Br. J. Pharmacol. (1988), **94**, 6–8

Inhibition by nedocromil sodium of early and late phase bronchoconstriction and airway cellular infiltration provoked by ovalbumin inhalation in conscious sensitized guinea-pigs

Penny A. Hutson, Stephen T. Holgate & [1]Martin K. Church

Immunopharmacology Group, Clinical Pharmacology and Medicine 1, Centre Block, Southampton General Hospital, Southampton SO9 4XY

Bronchial challenge of ovalbumin-sensitized conscious guinea-pigs induced a triphasic reduction in specific airways conductance (sGaw) with maximal reductions being observed at 2, 17 and 72 h accompanied by infiltration of the airways with neutrophils at 17 h and eosinophils at 17 and 72 h. Nedocromil sodium (10 mg ml^{-1}) inhaled before challenge blocked the 2 and 17 h sGaw responses and the neutrophil influx. When inhaled 6 h after challenge, nedocromil inhibited the 17 h sGaw response but not neutrophil influx, suggesting that these are unrelated. The sGaw response and eosinophil accumulation at 72 h was also inhibited by nedocromil given at this time. Our findings correlate with the clinical effects of nedocromil, suggesting the guinea-pig model may be useful for investigating the mechanism of action of anti-asthmatic drugs.

Introduction Inhalation of allergens by atopic asthmatic subjects induces episodes of reversible airflow limitation which may be divided into early asthmatic responses (EAR), occurring within 30 min, and late asthmatic responses (LAR), occurring 6–12 h later. Late responses may recur with diminishing intensity over several days and are accompanied by bronchial hyperresponsiveness (BHR) to a variety of non-specific stimuli. Pathologically, allergen provocation causes an influx into the airways of neutrophils followed by eosinophils (Metzger *et al.*, 1986), the eosinophils in particular being associated with LAR and BHR (Durham & Kay, 1985).

Nedocromil sodium is an anti-asthma drug developed because of its ability to reduce airway inflammation (Auty, 1986). In man, nedocromil protects against both EAR and LAR following bronchial provocation and is efficacious in clinical asthma (reviewed by Holgate, 1986). Evidence for the anti-inflammatory effects of this drug derives from its ability to inhibit allergen-induced LAR and BHR in sheep (Abraham *et al.*, 1986) and to prevent activation of human neutrophils and eosinophils *in vitro* (Moqbel *et al.*, 1986).

We describe the effects of nedocromil on EAR and LAR and on the intraluminal accumulation of inflammatory cells provoked by bronchial challenge in conscious sensitized guinea-pigs (Hutson *et al.*, 1988).

Methods Male Dunkin Hartley guinea-pigs (450–500 g) were sensitized by inhalation of ovalbumin (1%) for 3 min on days 0 and 7 (Hutson *et al.*, 1988). On day 14, they were challenged by inhalation of ovalbumin (2%) for 5 min under cover of mepyramine maleate (10 mg kg^{-1}) injected intraperitoneally 30 min beforehand. Specific airways conductance (sGaw) was determined in conscious guinea-pigs by whole body plethysmography both before challenge (baseline) and at repeated times up to 72 h thereafter (Hutson *et al.*, 1988). Results are expressed as mean ± s.e. mean of percentage changes in sGaw values from baseline which was 0.066 ± 0.003 ($cmH_2O\ s)^{-1}$ in the 96 animals used throughout this study.

Bronchoalveolar lavage (BAL) was performed with 2 × 5 ml volumes of sterile saline, recovery of which was ~80% (Hutson *et al.*, 1988). Total nucleated cell counts were performed in a Neubauer haemocytometer and differential counts on cytospin preparations stained with May-Grünwald-Giemsa. Results are expressed as the total number of cells recovered from each animal following BAL.

The significance of differences between treatment groups was assessed by Student's *t* test for unpaired data.

Results Bronchial challenge induced a triphasic reduction in sGaw with maximal reductions being observed at 2, 17 and 72 h (Figure 1). Examination of

[1] Author for correspondence at: Clinical Pharmacology, Centre Block, Southampton General Hospital, Southampton SO9 4XY.

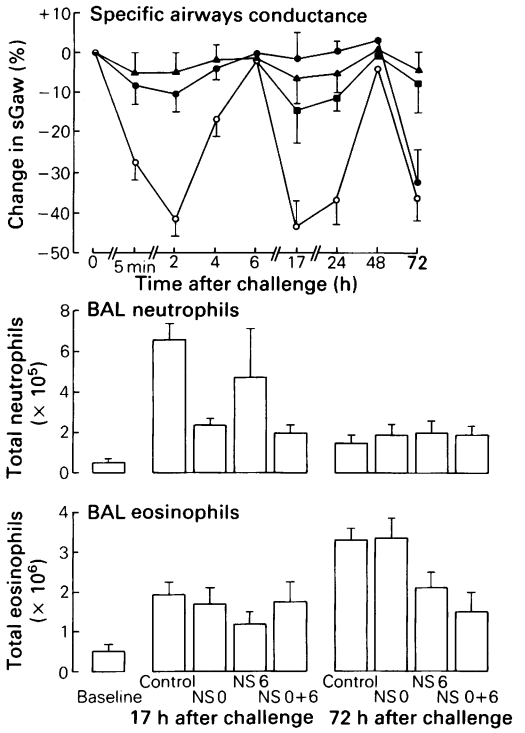

Figure 1 Effect of nedocromil sodium on changes in specific airways conductance (sGaw) and cellular infiltration of the airways induced in conscious guinea-pigs by inhalation of ovalbumin. Each result is the mean for groups of 16 animals up to the 17 h time point and 8 animals thereafter for sGaw and groups of 8 animals for bronchoalveolar lavage (BAL) analysis; vertical lines show s.e. mean. Nedocromil sodium (10 mg ml^{-1}) was inhaled for 2 min at 15 min before ovalbumin challenge (●, NS 0), 6 h after challenge (■, NS 6) or at both times (▲, NS 0 + 6). Groups of control animals inhaled 0.9% NaCl saline at the same time points. As the values in these groups were not statistically different, mean control values are used throughout the figure (○). In the sGaw graph, s.e. mean lines have been excluded at the 6 and 48 h time points for clarity. Histograms show total neutrophil and eosinophil numbers in BAL fluid from unchallenged animals (baseline) and from animals 17 and 72 h after challenge.

BAL fluid (Figure 1) at 17 and 72 h revealed 13.2 fold ($P < 0.001$) and 3.0 fold ($P < 0.005$) increases in neutrophils from a baseline at $0.05 \pm 0.01 \times 10^6$ cells ($n = 24$). Eosinophil numbers rose more slowly achieving 4.0 fold ($P < 0.001$) and 6.7 fold ($P < 0.001$) increases above a baseline of $0.49 \pm 0.09 \times 10^6$ cells at 17 and 72 h respectively. No significant changes were observed in macrophage or lymphocyte numbers.

Inhalation of nedocromil, 10 mg ml^{-1} for 2 min, produced no significant changes in baseline sGaw. When inhaled 15 min before challenge, nedocromil significantly ($P < 0.001$) protected animals against the 2 h (EAR) and 17 h (LAR) falls in sGaw but not the later late response (LLAR) occurring at 72 h. The rise in BAL neutrophils observed at 17 h was also inhibited ($P < 0.001$) but no effects were seen on eosinophil numbers. Inhaled as a single dose 6 h after challenge, nedocromil inhibited ($P < 0.001$) both the LAR and LLAR. No significant effects on neutrophil numbers were observed but the rise in BAL eosinophils at 72 h was reduced ($P < 0.025$). When inhaled both 15 min before and 6 h after challenge, nedocromil significantly ($P < 0.001$) inhibited the EAR, LAR and LLAR. Examination of the BAL fluid showed neutrophil numbers at 17 h and eosinophil numbers at 72 h both to be significantly ($P < 0.001$) lower than control.

Discussion This study shows that nedocromil is capable of inhibiting both early and late asthmatic responses and that influx of inflammatory cells into the airways of guinea-pigs following bronchial allergen challenge.

The observation that neutrophil accumulation at 17 h was inhibited only when nedocromil was inhaled before challenge strongly suggests that it is consequential upon an intact EAR. Furthermore, the ability of nedocromil inhaled after the EAR to inhibit the LAR, but not neutrophil accumulation, would suggest that these events are unrelated. Subsequent experiments in which guinea-pigs were depleted of neutrophils (unpublished observations) support this hypothesis.

In contrast, eosinophil infiltration appears to be independent of the presence of an intact EAR. The relationship between the 17 h LAR and intraluminal eosinophil content is unclear as eosinophil numbers at 17 h are unaffected even in the absence of a demonstrable LAR. There does, however, appear to be a temporal relationship between the ability of nedocromil to inhibit eosinophil accumulation in BAL at 72 h and its ability to block the fall in sGaw at this time.

In conclusion, the actions of nedocromil against the pulmonary effects of allergen challenge in guinea-pigs correlate with its clinical activity. We suggest that this model provides useful information for investigating the mechanisms of action of anti-asthmatic drugs.

This study was supported in part by a MRC programme grant. P.A.H. is a SERC-CASE award student with Roussel Laboratories. We thank Fisons Pharmaceuticals for supplying nedocromil sodium.

References

ABRAHAM, W.M., STEVENSON, J.S. & SIEKZAK, M.S. (1986). Preliminary report on the effect of nedocromil sodium on antigen-induced early and late reactions in allergic sheep. *Eur. J. Respir. Dis.*, **69** (Suppl. 147), 192–195.

AUTY, R.M. (1986). The clinical development of a new agent for the treatment of airway inflammation, nedocromil sodium (Tilade). *Eur. J. Respir. Dis.*, **69** (Suppl. 147), 120–131.

DURHAM, S.R. & KAY, A.B. (1985). Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin. Allergy*, **15**, 411–418.

HOLGATE, S.T. (1986). Clinical evaluation of nedocromil sodium in the treatment of asthma. *Eur. J. Respir. Dis.*, **69** (Suppl. 147), 149–159.

HUTSON, P.A., CHURCH, M.K., CLAY, T.P., MILLER, P. & HOLGATE, S.T. (1988). Early and late phase bronchoconstriction following allergen challenge of non-anaesthetized guinea-pigs: I. The association of disordered airway physiology to leucocyte infiltration. *Am. Rev. Respir. Dis.*, (in press).

METZGER, W.J., RICHERSON, H.B., WORDEN, K., MONICK, M. & HUNNINGHAKE, G.W. (1986). Bronchoalveolar lavage of allergic asthmatic patients following allergen provocation. *Chest*, **89**, 477–483.

MOQBEL, R., WALSH, G.M. & KAY, A.B. (1986). Inhibition of human granulocyte activation by nedocromil sodium. *Eur. J. Respir. Dis.*, **69** (Suppl. 147), 227–229.

(Received November 18, 1987
Accepted January 28, 1988)