THE ROLE OF PLATELET ACTIVATING FACTOR (PAF) BRONCHIAL IN AIRWAY HYPERRESPONSIVENESS

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SUMMARY

PAF is a biologically active lipid mediator of inflamation when given by inhalation it causes bronchoconstriction, airway epithelial damage and increases vascular permeability. PAF also activates eosinophils to release the major basic protein and eosinophils cationic protein (ECP). Inhaled PAF has been reported to increase airway responsiveness to methacholine in normal subjects over several weeks. We have examined the effect of inhaled PAF (96ug) on bronchial responsiveness to methacholine at day 1, 3 and 7 after PAF challenge in 6 non-atopic and 7 atopic healthy volunteers. PAF challenge and methacholine responsiveness was repeated on 2 more occasions allowing at least 4 weeks between cycles. The maximum mean (sem) % falls in SGaw in the 1st , 2nd and 3rd PAF challenge in non-atopic subjects were 47.3 (8.7), 49.5 (9.3) and 47.2 (8.2) Respectively. and in atopic subjects 41.7 (6.2), 48.0 (6.1) and 49.3 (6.3) respectively.The changes in SGaw were comparable in 3 cycles in both groups. The geometric mean PC SGaw was 9.2/mg/ml in non-atopic subjects and 1.16mg/ml in atopic subjects before PAF inhalation. However PAF did not alter the mean PC SGaw in both groups. Our results suggests that PAF is a potent bronchoconstrictor but does not induce airways hyper responsiveness in non atopic and atopic subjects.

INTRODUCTION

Bronchial hyperresponsiveness is defined as an exaggerated airway narrowing that occurs in response to inhalation of variety of stimuli and is an important component of asthma (defined as a disease characterized by periodicity of symptoms of cough and wheezing, reversible obstruc-

tive ventilatory defect and increased airway responsiveness.[1] Although all the stimuli used, demonstrate bronchial hyperresponsiveness resulting in some degree of narrowing in normal subjects, it is the excessive narrowing at very much lower dose or concentration that characterize nonspecific bronchial hyperresponsiveness.

Platelet activating factor (PAF) is a biologically active inflammatory mediator, like histamine, prostaglandin and leukotrines. It has been demonstrated to play an important role in allergic disease.[2] Lung seems to be the main target organ for its action where It causes bronchoconstriction[3,4,5,7], induces bronchial epithelial damage[8] and increased bronchopulmonany vascular permeability. Though it has been reported that PAF increases bronchial responsiveness to metha-choline in normal as well as atopic subjects[4,7], some studies[9,10,11] failed to confirm these finding. In this study we have investigated the effect of inhaled PAF inducing hyperresponsiveness by comparing atopic and non atopic symptomatic subjects. Furthermore we here studied whether this phenomenon is reproducible.

MATERIAL AND METHODS

SUBJECTS

Thirteen subjects participated in the study, which was approved by the west of Scotland Hospital Ethical Committee, western infirmary Glasgow. Written consent was obtained in each case. The subjects were all non smokers and were divided into two groups. The first group comprised, seven of them, who were atopic. In the second Group there were six nonatopic healthy volunteers. The atopic subjects were all female age 21-38 (average 29.3 yr), height between 157-169 (average 164 cm) and weight 58-68 (average 64.7 kg). They were all skin tested to standard antigens, i.e. house dust, house dust mites, cat, dog, feather, aspergillus grass, pollens and negative control. The atopy was defined by a positive skin reaction of greater then 3mm diameter to at lost two antigens. The atopic subjects were asymptotic at the time of the study which was carried out before the pollen season.

THE DEMOGRAPHIC DATA OF THE ATOPIC SUBJECTS

Name	Age (yrs.)	Sex	Height (cm)	Wt (Kg)	Skin test (+ to)
SG	32	F	168	64	Pol. HD.
MW	21	F	158	6	HD. Pol. Cat. Flower
JS	28	F	164	65	Cat. HD.
CR	38	F	165	66	HD.Cat. Pol.
CJ	31	F	167	68	Cat.Gr. Fea. H.D.M. Pol.
MM	27	F	169	64	HDM.Gr. Hors. Cat. Asp. Pol.
LM	28	F	157	58	Gr. HD. Fea.
Mean	29.3		164	64.7	

HD = House Dust Pol = Pollen
HDM = House dust mites Gr = Grass
Asp = Aspergillous Fea = Feather

TABLE – 1

The six nonatopic (negative skin reaction to the above mentioned antigens) subjects comprised three males and three

THE DEMOGRAPHIC DATA OF THE NON-ATOPIC SUBJECTS

Name	Age (yrs.)	Sex	Height (cm)	Skin test (+ to)
JM	38	F	170	–
EH	34	F	168	–
CO	22	M	170	–
JJ	27	F	163	–
DS	21	M	187	–
MI	36	M	168	–
Mean	29.3		164	

TABLE – 2

SHOWING DETAIL ABOUT THE PROTOCOL OF PAF PROJECT

Study	PAF Inhalation	Methacoline Challenge
1	–	+
2	+	–
3	– Post PAF Day 1	+
5	– Post PAF Day 3	+
9	– Post PAF Day 7	+
12	– Post PAF Day 10	+
+/- =	Methacoline or PAF challene given/not given	

TABLE – 3

females, age 22-38 (average 29.7 yr), height 163-163-187 (average 170.7 cm) and weight (60-73 average 66.9 kg).

METHACHOLINE

Methacholine chloride solutions (Sigma Chemical Company Limited, Fancy Road Pool Dorset BH 17 7NH), ranging from 0.0625 mg/ml to 64 mg/ml, was given via Wright's nebuliser.

Bronchial responsiveness was calculated from a series of methacholine challenge tests, starting with a smallest concentration of 0.0625 mg/ml of methacholine given via the Wright's nebuliser. Successively greater concentration in two fold increments were used to the maximum concentration when SGaw (specific airway conductance) fell by 35% of the lowest post saline starting value. The dose response curve was then plotted on a semilog paper and the concentration of methacholine that decreased SGaw to 35% (PC 35) was determined by linear interpolation.

PAF & ITS INHALATION

Synthetic PAF c-16 (Casecade Biochem Limited the innovation Center University Of Reading Berkshire) was delivered by the Acorn nebuliser attached to a dosimeter. This is a breath actuated device (Nebucheck P.K Morgan Gillingham Kent) driven by compressed air at a pressure of 2.5 Kpa and an output of 12μg/breath with total dose of 96μg of PAF was inhaled by each subject at one sitting. The response to PAF was measured by measuring specific airway conductance (SGaw by the Master Lab Body Plethysmograph jaeger (Medical Electronic and Data Processing System Leics).

PROTOCOL & METHOD

The protocol aimed to examine the effects of inhaled PAF, on the bronchial airway responsiveness to methacholine by checking specific airway conductance SGaw in the volume constant body plethysmograph and to see whether this phenomenon was reproducible in atopic and nonatopic subjects.

This involved three cycles at least four weeks apart in both groups. Each cycle consisted of three to five visits each lasting for about 45 minutes. At the beginning of each cycle a baseline bronchial responsiveness was performed by giving doubling concentration at which the SGaw (specific airway conductance) falls by 35%. On next day, a fixed dose of 96 ug PAF was given by acorn nebuliser as explained in the previous section. Specific airway conductance (SGaw) was measured by the plethysmograph before and at 0,2,3,5,7,10,15,20, and 45, minutes after PAF inhalation 24 hours later i.e. on 1st post PAF day bronchial airway responsiveness was measured by methacholine challenge test. The same challenge test was repeated on 3rd post PAF day, 7th post PAF and so on until the bronchial reactivity to methacholine came back to baseline level. Responsiveness was repeated on 2 occasions allowing at least 4 weeks between cycle (Table 3).

STATISTICAL ANALYSIS

All values are listed as mean and standard error of the man (sem) unless stated as G Mean (geometric mean). The analysis was performed by the Minitab

Statistics System Fundamental version. The PC35 value was calculated by computerized programme for PC. The comparison between Pre PAF was performed by paired t-test and a p value of <0.05 was considered to be statistically significant.

RESULTS:

PAF CAUSES BRONCHOCONSTROCTION IN BOTH ATOPIC AND NONATOPIC SUBJECTS

PAF when given by inhalation in a fixed dose of 96 ug caused marked bronchoconstriction in both atopic and non atopic subjects in each PAF day of the three cycles. The maximum mean (sem) percentage falls in SGaw from post saline baseline value in nonatopic subjects were 47.3 (8.67), 49.5 (9.26) and 47.17 (8.19) in the three cycles respectively. There was no significant difference between the three cycles suggesting that the PAF was equally effective in all cycles. The mean baseline SGaw in the same subjects on the study days of the three cycles were comparable with no statistically significant difference.

A similar response to PAF was also seen in the atopic subjects with the maximum mean (sem) percent fall in SGaw 41.7 (6.15), 48.0 (6.12) and 49.29 (6.33) respectively in the three cycles.

The mean Pre and Post basesaline SGaw on the methacholine challenge days were comparable and there was no significant difference. Both atopic and nonatopic subjects who had shown a significant fall in SGaw also became wheezy. Two of the nonatopic subjects who were less wheezy were moderately flushed after PAF inhalation. After PAF inhalation neither atopic nor nonatopic subjects developed excessive airway secretions. Other characteristics of the aerosolised PAF included a rapid onset of action (1-3 min) and short duration (15 – 45 min) and the absence of late response.

EFFECT OF PAF ON METHACHOLINE RESPONSIVENESS:

There was no statistically significant difference between the mean PC 35 SGaw methacholine before and after PAF challenge on day 1, day 3 and day 7 in the three cycles in nonatopic subjects (Table 4.1, 4.2, 4.3). However two subjects (JM, DS Figure 1.1 – 1.3) of the nonatopic healthy volunteers did show increased bronchial responsiveness. Amongst the

PC35 SGAW BEFORE AND AFTER INHALATION IN NON-ATOPIC SUBJECTS IN THE FIRST CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF		
		D1	D3	D7
JM	3.2	2.2	2.7	2.8
JJ	4.3	9.9	6.8	4.3
EH	9.7	14.1	8.3	22
CO	3.9	3.7	4.1	5.5
DS	5	3.6	5.4	5.5
MI	29.5	23.9	28.4	24.2
G. mean	6.51	6.79	6.76	7.6

TABLE – 4.1

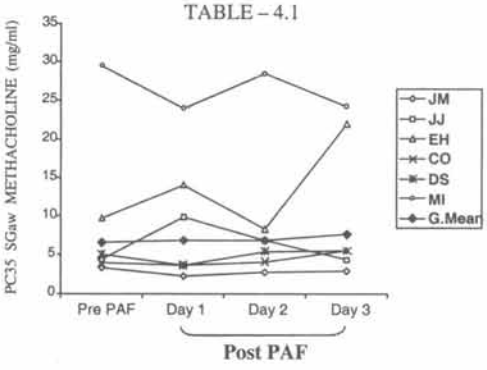

Fig. 1.1: PC35 SGaw Methacholine before and after PAF challenge in nonatopic subjects in the first cycle. p > 0.05 NS

PC35 SGAW BEFORE AND AFTER INHALATION IN NON-ATOPIC SUBJECTS IN THE SECOND CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF		
		D1	D3	D7
JM	4.3	3.4	3.9	4.8
JJ	4.3	7.9	4.5	3.2
EH	22	24.1	12.6	10.5
CO	5.5	4.6	6.6	3.04
DS	5.5	3.2	6.4	5.6
MI	24.5	30	17	23.8
G. mean	8.16	8.1	7.36	6.42

TABLE – 4.2

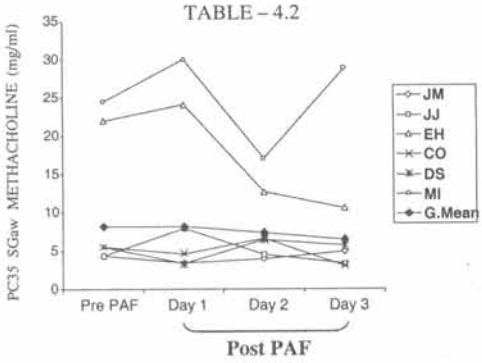

Fig. 1.2: PC35 SGaw Methacholine before and after PAF challenge in non-atopic subjects in the second cycle. p > 0.05 NS

PC35 SGAW BEFORE AND AFTER INHALATION IN NON-ATOPIC SUBJECTS IN THE THIRD CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF		
		D1	D3	D7
JM	4.8	3.4	3.7	5.7
JJ	8.4	4.2	4.01	3.7
EH	10.5	9.3	10.5	11
CO	4.6	4.3	4.03	5.9
DS	5.6	3.9	3.4	4.3
MI	25.8	27.1	31.1	28.2
G. mean	8.1	6.3	6.4	7.4

TABLE – 4.3

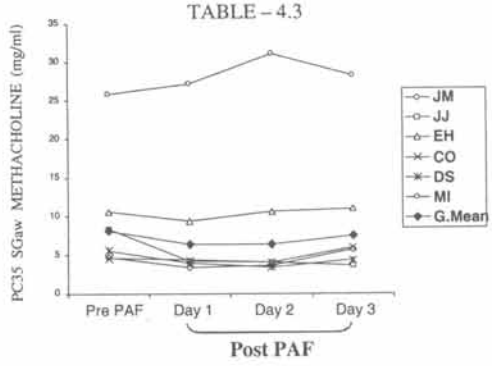

Fig. 1.3: PC35 SGaw Methacholine before and after PAF challenge in non-atopic subjects in the third cycle. p > 0.05 NS

atopic asympto-matic subjects there was no statistically significant difference of PC 35 SGaw of methacholine before and after PAF challenge on day 1, day 3 and day 7 of the three cycles (Table 5.1, 5.2, 5.3). An occasional Subject (CJ) shows some increase bronchial responsiveness (Figures 1.1 – 1.3).

The mean baseline SGaw and geometric mean PC 35 SGaw Methacholine before and after PAF challenge on the Study days of the three Cycles in both atopic and non-atopic subject were comparable.

The PC35 SGaw values were much smaller in atopic than that of nonatopic subjects. This suggests that bronchial airway in atopic subjects is more hyper-responsive to methacholine than nonatopic subjects.

WHETHER BRONCHIAL AIRWAY RESONSIVENESS IS REPRODUCIBLE?

Our study failed to show statistically significant increase in bronchial responsiveness to methacholine after PAF challenge. However the two nonatopic subjects (JM, DS), who showed a moderate degree of increase (<2 fold increase). In bronchial responsiveness in the two cycles, the temporal relationship in the subsequent cycles was no clear (Fig 1,1–1.3).

PC35 SGAW BEFORE AND AFTER INHALATION IN ATOPIC SUBJECTS IN THE FIRST CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF		
		D1	D3	D7
CJ	3.8	3.4	2.3	4.4
SM	2.6	4.7	3.8	6.4
MM	0.9	1.3	2.3	0.8
JS	2.9	2.8	3.9	2.6
MW	0.4	0.3	0.1	0.3
CR	0.3	0.5	0.3	0.4
LM	0.3	0.1	0.3	0.13
G. mean	0.99	0.98	0.95	0.99

TABLE – 5.1

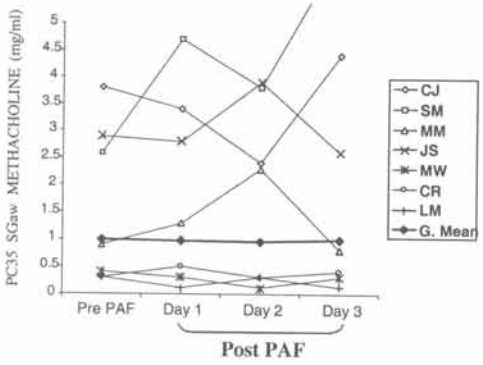

Fig. 2.1: PC35 SGaw Methacholine before and after PAF challenge in atopic subjects in the first cycle. p > 0.05 NS

PC35 SGAW BEFORE AND AFTER INHALATION IN ATOPIC SUBJECTS IN THE SECOND CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF		
		D1	D3	D7
CJ	2.8	2.8	3.5	3.8
SM	2.5	4.1	2.8	2.6
MM	0.9	1.3	0.7	0.5
JS	1.3	1.5	1.9	2.9
MW	0.2	0.2	0.4	0.4
CR	0.3	0.65	0.42	0.32
LM	0.13	0.12	0.08	0.33
G. mean	0.67	0.86	0.78	0.89

TABLE – 5.2

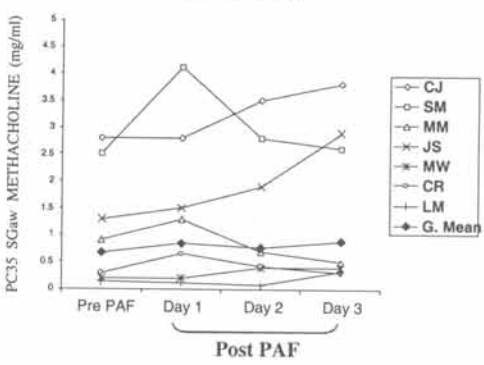

Fig. 2.2: PC35 SGaw Methacholine before and after PAF challenge in atopic subjects in the second cycle. p > 0.05 NS

DISCUSSION

Although the aetiology of increased bronchial airway responsiveness is not well defined. Its presence as a component of clinical asthma is firmly established. It has also been established that changes in airway reactivity, either through seasonal allergen exposure or when attenuated by therapy[17], are closely related to the clinical expression of asthma. What is not certain however, is what mediators are involved in the increase in nonspecific airway responsiveness.

Platelet activating factor is one of the chemical mediators that may participate in the inflammatory process underlying asthma. Cuss et al[3], R. Luis[20] have suggested that PAF may be involved in the changes in bronchial responsiveness. They demonstrated enhanced airway responsiveness in normal subjects and asthmatics respectively for several weeks after PAF inhalation. This was in part confirmed by Rubin et al[4] who reported an increase in bronchial airway responsiveness in normal subjects, but not in asthmatics 1 hour after PAF challenge. Although nonspecific bronchial

PC35 SGAW BEFORE AND AFTER INHALATION IN ATOPIC SUBJECTS IN THE THIRD CYCLE. P > 0.05 NS

Name	Pre PAF	Post PAF			
		D1	D3	D7	D10
CJ	4.4	4.5	3.1	4.5	
SM	6.4	4.6	2	1.6	2.99
MM	0.8	0.4	0.2	0.5	
JS	2.6	2.9	3.8	3.4	
MW	0.3	0.1	0.1	0.4	
CR	0.4	0.31	1.14	0.2	0.38
LM	0.13	0.1	0.4	0.1	
G. mean	1.15	0.79	0.75	0.89	

TABLE – 5.3

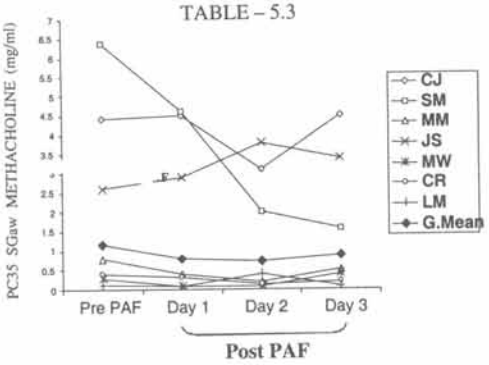

Fig. 2.3: PC35 SGaw Methacholine before and after PAF challenge in atopic subjects in the third cycle. p > 0.05 NS

responsiveness is increased in normal subjects, the fact that Rubin et al showed that asthmatic patients did have increased bronchial responsiveness following PAF inhalation make it a unique mediator. All other known mediators cause immediate bronchoconstric-tion in asthmatic patients but in few normal subject. Importantly it has been reported to induce increase in nonspecific bronchial airway responsiveness, but mainly in no asthmatic subjects Stanton et al[15] also reported increase in airway responsiveness following PAF inhalation, which were poorly sustained and not reproducible.

Our study showed contrasting evidence to the concept that PAF can increase bronchial responsiveness in normal subjects, as shown by Cuss et al[3] and Rubin et al[4]. The fact that PAF inhalation had no effect on bronchial airway responsiveness in our study supports the finding of Russell et al[10], Hoop et al[11], Jenkins et al[14] Lai et al[16], and others[18].

It is not known however, if there is a threshold dose of inhaled PAF necessary to induce changes in airway responsiveness. For their study Russell et al[10] used five breaths of 200mg/L (30 ug delivered). Cuss et al[3] used a mean dose of inhaled PAF of 68 ug (27.5-145 ug), given as five single breaths over 1 hour. Rubin et al[4] used a single breath of 1000 μg/L (Delivered a dose of 23 ug). In our study all subjects inhaled eight breaths of 200mg/L (96 ug delivered). The difference in the results is not likely to be due to a discrepancy in the amount of inhaled PAF, as the dose was sufficient to cause marked broncho-constriction. A recent report by Wardlaw et al[6] suggests that larger doses of inhaled PAF then used may be necessary to induce changes in nonspecific airway responsiveness. It is also important to know that in the studies of Cuss et al (used pFEF 60-80%) and Rubin et al (used SGaw & Vp 30), the workers used a measurement of minimal changes in airway caliber to determine the changes in airway responsiveness. This is necessary because normal subjects often do not have marked changes to methacholine using the measurement of forced expiratory volume in one second. We used SGaw to measure the airway responsiveness and a similar measurement was used by Robin et al. Although these tests are very similar, they also have a larger variability than FEV1.

Patient selection is another variable to be considered. All subjects studied by Cuss et al showed bronchoconstriction after PAF inhalation with a greater fall than 40% in

VP30. It is not clear whether their subjects were selected using these criteria. It is probable that not all subjects are similar in their response to the PAF. The Normal subjects studied by Rubin et al were less bronchial responsive to PAF compared to the subject used by Cuss et al. It is conceivable that subjects with large airway response are more likely to have a prolonged change in airway responsiveness.

Our result on inhaled PAF in atopic subjects support the work of Rubin et al who fail to show an increase in bronchial responsiveness in asthmatics, I hour after PAF challenge. Chung and Barnes[17] have reported that in eight mild asthmatics there was no increase in airway responsiveness as a group, up to seven days following PAF inhalation, however, selected asthmatic subject did have increased airway responsiveness. In our study occasional atopic subjects had a moderate increase in bronchial airway responsiveness on post PAF day 1 and day 3 but a temporal relationship in subsequent cycles was less clear.

Airway hyperresponsiveness and airway eosinophilia are hallmarks of asthma. The studies of PAF induced bronchial hyper-responsiveness may give further insight into the pathogenesis of asthma, as PAF has many properties that make it a mediator of interest in the aetiology of asthma. The studies so for showed that inhaled PAF no doubt causes bronchoconstriction[18,19] but opinion still differs whether it cause increase bronchial airway hyperresponsiveness. Recently Hozawa S et al[22] suggested that PAF is an important mediator involved in bronchial hyperresponsiveness of bronchial asthma in human by using an oral PAF antagonist Y 24180 which significantly improved the PC 20-FEVI.

Clearly further studies are required to clarify the potential role of PAF in the pathogenesis of hyperresponsiveness and to determine why there is differences in the results between various studies in the effect of PAF in normal healthy people, despite showing similar bronchoconstriction and cardiovascular responses. Also studies are needed to address the question whether the hyperresponsiveness is reproducible.

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