

Effects of Cromolyn on Codeine-Induced Histamine Release *in Vivo*

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Cromolyn has been shown to inhibit histamine release from mast cells induced by various stimuli *in vitro*. However, the local effects of cromolyn on codeine-induced wheal and flare skin reactions are not well understood. Intradermal injection of codeine induced prominent whealing in almost all humans. We studied the effect of local cromolyn injection on codeine-induced skin reactions, histamine release, and ultramicroscopic changes in mast cells in 10 volunteers. The finding in this study showed that injection of a 2% cromolyn solution before or together with the codeine injection does not affect the subsequent skin reactions, histamine release and ultramicroscopic changes of mast cells.

Key words: cromolyn; codeine; histamine; mast cell.

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Histamine is released from mast cells in response to both immunological and non-immunological stimuli. Investigations during the past two decades have shown that disodium cromoglycate is capable of inhibiting the *in vitro* and *in vivo* release of mediators from mast cells in IgE-mediated and non-IgE-mediated reactions (2, 3, 9). However, little is known about the effects of this agent on wheal and flare reactions in human skin. Codeine has been shown to induce histamine release *in vivo* (4, 10, 14). This study describes the result of cromolyn effect on codeine-induced mast cell alteration, histamine release and skin whealing responses.

MATERIAL AND METHODS

The design of the study was approved by the clinical research and human use committee of the William Beaumont Army Medical Center.

Histamine dihydrochloride (Calbiochem, San Diego, CA). Phosphate buffered saline pH 7.4 (PBS) was used as a diluent for the preparation of cromolyn and codeine solutions. Cromolyn 2% solution prepared by dissolving commercial cromolyn capsules (Fison) in PBS. A 1% codeine solution was prepared from the codeine-phosphate reagent supplied for injection therapy (Wyeth).

Measurement of whealing responses of skin

Ten volunteers were skin tested intradermally with 0.02 ml of: a) 1% codeine solution, b) buffered saline (control solution), c) a solution containing a final concentration of 1% codeine and cromolyn 2%, and d) cromolyn 2% alone.

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Twenty minutes later, the whealing response of each skin test was determined by a zero setting compensating polar planimeter (Leitz) and expressed as an area in square millimeters. In order to determine whether prior injection of cromolyn could block the whealing response to a subsequent codeine challenge, the codeine concentration (ED_{20}) required to elicit an approximate whealing response of 20 mm^2 was determined first in five volunteers. Then, cromolyn 2%, 0.04 ml, was injected at three different sites over the outer aspect of the upper arm at least 8 cm apart. Site A was challenged with codeine ED_{20} dose 30 min later; Site B challenged 60 min later; and Site C 120 min later. The skin whealing response was measured 20 min later.

Measurement of histamine release into skin chambers

Four blisters each with 1 cm diameter base were induced simultaneously (2 on each forearm) in five subjects as described previously by us (11); each blister was then unrooted aseptically. A specially designed plastic collection chamber with entry and exit ports was placed directly over the denuded base and kept in place by appropriate taping. Each chamber was filled with PBS. Five minutes later the fluid was removed, the chamber irrigated, and filled with 0.3 ml of either: a) *Chamber A* – 1% codeine solution; b) *Chamber B* – PBS alone (negative control); c) *Chamber C* – a solution containing a final concentration of codeine 1% and cromolyn 2%; and d) *Chamber D* – cromolyn 2%. All chamber fluids were removed after 30 min incubation and stored in coded polyethylene tubes at $-20^\circ C$ until studied. Histamine was analyzed by the micro-enzymatic method of Levy & Widra (7).

Ultramicroscopic studies of skin test sites

The effects of exogenous cromolyn on codeine-induced mast cell alterations were studied in two of these subjects. Repeat skin tests were carried out as mentioned above, and skin biopsies were obtained 10 min later at the sites of intradermal injections of either a) codeine, b) control PBS

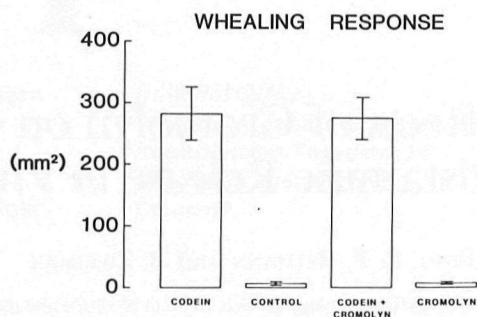


Fig. 1. Skin whealing response (mean $\pm$ SEM) at the sites of injections of codeine, PBS (control), codeine plus cromolyn mixture and cromolyn alone. Codeine-induced skin whealing response was not inhibited by cromolyn.

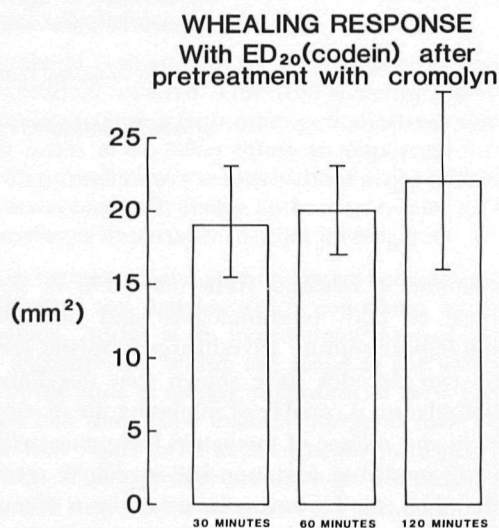


Fig. 2. Time study of the whealing response (mean $\pm$ SEM) to codeine ED_{20} when cromolyn was injected at 30, 60, and 120 min before the codeine. Pretreatment of the skin sites with cromolyn did not modify the codeine-induced whealing response.

solution, c) codeine, cromolyn, or d) cromolyn alone. All biopsied sections were coded before evaluation and embedded in plastic for ultrathin sectioning and electron microscopy.

Classifications were based on examination of 16–20 mast cells in multiple replicative ultrathin sections (13).

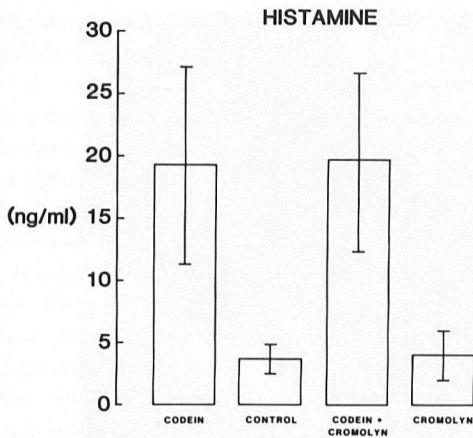


Fig. 3. Histamine levels (mean $\pm$ SEM) in skin chamber fluids after 30 min incubation with codeine, PBS (control), codeine plus cromolyn and cromolyn alone. Codeine-induced histamine release was not inhibited by cromolyn.

RESULTS

Cromolyn effects on skin test

As shown in Fig. 1, the mean whealing response to intradermal injection of codeine plus cromolyn injection was very similar to the mean whealing response at the codeine injected sites in all subjects: Mean $\pm$ SEM mm² was (278 ± 33 vs 283 ± 42). The whealing response at the skin sites injected with either a) control buffered saline or b) cromolyn alone were very small (8 ± 4 vs 9 ± 3). Furthermore, prior injection of cromolyn into the skin testing sites at intervals up to 2 h prior to codeine did not modify the codeine-induced whealing response in any of the five subjects (Fig. 2).

Cromolyn effects on codeine-induced histamine level

A qualitative, very similar pattern of events was seen in all five subjects (Fig. 3). Skin chamber fluid histamine levels at sites exposed 30 min

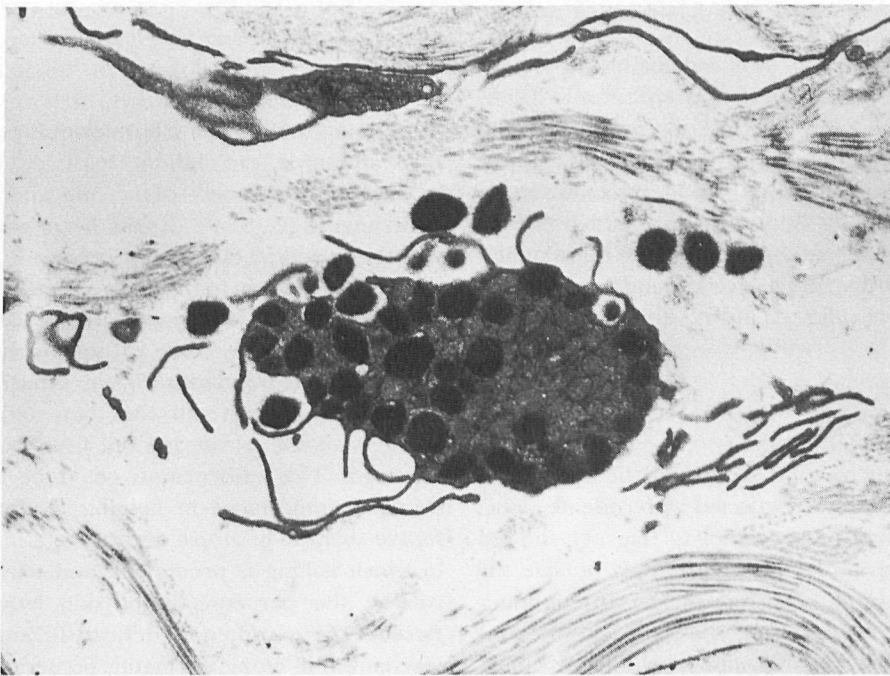


Fig. 4. Electron micrograph of a mast cell from the skin site challenged with codeine or codeine plus cromolyn, showing release of granules from the cell into the intercellular space ($\times 6000$).

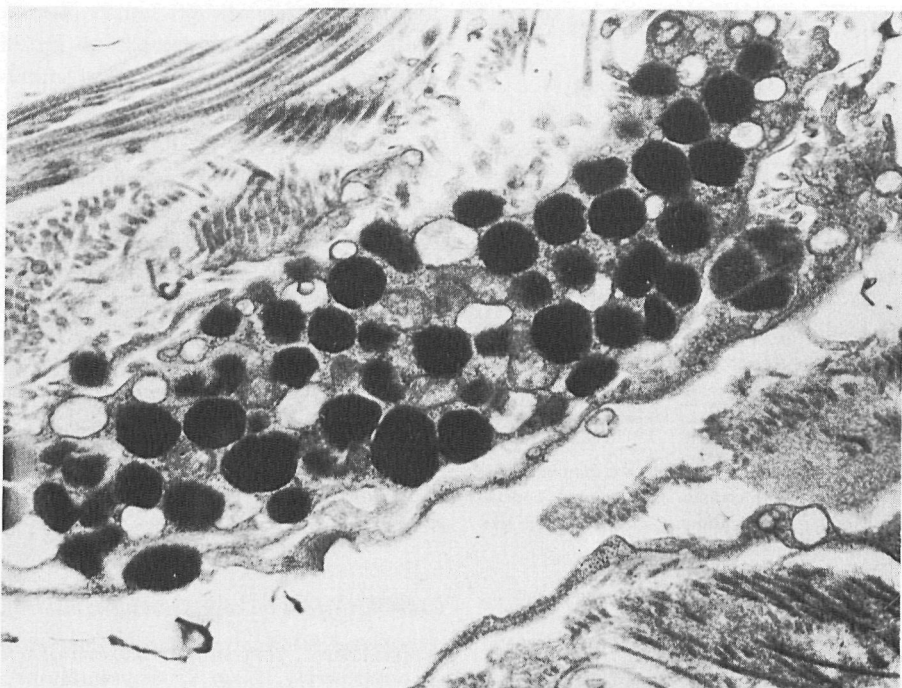


Fig. 5. Electron micrograph of a typical mast cell from the skin site challenged with normal saline or cromolyn demonstrating mast cell granules are confined in the cell cytoplasm ($\times 10,500$).

earlier to cromolyn plus codeine (m $\acute{e}$ an $\pm$ SEM 19.6 ± 7.2 ng/ml) were not significantly different than the histamine levels at sites exposed to codeine alone (19.4 ± 7.9 ng/ml).

The histamine levels in skin chambers to which cromolyn alone had been added 30 min previously (4.2 ± 0.86 ng/ml) were quite similar to the low histamine levels found in the negative control PBS chamber fluids (3.6 ± 1.0 ng/ml).

Skin biopsy studies

Electron microscopic studies of the upper arm skin of two subjects injected with codeine alone, and codeine plus cromolyn (Fig. 4) showed mast cell granules overwhelmingly outside the cytoplasm and scattered in the surrounding stroma. By comparison, mast cells in sites injected with cromolyn alone or control saline, showed majority of the granules confined in the cell cytoplasm (Fig. 5).

DISCUSSION

Studies in a number of laboratories have shown that cromolyn can inhibit mast cell-derived mediators in a variety of *in vitro* and *in vivo* experiments (2, 3, 9). It has been postulated that the beneficial effects of cromolyn in asthma may be at least in part due to this effect on mediator release (1). Although topical cromolyn has been utilized by some groups in the treatment of atopic dermatitis (5), the effects of this agent on skin mast cell alteration and subsequent mediator release has not been well characterized. This information could be of considerable importance in helping to design effective therapy in atopic dermatitis, a condition in which itching is prominent, and may play a role in the perpetuation of skin lesions (6). Because there is often no defined IgE-mediated mechanism in atopic dermatitis occurring in the adult, we chose to investigate the effects of cromolyn on the non-IgE-mediated mast cell

alterations and subsequent events that are induced in human skin by codeine.

Our findings that local application of cromolyn did not alter the expected codeine-induced changes in human skin, are similar to the *in vitro* and *in vivo* finding of the effect of cromolyn on the IgE-mediated hypersensitivity reactions of the human skin (8, 12).

It is possible, that the lack of cromolyn effect was due to inadequate absorption of this agent from the skin chamber fluid through the base of the skin blister into the dermis. However, we think that this is unlikely to be a major factor, since intradermal injection of the same solution did not inhibit codeine-induced mast cell alterations or whealing responses. Although the cromolyn powder available for our study contained lactose as a filler substance, we have not found that lactose itself affects skin test reactivity. We think the most likely explanation is that cromolyn in the concentration used affects skin mast cells *in vivo* less than observed in the *in vivo* lung models previously utilized by others (1, 9). Attempts to use higher cromolyn concentrations have been limited by a non-specific irritative effect in the skin. Studies using vehicles which permit longer *in vivo* exposures of the skin mast cell to cromolyn, or an analogue of this agent, may uncover therapeutically relevant effects in skin disorders.

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