

Failure of Verapamil in Inhibiting Allergen-Induced Histamine Release *in Vivo*

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In order to evaluate the effect of Verapamil on human cutaneous anaphylactic responses, ragweed-sensitive individuals were skin tested with ragweed, ragweed plus Verapamil, and Verapamil alone. In addition, using a skin chamber technique, the effect of Verapamil on antigen-induced histamine release *in vivo* was investigated. The results suggest that Verapamil, applied locally in non-irritant doses, does not affect Type I hypersensitivity skin reactions.

Key words: histamine; inhibition; *in vivo*; Verapamil.

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Calcium antagonists are a group of therapeutic agents which block calcium ion influx into cells. These agents inhibit smooth muscle contraction, mast cell degranulation, and mucous gland secretion (9). With these pharmacologic properties, calcium channel blocking agents might be of use in treating Type I hypersensitivity reactions. This study describes the effects of locally applied, non-irritant concentrations of Verapamil on antigen-induced mast cell alterations and histamine release in the skin of atopic subjects.

MATERIAL AND METHODS

The design of the study was approved by the Clinical Investigation and Human Use Committees of William Beaumont Army Medical Center, El Paso, Texas.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Subjects

Ten ragweed-sensitive individuals participated in the study. Informed consent was obtained from all volunteers. Ragweed sensitivity was based on a typical seasonal history of allergic rhinitis, with or without asthma, and a 250 mm² or greater whealing response at a skin site, 15 min after the intradermal injection of 0.02 ml of ragweed extract 500 PNU/ml.

Reagents

Phosphate buffered saline pH 7.4 (PBS) was used as the diluent for the ragweed extract and as a control solution. Various concentrations of Verapamil solution were prepared from commercially available Verapamil hydrochloride, 2.5 mg/ml (Knoll Pharmaceutical, U.S.A.).

Measurements of whealing response

Ten atopic subjects underwent intradermal skin testing with 0.02 ml each of these four solutions: 1) ragweed 500 PNU/ml; 2) a mixture containing a final concentration of ragweed 500

WHEALING RESPONSE

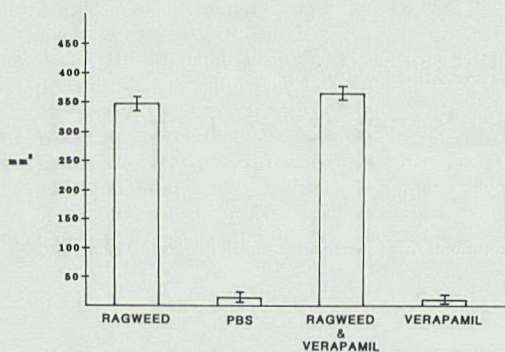


Fig. 1. Skin whealing response (mean $\pm$ SEM) at the sites of injection of ragweed, PBS, ragweed plus Verapamil or Verapamil alone. Ragweed-induced skin whealing response was not inhibited by Verapamil.

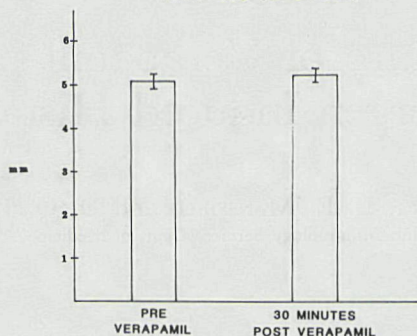
WHEALING RESPONSE
WITH ED₅ (RAGWEED)

Fig. 2. Time study of whealing response (mean $\pm$ SEM) to ragweed ED₅ when Verapamil was injected 30 min before the ragweed. Pretreatment of the skin site with Verapamil did not modify the ragweed-induced whealing response.

PNU/ml and Verapamil 25 μ g/ml; 3) Verapamil 25 μ g/ml; and 4) PBS as a negative control. After 15 min the whealing response at each skin test site was outlined on a sheet of tracing paper. The total wheal area was measured (mm²) with an electronic planimeter (13).

Additional skin testing was performed to investigate whether prior intradermal injection of Verapamil would inhibit the whealing response to ragweed antigen, injected subsequently at the same skin site. Initially, serial-dilution skin testing with ragweed antigen was carried out in five volunteers to determine concentration causing a 5 mm in diameter whealing response (thereafter called the ED₅) for each subject. Then 0.75 μ g of Verapamil was injected at the upper/outer aspect of the arm. Thirty minutes later the ragweed ED₅ doses were injected at the same site. The size of the wheal was measured 20 min later, in the manner described above.

Measurement of histamine release into skin chambers

Four blisters, each with 1 cm diameter base, were induced simultaneously (two on each forearm) in four subjects as described previously by us (14). A plastic collection chamber with entry and exit ports was placed directly

over the blister base. Each chamber was filled with PBS. Five minutes later the PBS was removed.

The chamber was irrigated with PBS and refilled with 0.5 ml of one of the following solutions: Chamber A - ragweed solution 1000 PNU/ml; Chamber B - PBS alone (negative control); Chamber C - a solution containing a final concentration of ragweed 1000 PNU/ml and Verapamil 25 μ g/ml; and Chamber D - Verapamil 25 μ g/ml. The chamber fluids were removed after 30 min incubation, and stored in coded polyethylene tubes at -70°C until analyzed. Histamine was analyzed by a fluorometric method (5).

Histology study of skin test sites

The effects of Verapamil on ragweed-induced mast cell alterations were studied in two subjects. Skin site biopsies were obtained 10 min after injection of the following: 1) ragweed 500 PNU/ml; 2) control PBS solution; 3) ragweed 500 PNU/ml plus Verapamil 25 μ g/ml, 4) Verapamil 25 μ g/ml alone. Biopsy specimens were coded, stained with Giemsa and examined under light microscope. The evaluations were categorized based on examination of 16-20 mast cells (15).

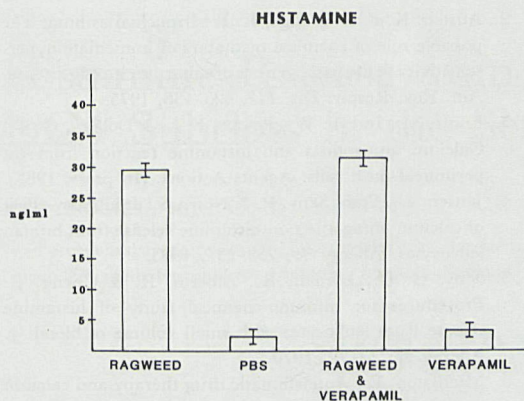


Fig. 3. Histamine levels (mean $\pm$ SEM) in skin chamber fluids after 30 min incubation with ragweed, PBS, ragweed plus Verapamil, and Verapamil alone. Ragweed-induced significant release of histamine, which was not inhibited by Verapamil.

RESULTS

The effects of Verapamil on ragweed skin testing are shown in Fig. 1. The mean wheal size at the ragweed plus Verapamil site was similar to that of the ragweed antigen site alone in the 10 subjects: (346 ± 17 versus 367 ± 26 mm²). Small whealing responses were seen at the skin sites injected with the PBS solution or with Verapamil solution (10 ± 4 versus 9 ± 3 mm²). Pretreatment of the skin sites with Verapamil did not modify the ragweed-inducing whealing response (Fig. 2).

Verapamil's effects on ragweed-induced histamine release

All four subjects had similar results. There was no difference between histamine levels from blister chambers to which ragweed or ragweed plus Verapamil had been added (mean $\pm$ SEM 29 ± 5 ng/ml vs 31 ± 5 ng/ml). PBS and Verapamil induced a small insignificant amount of histamine release (2.8 ± 0.2 vs 3 ± 0.4) (Fig. 3).

Skin biopsy studies

The photomicrographs of mast cells obtained from tissue sites injected with ragweed or

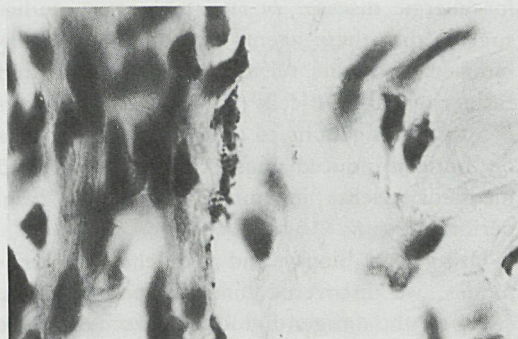


Fig. 4. Photomicrograph of a mast cell obtained from tissue sites injected 10 min earlier with ragweed alone or ragweed plus Verapamil, showing evidence of granular exocytosis $\times 1000$.

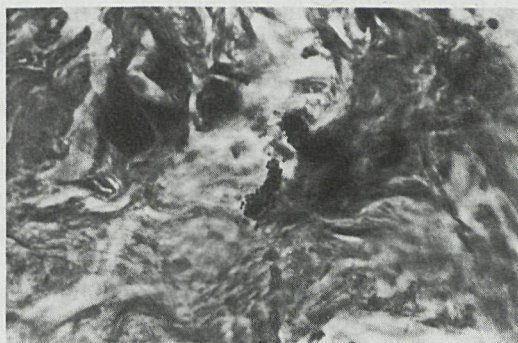


Fig. 5. Photomicrograph of a typical mast cell 10 min after injection with Verapamil or PBS. Showing little evidence of degranulation $\times 1000$.

ragweed plus Verapamil showed extensive evidence of granule release (Fig. 4). The mast cells in sites injected with Verapamil alone, or PBS alone, had granules confined to the cell cytoplasm (Fig. 5).

DISCUSSION

It would seem desirable to treat allergic disease with agents that prevent the release of mast cell derived mediators stimulated by IgE-allergen interaction. Calcium ion appears to be an absolute requirement for the release of mediators from sensitized basophils/mast cells challenged with specific antigen (1, 2, 6, 10). The recent availability of calcium channel blocking agents may offer a newer treatment modality

for allergic disease. *In vitro* laboratory studies suggest that these agents can inhibit allergen-induced histamine release from basophils and rat peritoneal mast cells (3, 4, 12). No previous *in vivo* investigations of calcium blocker effects on antigen-induced mast cell alteration and mediator release in human skin have been performed.

Using skin biopsy and skin chamber techniques, we discovered that Verapamil has no effect on the antigen-induced histamine release and cell alterations of human skin mast cells. This observation is consistent with the findings of Middleton, who found no effects of Verapamil on *in vitro* human basophil histamine release model (7). In addition, other investigators have reported that calcium channel blocking agents did not inhibit allergen-induced asthma in humans (8, 11).

It does not seem likely that the apparent lack of effects by Verapamil on the skin mast cell reflects inadequate concentration. Pretreatment of the skin sites with Verapamil up to 0.75 μ g, also did not inhibit the allergen-induced skin reaction. It is conceivable, but we believe unlikely, that higher concentrations of Verapamil might inhibit the reactions. However, doses above 1 μ g of Verapamil result in significant nonspecific irritant effects on the skin. Presently, it is too early to conclude that drugs which modulate calcium metabolism will not play a significant role in the management of allergic dermatosis. Perhaps an investigation using an isolated pure skin mast cell population might yield different conclusions.

Further studies are planned to investigate the effects, if any, of the other available calcium channel blocking agents on the allergen-induced mast cell alterations and histamine release in the human skin.

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