nitric oxide production.[13–15] An oxidant–antioxidant imbalance could induce pulmonary injury by direct oxidative assault to epithelial cells[16] and in experimental acute pulmonary inflammation sequestered pulmonary neutrophils are primed to release ROS.[17] Increases in inflammatory leukocytes in the bronchoalveolar space and those sequestered in the pulmonary microcirculation during asthma attacks may result in an increased oxidant burden both locally, in the lungs, and systemically, reflected as increase in systemic markers of oxidative stress,[13,14] as has been shown to occur in acute asthma.[3] As inflammation is associated with an increased generation of ROS, and the biochemical environment in the asthmatic airways is favourable for oxidant-mediated reactions, it has been proposed that an oxidative stress could be relevant to the pathogenesis of asthma.[17] Inflammatory cells recruited to the asthmatic airways have an enhanced capability to produce ROS[18] and there is also evidence of systemic oxidative stress in asthmatics.[3] Several asthma mediators, including lipid mediators such, as PAF,[19] chemokines,[20,21] adhesion molecules,[22] and eosinophil granule proteins,[23] are potential stimuli or promoters of ROS production. Fortunately, the lungs have an extensive and potent antioxidant defence system, present both extra- and intracellularly, which protects lung cells from oxidant damage.[18]

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