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SPECIAL REPORT

Nedocromil sodium inhibits histamine-induced itch and flare in human skin

[1]Poonam Ahluwalia, [1]James I. McGill & *[,1]Martin K. Church

[1]Dermatopharmacology Unit, Allergy and Inflammation Sciences, South Block 825, Southampton General Hospital, Southampton SO16 6YD

This study was designed to test the hypothesis that nedocromil sodium inhibits sensory nerve function to reduce flare and itch in human skin. Nedocromil sodium (2%) or water (control) was introduced into the volar forearm skin of eight non-atopic volunteers by iontophoresis (8 mC) and histamine (20 μl of 1 μM and 300 nM) injected intradermally 10 min later at the same site. Itch was assessed on a visual analogue scale every 20 s for 5 min. Weal and flare areas and mean blood flux within the flare were assessed by scanning laser Doppler imaging at 10 min. The results showed that nedocromil sodium reduced itch scores, totalled over 5 min, by $\sim$74.0% ($P<0.005$) and flare areas by $\sim$65% ($P<0.03$). Neither weal areas nor blood flux within were reduced. These data demonstrate that nedocromil sodium is effective in reducing neurogenic itch and flare in the skin. We suggest that its mechanism of action is modulation of sensory neurone activation or conduction in the skin.

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Abbreviations: VAS, visual analogue scale

Introduction The cromones, sodium cromoglycate and nedocromil sodium, are widely used in the treatment of bronchial asthma and rhinoconjunctivitis. Although their mechanism of action was initially considered to be mast cell stabilization (Cox, 1967), it has since become apparent that the drugs also have anti-inflammatory actions (Auty, 1986; Holgate *et al*., 1991) and the ability to modulate sensory nerve function (Chung, 1996; Dixon *et al*., 1980; Jackson *et al*., 1992; Sheppard *et al*., 1981). It is the last of these actions that is the focus of this study.

The model used to study the potential inhibitory effects of nedocromil sodium on neurogenic inflammation was the histamine-induced weal and flare response. This 'triple response' first described over 70 years ago (Lewis, 1927), consists of both local vascular and more widespread neurogenic effects. The local effects, both considered to be caused by the actions of histamine on the local vascular bed, are an initial vasodilatation at the site of histamine injection and then leakage of plasma proteins from post-capillary venules to form a weal (Raud *et al*., 1988). The flare results, not from the spread of histamine, but from the vasodilator effects of neuropeptides, released as a result of the stimulation of axon reflexes within the dermis by histamine, which itself diffuses only two to three millimetres from its site of injection (Petersen *et al*., 1997; Torebjork, 1974).

Trials of topical cromones in clinical or experimental inflammation in the skin have provided conflicting results. An early trial with 10% sodium cromoglycate in children with atopic eczema gave encouraging results (Haider, 1977), particularly with respect to the amelioration of inflammation and itch. In contrast, many subsequent trials have proved disappointing e.g. (Pike *et al*., 1988). One possible explanation is the poor and inconsistent penetration of cromones into the skin. Given their polar nature (p$K_a \sim 2$), penetration of these drugs through the epidermis with conventional vehicles is very poor, one study reporting a mean efficiency of absorption of 0.44% (range 0.01–2.75%) from an oil in water formulation containing 4% sodium cromoglycate (Ariyanayagam *et al*., 1985).

Our study was designed to test the hypothesis that nedocromil sodium inhibits sensory nerve function to reduce flare and itch in human skin. To ensure adequate penetration of drug into the dermis, its passage was facilitated by iontophoresis and 10 min allowed to lapse between iontophoresis and histamine injection to allow any potential effects of nedocromil sodium on the nerve to become optimal.

Methods Eight non-atopic subjects (one male, seven females, mean age 23 years) participated in this single blind study. The study was approved by the Southampton and South West Hampshire Ethics Committee (121/99) and signed informed consent obtained from all subjects.

Iontophoresis Nedocromil sodium (a gift from Rhone-Poulenc Rorer) was introduced into the skin using iontophoresis (MIC1-e, Moor Instruments Ltd, Axminster, Devon, U.K.). The chamber was fixed to the skin, filled with a 2% solution of nedocromil sodium in reverse osmosis purified water (water) and a total charge of 8 mC applied (200 μAmps/s for 40 s). It was calculated that this charge would introduce 1.71 μg of nedocromil sodium into the skin over an area of approximately 0.8 cm^2 (a circle of 1 cm diameter). At control sites, the iontophoresis chamber was filled with reverse osmosis purified water.

Histamine injection Histamine (Sigma, Poole, Dorset, U.K.) dissolved in sterile saline (0.9% NaCl) was injected

*Author for correspondence; E-mail: mkc@soton.ac.uk

intradermally (20 μl of 1 μM or 300 nM) using a 27 gauge needle and U-100 insulin syringe (Myjector, Terumo Europe NV, Leuven, Belgium) into the centre of the iontophoresis sites 10 min after iontophoresis.

Scoring of itch The intensity of itch was graded by subjects on a 100 mm visual analogue scale (VAS) at 20 s intervals for 5 min after the injection of histamine.

Scanning laser doppler imaging Weal and flare areas and the mean blood flux, indicative of dermal perfusion up to a depth of 1 mm, was assessed in the skin using scanning laser Doppler imaging (Moor LDI, Moor Instruments Ltd, Axminster, Devon, U.K.). Ten minutes after histamine injection, an area of skin of 5 cm square was scanned giving $\sim$16,000 data points for analysis. Our experience has shown that the changes in weal and flare areas may be measured to an accuracy of ± 0.05 cm^2 and changes in perfusion to $\pm 5\%$ (Clough *et al*., 1998).

Protocol Four sites, two on the volar surface of each forearm, were selected for study. The sites were studied sequentially. At each site, nedocromil sodium or water was introduced in random order by iontophoresis followed 10 min later by an injection of 300 nM or 1 μM histamine. Weal flare and itch responses were then assessed as described above.

Statistics All data are shown as means $\pm$ s.e.mean for observations in eight subjects. Results in nedocromil sodium and water treated sites were compared using a Student's *t*-test for paired data and $P<0.05$ taken as statistically significant.

Results Whether the introduction of nedocromil sodium into the skin or the process of iontophoresis itself caused local inflammation was assessed by single point laser Doppler fluximetry using a probe built into the iontophoresis head. Assessments of blood flux were taken before and 2 min after iontophoresis. The blood flux readings varied between 20–30 flux units before iontophoresis and did not change significantly following iontophoresis. ($P=0.996$ for water and $P=0.759$ for nedocromil sodium compared to basal fluxes).

Itch The time course of itch (Figure 1), shows a marked reduction by nedocromil sodium compared with controls. Summed over the 5 min assessment period (Figure 2A), the total itch scores were reduced by 73.6% ($P=0.008$) with 1 μM histamine and 74.0% ($P=0.005$) with 300 nM histamine in the nedocromil treated sites.

Flare The analysis of flare areas is shown in Figure 2B. With 1 μM histamine, nedocromil sodium reduced the mean flare area from 12.4 ± 3.4 to 4.2 ± 0.8 cm^2 (65.8% reduction, $P=0.028$) whilst with 300 nM histamine, the mean flare areas was reduced from 5.2 ± 1.2 to 1.9 ± 0.6 cm^2 (63.7% reduction, $P=0.010$).

Blood flux Nedocromil sodium had no significant effects on the mean blood flux within the total area of the flare. With 1 μM histamine, the mean blood fluxes in the presence and absence of nedocromil sodium respectively were 457 ± 47 perfusion units and 430 ± 33 perfusion units (6.1% reduction, $P=0.504$). With 300 nM histamine, the corresponding values were 366 ± 45 perfusion units (PU) and 309 ± 26 PU (15.5% reduction, $P=0.336$). The values for baseline flux before histamine injection were 122 ± 3, 117 ± 5, 120 ± 3 and 114 ± 3 PU respectively for the four groups.

To assess whether nedocromil sodium reduced the mean blood flux within its area of iontophoresis, values were also calculated for this area only. The results showed no significant differences. With 1 μM histamine, the mean blood flux in the presence and absence of nedocromil sodium respectively were 861 ± 70 and 820 ± 87 PU (4.8% reduction, $P=0.631$) whilst with 300 nM histamine, the corresponding

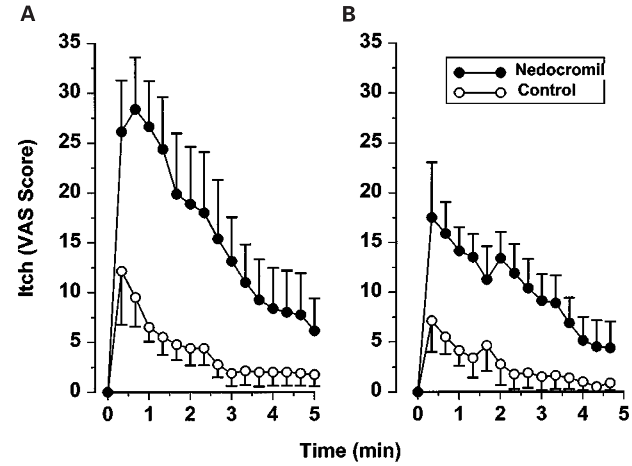

Figure 1 Histamine-induced itch in the presence and absence of nedocromil sodium. Itch was recorded on a 100 mm VAS every 20 s for 5 min in eight subjects. Water (control) and nedocromil sodium were introduced into the skin by iontophoresis. Histamine (20 μl) at (A) 1 μM and (B) 300 nM was injected 10 min later.

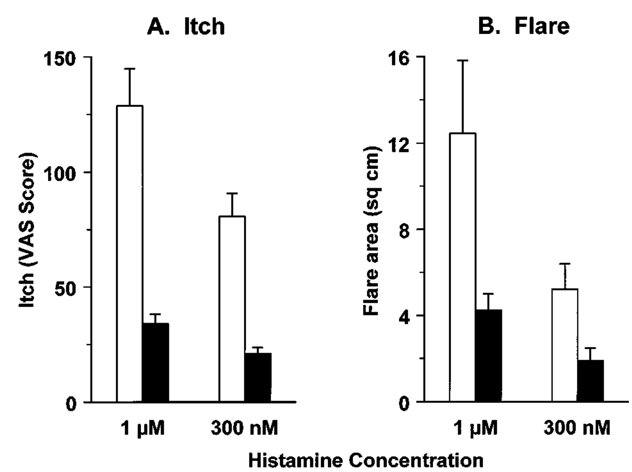

Figure 2 Histamine-induced itch and flare in the presence and absence of nedocromil sodium. (A) Itch is presented as the total VAS score summed over 5 min and (B) the flare area assessed by scanning laser Doppler imaging in eight subjects. Water (control, open bars) and nedocromil sodium (closed bars) were introduced into the skin by iontophoresis. Histamine (20 μl) was injected 10 min later. Reductions in both itch and flare in the nedocromil treated sites were statistically ($P<0.05$) significant with both concentrations of histamine.

values were 512 ± 71 and 506 ± 65 PU (1% reduction, $P = 0.952$).

Weal Following the injection of 1 μM histamine, there was a small increase in the mean weal area from 0.11 ± 0.02 to 0.14 ± 0.02 cm^2 (27.3% increase, $P = 0.007$) in the nedocromil treated area compared with control. With 300 nM histamine, the difference was not statistically significant, the respective areas being 0.07 ± 0.02 to 0.08 ± 0.02 cm^2 (14.3% increase, $P = 0.73$).

Discussion The results showed that nedocromil sodium significantly reduced histamine-induced itch by ~74% and flare by ~64% while there was no significant effect on blood flux within the flare and no reduction in weal area.

There are several possible mechanisms by which nedocromil sodium exerts its inhibitory effects on the itch and flare. First, an inhibitory effect of nedocromil sodium on mast cell degranulation is highly unlikely as the weal and flare response was induced by histamine rather than a mast cell degranulating agent. Furthermore, studies *in vitro* have shown nedocromil sodium not to inhibit degranulation of human skin mast cells (Church *et al*., 1991). Second, an antihistaminic effect can be ruled out because nedocromil sodium is not a histamine H_1-receptor antagonist (Auty, 1986). Also, the drug did not inhibit the weal response as would have been expected if it had antihistaminic effects. Indeed, with the higher concentration of histamine, there was a statistically significant increase in the area of the weal, an observation similar to that previously reported (Humphreys *et al*., 1987).

The third possibility to be considered is that nedocromil sodium interferes with the release or actions of neuropeptides such as calcitonin gene related peptide (CGRP). Such a suggestion was made following observations that nedocromil sodium inhibited sensory nerve fibre activation induced by capsaicin and bradykinin, but not by mechanical or electrical stimulation (Javdan *et al*., 1995; Myers *et al*., 1996). Also, observations that cromones inhibit neurokinin-induced bronchoconstriction (Crimi *et al*., 1992; Crossman *et al*., 1993) led to the suggestion of a receptor antagonist effect of the drugs although there is no direct evidence to support this conclusion. Indeed, the structural and charge differences between tachykinins and cromones make receptor antagonism highly unlikely. Also there is also no chemical interaction between cromones and neuropeptides (Crossman *et al*., 1993). Our results suggest that nedocromil sodium does not interfere with the release or actions of neuropeptides as a reduction in the mean blood flux within its area of iontophoresis compared with the surrounding area would have been expected if this was the case. It was not.

Thus, the most likely way in which nedocromil sodium reduces flare and itch in this model is an inhibition of sensory nerve activation or transmission. Such an effect has been suggested for cromones from a clinical study in which sodium cromoglycate caused a feeling of warmth when infused intravenously (Collier *et al*., 1983). This hypothesis is supported by the observation that nedocromil sodium induces a long-lasting chloride-dependent nerve depolarization, thereby reducing the sensitivity of the nerve to subsequent action potentials (Jackson *et al*., 1992).

At the clinical level, it is clear that nedocromil sodium is effective in reducing neurogenic itch in the skin when its penetration through the stratum corneum is facilitated by iontophoresis. The development of appropriate vehicles which will allow the penetration of this polar cromone into the skin may thus provide a unique treatment for itching in dermatological disease.

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