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Attenuation of platelet-activating factor induced bronchoconstriction by nedocromil sodium

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Attenuation of platelet-activating factor induced bronchoconstriction by nedocromil sodium. J.P. Hayes, K.F. Chung, P.J. Barnes.

ABSTRACT: We assessed the effect of nedocromil sodium on bronchoconstriction and airway responsiveness induced by platelet-activating factor (PAF) in eight normal subjects, in a double-blind, placebo-controlled cross-over study. Subjects inhaled PAF by a dosimeter method in 5 doses of 18 μg each, separated by an interval of 15 min, (total dose of 90 μg). Airway calibre was measured by partial expiratory flow at 30% of vital capacity ($\dot{V}p_{30}$) before and at 1, 3, 5, 10 and 15 min after each dose of PAF. The bronchoconstrictor response was assessed by measuring the area under the curve of the percentage fall in $\dot{V}p_{30}$ over time.

There was a significant reduction in PAF-induced bronchoconstriction after nedocromil sodium (1,225±392 arbitrary units; mean±SEM) compared to placebo (2,395±598; $p<0.01$). There was no significant difference in the fall in peripheral neutrophil count measured at 5 min after PAF with nedocromil sodium (48.5±9.5%) compared to placebo (43.3±6.8%).

In conclusion, nedocromil sodium significantly attenuates PAF-induced bronchoconstriction but not the peripheral neutropenia in normal subjects. Since PAF is not a direct constrictor of human airway smooth muscle, this effect of nedocromil sodium may indicate inhibition of release of bronchoconstrictor mediators.

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Nedocromil sodium is a pyranoquinalone dicarboxylic acid which has recently been introduced in the treatment of asthma. It has several properties which suggest that it has an anti-inflammatory role in asthma. Thus, it inhibits the release of mediators from activated human cells *in vitro,* including mast cells, eosinophils, platelets and macrophages [1]. In addition, it inhibits early and late responses to allergen challenge in asthmatics [2]. In order to further evaluate the possible mechanism of action of nedocromil sodium, we investigated its effect on the airway responses to inhaled platelet-activating factor (PAF) in normal volunteers. PAF induces bronchoconstriction and bronchial hyperresponsiveness in normal volunteers [3]. PAF is known to be a potent activator of inflammatory cells, such as neutrophils and eosinophils *in vitro* [4], but does not directly contract airway smooth muscle, and it has been suggested that PAF may induce its effects through activation of, and release of, mediators from inflammatory cells [5]. Previous studies have found a positive correlation between the number of neutrophils recovered in samples of lavage fluid and the fall in lung function after inhalation of PAF. We postulated that nedocromil sodium, from its profile of activity, could attenuate PAF-induced airway effects and the associated peripheral neutropenia *in vivo* in normal subjects.

Materials and methods

Subjects

Eight healthy normal volunteers (5 males and 3 females, aged 19–32 yrs) gave written informed consent to participate in this study, approved by the Ethics Committee of the Royal Brompton and National Heart Hospital. All subjects were nonsmokers and gave no history of asthma or of respiratory infection for at least 4 weeks before entering the study.

They abstained from caffeine containing beverages for at least 12 h prior to attending the laboratory. Subjects were assessed for atopy by examining skin responses to common allergens and all were non-atopic. Subjects with a provocative concentration of methacholine causing a 40% fall in baseline airway calibre (PC_{40}) of less than 4 $mg \cdot ml^{-1}$ were excluded from the study (table 1). In all subjects, forced expiratory volume in one second and forced vital capacity measurements were greater than 80% predicted.

Table 1. – Characteristics of normal subjects including baseline lung function

Subject no.	Age yrs	Sex	Atopy	$\dot{V}p_{30}$ l·min^{-1} Period 1	Period 2	PC_{40} mg·ml^{-1}	FEV_1 % pred	FVC % pred
1	32	F	-	105	110	5.2	96	104
2	19	M	-	204	194	52	106	110
3	26	M	-	223	196	5.8	105	108
4	24	M	-	241	256	24.7	96	104
5	19	F	-	92	73	6.6	87	94
6	21	M	-	234	219	47	102	109
7	20	F	-	121	109	20	90	94
8	22	M	-	168	198	42	89	103

PC_{40}: provocative concentration of methacholine needed to cause a 40% fall in baseline $\dot{V}p_{20}$; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; $\dot{V}p_{30}$: partial expiratory flow at 30% of vital capacity.

Protocol

This protocol is similar to that used previously in our laboratory [5]. Each subject was studied during two periods, separated by a period of at least 4 weeks. Each subject inhaled either 8 mg of nedocromil sodium (4 puffs; 2 mg·puff^{-1}) or matched placebo, by metered dose inhaler, in a randomized, double-blind, cross-over fashion. This was followed 30 min later by inhalation of PAF from a nebulizer (mass median particle size 5 μ; output 6 μl·breath^{-1}) connected to a dosimeter (Mefar Elecromed, Brescia, Italy). Each subject inhaled two breaths of PAF aerosol (1.5 mg·ml^{-1}; 18 μg), up to five times in succession every 15 min (total dose 90 μg). For each dose, the subject inhaled twice from a nebulizer attached to the dosimeter driven by compressed air at a pressure of 152 kPa. For each nebulization (which lasted 1.0 s) the subject inhaled 9 μg PAF slowly, from functional residual capacity to total lung capacity, with breath-holding for 10 s before exhaling. Airway calibre was measured at 1, 3, 5, 10 and 15 min after each dose of PAF. A blood sample for measurement of total white cell and platelet counts was taken immediately before inhaling PAF and at 5, 15 and 60 min after inhaling the first dose of PAF.

Measurement of airway calibre

Airway calibre was measured from standardized partial expiratory flow-volume curves at 30% vital capacity ($\dot{V}p_{30}$) [6] with a rolling spirometer (Vitalograph, Buckingham, UK) and a Hewlett-Packard microcomputer (Collingwood Measurements Leicester, UK). Subjects initially performed a full vital capacity, and measurements of flow were made at $\dot{V}p_{30}$. Flow volume manoeuvres were performed by expiration from just above tidal inspiration to residual volume, followed by inhalation to total lung capacity before breathing out normally. All subjects were trained in partial flow measurements on the first visit. Changes in airway calibre were expressed as a percentage of baseline measurements taken after inhalation of a control solution (mean of three readings).

Chemicals

C_{16}-PAF was obtained from Bachem, Switzerland and stock solutions of 10 mg·ml^{-1} were made in absolute alcohol and stored at -70°C. This was diluted in 2.5% human serum albumin (Immuno AG, Vienna, Austria) in saline to 1.5 mg·ml^{-1}. Methacholine (Sigma UK) was diluted in saline in doubling concentrations from 2 to 128 mg·ml^{-1}.

Statistical analysis

The paired Student's t-test was used to compare area under the curve measurements following inhalation of PAF and the percentage fall in the neutrophil count in peripheral blood. A p-value <0.05 was considered statistically significant.

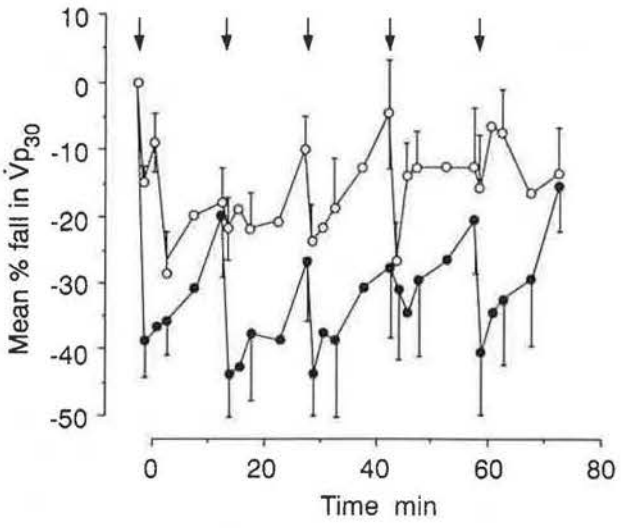

Fig. 1. – Effect of placebo (●) and nedocromil sodium (○) on the partial expiratory flow at 30% vital capacity ($\dot{V}p_{30}$) (mean±SEM) after inhalation of platelet-activating factor (18 μg) every 15 min as indicated by the arrows. Placebo or nedocromil (8 mg) was inhaled 30 min pior to baseline measurements at time 0.

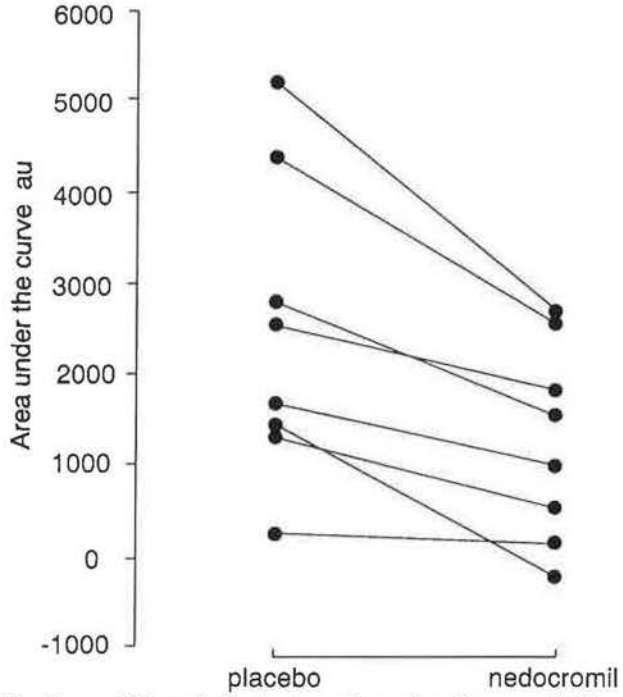

Fig. 2. – Effect of placebo or nedocromil sodium on platelet-activating factor-induced bronchoconstriction expressed as area under curve of percentage fall in $\dot{V}p_{30}$ with time, in arbitrary units (au) of area. Individual responses are shown. Nedocromil significantly inhibited bronchoconstriction ($p<0.01$). $\dot{V}p_{30}$: partial expiratory flow at 30% vital capacity.

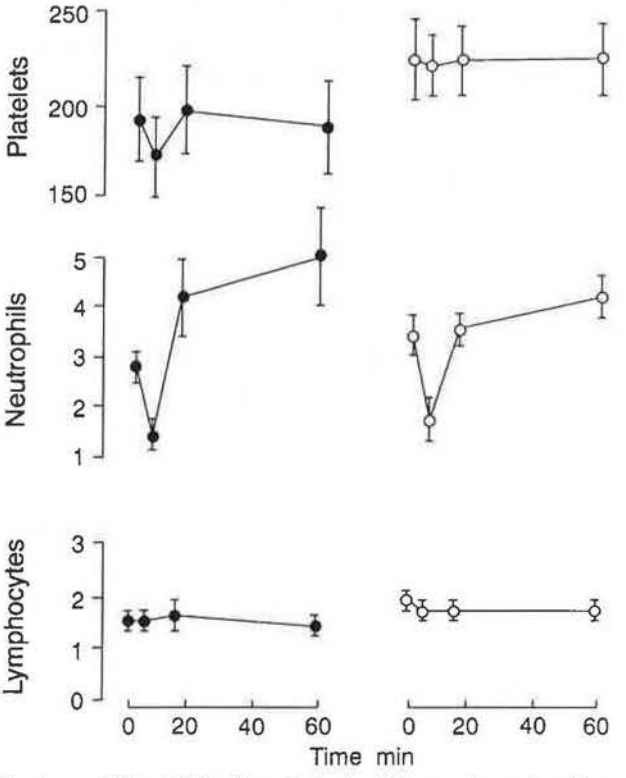

Fig. 3. – Cells $\times 10^9 \cdot l^{-1}$ effect of placebo (●) or nedocromil sodium (○) on the change in circulating cell counts (upper panel: platelet; middle panel: neutrophil; lower panel: lymphocyte) after inhalation of platelet-activating factor (mean±SEM). Nedocromil had no significant effect on the transient neutropenia observed at 5 min. Data shows mean±SEM.

Results

All subjects experienced a fall in $\dot{V}p_{30}$ after inhaling PAF on each occasion. There was no overall significant difference between the baseline airway calibre or blood measurements on the two days. There was a significant reduction in PAF-induced bronchoconstriction after inhaling nedocromil sodium (1,225±392 units of area, mean±SEM) when compared to placebo (2,395±598, $p<0.01$) (figs 1 and 2). The total white cell count was reduced after both placebo (31.7±10.8%) and nedocromil (37.5±6.7%) at 5 min compared to baseline.

There was no significant difference in the fall in neutrophil count in the peripheral blood seen at 5 min after PAF inhalation (48.9±9.5%) with nedo-cromil sodium compared with placebo (43.3±6.8%) (fig 3). There was a rebound neutrophilia seen 60 min after PAF inhalation with both nedocromil sodium and placebo. Inhalation of PAF did not affect the platelet count or the lymphocyte count in the peripheral blood.

Discussion

We have shown that nedocromil sodium at a dose of 8 mg significantly attenuates PAF-induced bronchoconstriction in man but not the associated peripheral neutropenia. Our results are partly in agreement with those of Di Maria *et al.* [7], who found a partial but nonsignificant inhibition of PAF-induced bronchco-constriction. However, they did not assess the effect of PAF inhalation on airway calibre until 8 min after inhalation of PAF and, therefore, it is likely that any significant effect on immediate bronchoconstriction was missed. Nedocromil sodium inhibits bronchoconstriction in response to a number of bronchial challenges including bradykinin [8], neurokinin A [9], adenosine [10] and sulphur dioxide [11], which are known to act indirectly by the release of secondary mediators and neurotransmitters; nedocromil sodium by contrast, does not inhibit bronchial challenge of directly acting agents, such as methacholine or histamine. Overall, these observations suggest that nedocromil sodium inhibits mediator release. This action of nedocromil sodium is supported by *in vitro* studies demonstrating inhibition of the release of histamine, leukotriene C_4 (LTC_4) and prostaglandin D_2 (PGD_2) from mast cells obtained from bronchoalveolar lavage fluid of macaque monkeys infected with ascaris [12].

The mechanism by which PAF causes bronchoconstriction, both in asthmatic and normal subjects [3, 13, 14], is unknown, but it is likely to occur by an indirect mechanism, because PAF does not contract human airway smooth muscle preparations in the absence of platelets, and there is no relationship between the degree of bronchoconstriction induced by PAF and that induced by methacholine. It is unlikely that histamine mediates PAF-induced bronchoconstriction because ketotifen, a histamine antagonist, does not inhibit PAF-induced bronchoconstriction in man [15]. Thromboxane

A_2 has been shown to be involved in PAF-induced bronchoconstriction in dogs and guinea-pigs [16] but thromboxane A_2 antagonists do not inhibit this response in man [17]. Inhalation of PAF increases urinary excretion of leukotriene E_4 (LTE_4) in man [18] and, recently, leukotriene D_4 (LTD_4) antagonists have been shown to inhibit PAF-induced bronchoconstriction [19, 20]. Therefore, it is probable that nedocromil inhibits the release of sulphidopeptide leukotrienes induced by PAF. This is supported by the observation that nedocromil sodium inhibits the stimulated generation of LTC_4 from human eosinophils *in vitro* [21]. PAF-induced peripheral neutropenia has been reported in several previous studies [14, 15]. Our studies suggest that there is no link between peripheral neutrophils and the bronchoconstriction response to PAF, because nedocromil had no effect on the neutropenia, whilst inhibiting the bronchoconstrictor response. However, infiltrating neutrophils in the lungs may be relevant to the bronchoconstrictor response, as WARDLAW *et al.* [5] have shown a significant positive correlation between the number of neutrophils recovered in bronchoalveolar lavage fluid and the fall in $\dot{V}p_{30}$ after PAF inhalation. The maximal bronchoconstrictor effect is seen 3 min after inhalation, while the peripheral neutropenia is found 5 min after inhalation. Nedocromil may inhibit release of inflammatory mediators from a number of cells, including neutrophils and eosinophils, without inhibiting the recruitment of neutrophils into the airways.

In conclusion, we have demonstrated that nedocromil sodium significantly attentuates PAF-induced bronchoconstriction in normal subjects. The mechanism of this effect may be through inhibition of release of LTD_4 from resident cells within the normal airway.

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References

1. Moqbel R, Cromwell O, Walsh GM, *et al.* – Effects of nedocromil sodium (Tilade) on activation of human eosinophils and neutrophils and release of histamine from mast cells. *Allergy*, 1988; 43: 268–276.
2. Dahl R, Pedersen B. – Influence of nedocromil sodium on the dual asthmatic response after allergen challenge; a double-blind, placebo controlled study. *Eur J Respir Dis*, 1986; 69(Suppl. 147): 263–265.
3. Cuss FM, Dixon CMS, Barnes PJ. – Effects of inhaled platelet-activating factor on pulmonary function and bronchial responsiveness in man. *Lancet*, 1986; ii: 189–192.
4. Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. – Platelet-activating factor; a potent chemotactic and chemokinetic factor for human eosinophils. *J Clin Invest*, 1986; 78: 1701–1706.
5. Wardlaw AJ, Chung KF, Moqbel R, *et al.* – Effects of inhaled PAF in humans on circulating and bronchoalveolar lavage fluid: relationship the bronchoconstriction and changes in airway responsiveness. *Am Rev Respir Dis*, 1990; 141: 386–392.
6. Barnes PJ, Gribbin HR, Osmanliev D, Pride NB. – Partial flow-volume curves to measure bronchodilator dose-response curves in normal humans. *J Appl Physiol*, 1981; 50: 1193–1197.
7. Di Maria GU, Bellofiore S, Ciancio N, Ruggieri F, Mistretta A. – Nedocromil sodium prevents airway hyperreactivity induced by platelet activating factor (PAF) *Eur Respir J*, 1990; 3 (Suppl. 10): 694P.
8. Dixon CMS, Barnes PJ. – Bradykinin-induced bronchoconstriction: inhibition of nedocromil sodium and sodium cromoglycate. *Br J Clin Pharmacol*, 1989; 27 (6): 831–836.
9. Joos GF, Pauwels RA, Van der Straeten ME. – The effect of nedocromil sodium on the bronchoconstrictor effect of neurokinin A in asthmatics. *J Allergy Clin Immunol*, 1989; 83: 663–668.
10. Crimi E, Brusasco V, Brancatisano M, Losurdo E, Crimi P. – Effect of nedocromil sodium on adenosine- and methacholine-induced bronchospasm in asthma. *Clin Allergy*, 1987; 17: 135–141.
11. Dixon CMS, Fuller RW, Barnes PJ. – Effect of Nedocromil sodium on sulphur dioxide-induced bronchoconstriction. *Thorax*, 1987; 42: 462–465.
12. Wells E, Jackson CG, Harper ST, Mann J, Eady RP. – Characterisation of primate bronchoalveolar mast cells. II. Inhibition of histamine LTC_4 and PGD_2 release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells. *J Immunol*, 1986; 137: 3941–3945.
13. Chung KF, Dent G, Barnes PJ. – Effects of salbutamol on bronchoconstriction, bronchial hyperresponsiveness and leucocyte responses induced by platelet activating factor in man. *Thorax*, 1989; 44: 102–107.
14. Chung KF, Barnes PJ. – Effects of platelet-activating factor on airway calibre, airway responsiveness and circulating cells in asthmatic subjects. *Thorax*, 1989; 44: 108–115.
15. Chung KF, Minette P, McCusker M, Barnes PJ. – Ketotifen inhibits the cutaneous but not the airway responses to platelet-activating factor in man. *J Allergy Clin Immunol*, 1988; 81: 1192–1197.
16. Chung KF, Aizawa H, Leikauf GD, *et al.* – Airway responsiveness induced by platelet-activating factor: role of thromboxane generation. *J Pharmacol Exp Ther*, 1986; 236: 580–584.
17. Stenton SC, Haines AH, Palmer JB, Hendrick DJ, Walters EH. – The actions of GR32191B a thromboxane receptor antagonist on the effects of inhaled PAF on human airways. *Clin Exp Allergy*, 1990; 20: 311–317.
18. Taylor IK, Taylor GW, Fuller RW. – Platelet-activating factor (PAF) induced bronchoconstriction in asthmatics: role of cysteinyl leukotrienes. *J Appl Physiol*, 1991; 71: 1396–1402.
19. Spencer DA, Evans JM, Green SE, Piper PJ, Costello JF. – Bronchospasm induced by platelet-activating factor is reduced by a selective cysteinyl-leukotriene antagonist in man. *Thorax*, 1991; 46: 441–445.
20. Kidney JC, Ridge S, Chung KF, Barnes PJ. – Inhibition of PAF-induced bronchoconstriction by the oral leukotriene D_4 receptor antagonist, ICI 204,219 in normal subjects. *Am Rev Respir Dis*, 1991; 143: A811.
21. Bruijnzeel PL, Hamelink ML, Kok PTM, Kreukinet J. – Nedocromil sodium inhibits the A23187- and opsonized zymosan-induced leukotriene formation by human eosinophils. *Br J Pharmacol*, 1989; 96: 631–636.