

## Short communication

## Desloratadine prevents compound 48/80-induced mast cell degranulation: visualization using a vital fluorescent dye technique

**Background:** Desloratadine is a selective H<sub>1</sub>-antihistamine used in the treatment of allergic rhinitis and chronic idiopathic urticaria. Desloratadine inhibits the release of allergic inflammatory mediators *in vitro*. We studied the impact of desloratadine on mast cell degranulation due to activation and re-activation by the secretagogue, compound 48/80.

**Methods:** Rat peritoneal eluate containing 5–6% mast cells were activated by a low concentration of compound 48/80 in a medium containing the vital fluorescent dye, Sulforhodamine-B (SFRM-B, 200 µg/ml), which is engulfed by activated mast cells. The fluorescent image of activated mast cells was captured digitally and the total fluorescent area was analyzed when desloratadine was applied before or after compound 48/80.

**Results:** Mast cells were not activated by desloratadine (10<sup>-4</sup> M), SFRM-B (200 µg/ml), or diluent alone. A low concentration of compound 48/80 (0.125 µg/ml) induced fluorescence, while mast cells lost fluorescent images due to further degranulation on re-exposure to compound 48/80. Desloratadine (10<sup>-8</sup>–10<sup>-4</sup> M), inhibited compound 48/80-induced mast cell degranulation in a concentration-dependent manner. Desloratadine also reduced the loss of fluorescent images due to re-exposure to compound 48/80.

**Conclusions:** Desloratadine may have a mast cell stabilizing effect at low concentrations in response to repeated mast cell activation *in vitro*.

Y. H. Wang<sup>1</sup>, Y. Taché<sup>1,2</sup>, A. G. Harris<sup>3</sup>,  
W. Kreutner<sup>3</sup>, A. F. Daly<sup>4</sup>, J. Y. Wei<sup>1,2</sup>

<sup>1</sup>Department of Medicine, Division of Digestive Diseases, CURE Digestive Diseases Research Center and Center for Neurovisceral Sciences, and <sup>2</sup>Brain Research Institute, UCLA School of Medicine, Los Angeles, CA, USA; <sup>3</sup>Schering-Plough Corp., Kenilworth, NJ, USA; <sup>4</sup>Clinical Concepts Research Group, Gijon, Spain

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Dr Jen Yu Wei  
CURE Brain-Gut Research Group  
11301 Wilshire Boulevard  
Building 115, Room 124A  
Los Angeles, CA 90073  
USA

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Mast cells play a central role in the initiation and maintenance of signs and symptoms of allergic diseases, such as, asthma, allergic rhinitis, and certain forms of chronic urticaria (1). Cross-linking of mast cell-associated IgE by an allergen or an autoantibody induces FcεRI-mediated activation of a signaling cascade that leads to inflammatory mediator release (2). Mediators of the early phase allergic response, principally histamine, are released preformed from granules, while eicosanoids are rapidly synthesized *de novo* from membrane phospholipids (3). Initial activation via FcεRI appears to be mediated by interactions between protein tyrosine kinases and specific activation targets on the γ-subunit of FcεRI (4, 5). Antigen-induced histamine release via FcεRI is severely impaired in mast cells in which *Syk* function is disrupted by mutation of a tyrosine phosphorylation site (5). Mast cells can also be activated by nonIgE stimuli, such as, compound 48/80, which acts at the mast cell membrane to stimulate trimeric G-proteins and induces degranulation via phospholipase C and D pathways (6–8).

While these recent advances in the molecular pathophysiology of allergic inflammation point to the complex-

ity of cytokine-cell interactions, the focus is now returning to the role of histamine as a key orchestrator of the allergic response (9). Histamine in itself has pro-inflammatory effects, which include inducing the release of cytokines, chemokines and adhesion molecules, while histamine also modulates eosinophil and neutrophil chemoattraction [for reviews see (10, 11)]. Binding of histamine to the H<sub>1</sub> receptor activates the transcription factor nuclear factor κB (12, 13), which, in turn, promotes the transcription of inflammatory mediator RNA (14). Blockade of histamine by H<sub>1</sub>-receptor antagonists, therefore, may not only impact directly the initial vascular and neurogenic actions of histamine, but also could potentially down regulate cytokine-mediated allergic inflammation. Data from *in vitro* studies of H<sub>1</sub>-antihistamines support this suggestion. For example, at relatively low concentrations, desloratadine, has been shown to inhibit histamine and eicosanoid release (15) in addition to the secretion of cytokines [interleukin (IL)-3, IL-6, GM-CSF, tumor necrosis factor (TNF)-α] and chemokines (IL-8) from mast cells (16, 17).

We wished to study in more detail the potential anti-allergic effects of desloratadine on mast cell degranulation with a technique not previously used in studies of antihistamines. In earlier studies, we combined electrophysiological techniques and digital imaging of

**Abbreviations:** SFRM-B, sulforhodamine-B; DMSO, dimethyl sulfoxide; DL, desloratadine.

vital dye release to investigate mast cell-sensory nerve interactions (18). This model permits the visualization and recording of changes in images of mast cell uptake of a fluorescent dye, following activation by compound 48/80. We used this fluorescent dye imaging technique to study the effects of desloratadine on degranulation in naïve and compound 48/80 pre-activated rat peritoneal mast cells.

## Materials and methods

### Animals

Twenty-nine male Sprague-Dawley (SD) rats weighting ~250 g (Harlan Laboratories, San Diego, CA) were housed under controlled conditions of temperature (22–24°C) and illumination (12 h light cycle, starting at 6:00 A.M.) and maintained with Purina Chow and tap water *ad libitum* for 1 week prior to the experiments. The UCLA Chancellor's Animal Research Committee approved the experimental design of this study (ACR no. 079-22).

### Compounds and reagents

Sulforhodamine-B (SFRM-B) (Molecular Probes Inc., Eugene, OR; MW: 558) 200 µg/ml was dissolved and diluted in rat Ringers (NaCl, 140 mM; KCl, 5 mM; MgCl<sub>2</sub>·6H<sub>2</sub>O, 1 mM; Na<sub>2</sub>HPO<sub>4</sub>, 1.3 mM; Hepes, 5 mM; CaCl<sub>2</sub>·2H<sub>2</sub>O, 2 mM; D-glucose, 10 mM; pH 7.38 ± 0.02). All chemicals in the composition of the rat Ringers were purchased from Sigma Chemicals Co., St Louis, MO. Desloratadine (Schering-Plough, Kenilworth, NJ) was dissolved in dimethyl sulfoxide (DMSO; Sigma) and was further diluted in SFRM-B solution (200 µg/ml in rat Ringers) or rat Ringers alone to produce final desloratadine concentrations ranging from 10<sup>-8</sup> to 10<sup>-5</sup> M with 0.1% DMSO. A solution of 10<sup>-4</sup> M desloratadine containing 1% DMSO was also prepared. Compound 40/80 (Condensation product of *N*-methyl-*p*-methoxyphenethylamine with formaldehyde) (Sigma) was dissolved in rat Ringers or SFRM-B solution (200 µg/ml).

### Peritoneal mast cell preparation

Our study required intact uncompromised mast cells that could survive two challenges with compound 48/80. We elected to harvest rat peritoneal mast cells from intraperitoneal lavage and used this nonpurified preparation of mast cells, as we reported previously that the processing during mast cell enrichment can result in alterations in rat peritoneal mast cell membrane activity (19). Another advantage of using unpurified rat peritoneal mast cells is that the cells are intact and can be used immediately after the collection of the peritoneal lavage.

Rats were deprived of food (but not water) for 16–24 h, and then anesthetized with isoflurane USP (Abbott Laboratories, North Chicago, IL). Immediately after the animal was exsanguinated by decapitation, 5 ml of SFRM-B solution was injected intraperitoneally, and the abdomen was gently massaged for ~1 min. Three milliliters of peritoneal eluate cells (5–6% are peritoneal mast cells) were harvested, and aliquots of 0.25 ml, containing ~6 × 10<sup>5</sup> cells were plated on 35 mm tissue culture dishes (Falcon 3801, Becton Dickinson, Franklin Lakes, NJ). An additional SFRM-B solution was added with or without compound 48/80 or desloratadine to reach a total volume of 1 ml. The final concentrations of compound 48/80 were 0.125 or 0.25 µg/ml, while the concentrations of

desloratadine were 0, 10<sup>-8</sup>–10<sup>-4</sup> M. All procedures were completed within 10–15 min in a sterilized hood at room temperature (25 ± 2°C). The dishes were then placed in a water-jacketed mini CO<sub>2</sub> incubator (VWR, model 2310) at 37°C, and exposed to 5% CO<sub>2</sub> for 15 min.

### Experimental procedures

The underlying principle of the current studies is based on the known ability of peritoneal mast cells to engulf compounds including vital dyes from the surrounding extracellular medium following activation. Thus, peritoneal mast cells that are activated with compound 48/80 in the presence of SFRM-B engulf this dye and fluoresce. Upon a second activation by compound 48/80, viable fluorescent mast cells undergo degranulation and loss of the fluorescent dye into the extracellular medium, thus decreasing their fluorescence. The measurement of the uptake and loss of fluorescence can be used as a marker of the degree of mast cell response to activation stimuli, such as compound 48/80 (18).

### Effect of vehicle and desloratadine on basal mast cell activity

The highest concentration of desloratadine used in the current report was 10<sup>-4</sup> M. At this concentration, desloratadine was dissolved and diluted in a solution with a final concentration of 1% DMSO and 200 µg/ml SFRM-B. We examined whether or not SFRM-B (200 µg/ml), DMSO (1%) and desloratadine (10<sup>-4</sup> M) influenced basal peritoneal mast cell activity. This was achieved by plating aliquots of 0.25 ml peritoneal eluate cells on four tissue culture dishes. To dish 1, 0.75 ml of SFRM-B (200 µg/ml) was added and to dishes 2, 3 and 4, 0.75 ml SFRM-B (200 µg/ml) containing DMSO, desloratadine and compound 48/80 (final concentrations 1%, 10<sup>-4</sup> M and 0.125 µg/ml, respectively) were added. All dishes were then put into a water-jacketed mini CO<sub>2</sub> incubator (5% CO<sub>2</sub>) at 37°C, for 15 min. At the end of the incubation, 1 ml oxygenated rat Ringers was added to each dish after rinsing them three times with oxygenated rat Ringers, and then they were stored in a dark container. The bright-field and/or fluorescent images of mast cells from five (the center, left, right, top, and bottom) regions in each dish were acquired, measured and analyzed.

### Effect of desloratadine pretreatment on compound 40/80-induced peritoneal mast cell degranulation

Aliquots of 0.25 ml of peritoneal eluate cells were plated on seven 35-mm tissue culture dishes. An additional 0.75 ml of SFRM-B (200 µg/ml) containing desloratadine (final concentrations 0, 10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup>, and 10<sup>-4</sup> M) was added to dishes 1 to 6, respectively, while 0.75 ml of SFRM-B (200 µg/ml) was added to dish 7. All dishes were put into a water-jacketed mini CO<sub>2</sub> incubator (5% CO<sub>2</sub>), at 37°C, for 15 min. At the end of incubation period, dishes were rinsed three times with oxygenated rat Ringers and 48/80 was added to reach a final concentration of 0.125 µg/ml. The dishes were re-incubated, rinsed and analyzed as in study 1.

### Effects of desloratadine on mast cells activated by subsequent exposure to compound 48/80

Aliquots of 0.25 ml peritoneal eluate cells were plated on five 35 mm tissue culture dishes. An additional 0.75 ml of SFRM-B (200 µg/ml) containing 48/80 (final concentration 0.25 µg/ml) was added to dishes 1 to 5. All dishes were then put into a water-jacketed

mini CO<sub>2</sub> incubator (5% CO<sub>2</sub>) at 37°C, for 15 min to activate the mast cells, leaving them fluorescent. The dishes were taken out, rinsed with oxygenated rat Ringers three times. Desloratadine (dissolved in DMSO and further diluted in SFRM-B solution) was added to dishes 1 to 5. The final concentrations of DMSO and SFRM-B were 0.1% and 200 µg/ml respectively, while the DL concentrations were 0, 10<sup>-8</sup>, 10<sup>-7</sup>, 10<sup>-6</sup>, and 10<sup>-5</sup> M. Dishes were incubated for an additional 15 min, then taken out and rinsed with 0.5 ml oxygenated rat Ringers three times. Compound 48/80 (0.25 µg/ml) was then added a second time to each dish and all dishes were returned to the incubator for 15 min. At the end of the incubation period, dishes were rinsed, stored and analyzed as in studies 1 and 2 above.

#### Data acquisition and analysis

The rat peritoneal mast cell has a unique appearance compared with other peritoneal cells, and can be easily recognized using bright-field light microscopy due to its large size, clear nucleus and cytoplasm rich in granules. Images were visualized with a Leitz fluorescence microscope [Laborlux S], and captured by a Silicon-Intensifier Target tube camera [MTI SIT-68]. The microscope was equipped with a 10×eyepiece, one 10×EF Wetzlar and one 50×water-immersion fluorescence objectives, and an N2 cube (530–560 nm excitation, 580 nm suppression) specially used for displaying the fluorescent image of SFRM-B. The real-time video signals from the camera were displayed on an RGB analog monitor (Sony PVM-1434-MD), and sent to a video recorder (Sony SVO-140) and an IBM-formatted computer. Dishes were orientated at random on the microscope stage. The bright-field and/or fluorescent images were randomly captured from five regions (center, bottom, top, left, and right) of each dish. Image fields of 250 × 180 and 1200 × 900 µm<sup>2</sup> are covered by 500× and 100× magnifications, respectively. Sixteen frames of each field were digitized, summed, and saved as a tagged image file (TIF). Five bright-field and/or fluorescent TIFs (a total of 15 files) were obtained from each dish.

IMAGE-1 software (Universal Imaging Corp., Downingtown, PA) was used to collect the following data: (a) The total fluorescent area (µm<sup>2</sup>) of activated mast cell was summed and the data were converted to the total number of full fluorescent mast cells; (b) The total number of mast cells (fluorescent and nonfluorescent) was counted; (c) The percentage of full fluorescent cells (i.e. fully fluorescent cell number divided by the total mast cell number) was calculated; (d) The total fluorescent area was normalized by multiplying (a) by (c).

#### Statistical analysis

Data were presented as mean ± SEM of µm<sup>2</sup>. Statistical significance was assessed with ANOVA followed by the Tukey–Kramer multiple comparisons test. The ED<sub>50</sub> was estimated directly from the scattered-plot of concentration-dependence inhibition.

## Results

#### Peritoneal mast cell distribution and viability

Rat peritoneal eluate was composed of diverse cells including macrophages and eosinophils. While 5–6% of total nucleated cells from peritoneal eluate were mast cells, they were readily identified on light microscopy. As illustrated in Fig. 1A, peritoneal mast cells were highly reflectile at 100× magnification.

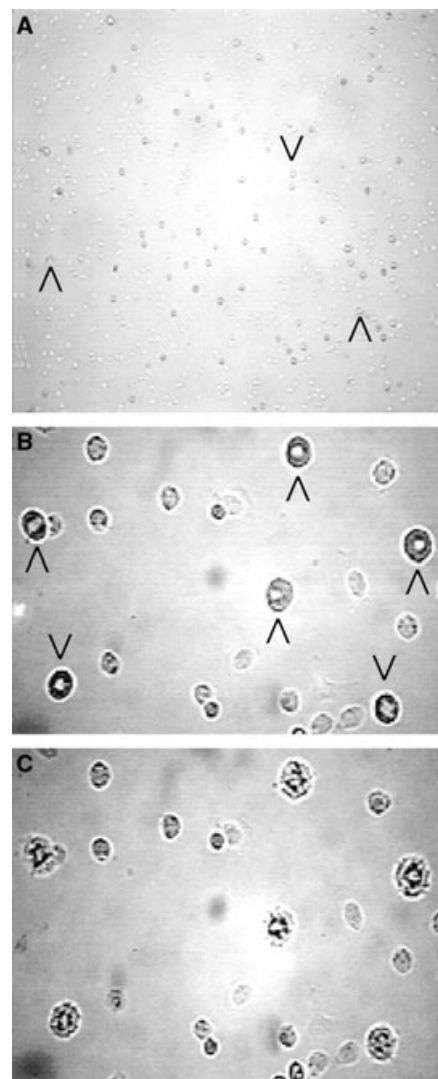


**Figure 1.** Peritoneal mast cells are highly reflectile with a smooth surface and outline under light microscope at 100× magnification (A). Appearance of peritoneal mast cells (arrows) from vehicle dish at the end of the experimental protocol (B). Following addition of compound 48/80, these cells degranulate (C), demonstrating that the length of the experimental procedure did not impact the viability of the cells. Magnification in (B) and (C): 500×.

Among the groups in the control and DL experiments, no significant differences in mast cell numbers per dish were noted (Table 1). Peritoneal mast cells were, therefore, evenly distributed throughout the dishes, thus ensuring the comparability of the data among the groups within each experiment.

In the control experiment, the vehicle dish (Vehicle, *n* = 6) was handled similarly to other experimental dishes except that it contained no desloratadine or compound 48/80. The appearance of such mast cells before a subsequent challenge with compound 48/80 is illustrated in Fig. 1B. The degranulation image of these mast cells

Table 1. Equal distribution of peritoneal mast cell numbers within experimental dishes

|                                                                      |              |              |                                 |              |                                             |              |
|----------------------------------------------------------------------|--------------|--------------|---------------------------------|--------------|---------------------------------------------|--------------|
| Control Experiment ( $F_{3,2,6} = 0.74, P > 0.05, N = 3$ )           |              |              |                                 |              |                                             |              |
| SFRM-B<br>(200 $\mu\text{g/ml}$ )                                    | DMSO<br>(1%) |              | Desloratadine<br>( $10^{-4}$ M) |              | Compound 48/80<br>(0.125 $\mu\text{g/ml}$ ) |              |
| 16 $\pm$ 1.2                                                         | 15 $\pm$ 0.0 |              | 17 $\pm$ 0.7                    |              | 16 $\pm$ 0.6                                |              |
| Compound 48/80 Activation ( $F_{6,5,30} = 2.21, P > 0.05, N = 6$ )   |              |              |                                 |              |                                             |              |
| Desloratadine (M)                                                    |              |              |                                 |              |                                             |              |
| 0                                                                    | $10^{-8}$    | $10^{-7}$    | $10^{-6}$                       | $10^{-5}$    | $10^{-4}$                                   | Veh          |
| 18 $\pm$ 1.1                                                         | 16 $\pm$ 0.9 | 16 $\pm$ 0.7 | 18 $\pm$ 1.3                    | 17 $\pm$ 1.3 | 19 $\pm$ 1.1                                | 16 $\pm$ 1.1 |
| Compound 48/80 Reactivation ( $F_{4,5,20} = 0.36, P > 0.05, N = 6$ ) |              |              |                                 |              |                                             |              |
| Desloratadine (M)                                                    |              |              |                                 |              |                                             |              |
| 0                                                                    | $10^{-8}$    | $10^{-7}$    | $10^{-6}$                       | $10^{-5}$    |                                             |              |
| 20 $\pm$ 1.1                                                         | 21 $\pm$ 1.7 | 20 $\pm$ 1.0 | 22 $\pm$ 1.5                    | 20 $\pm$ 1.1 |                                             |              |

Each value is the mean  $\pm$  SEM of the number of peritoneal mast cells in each dish.

after challenge with compound 48/80 is shown in Fig. 1C. The integrity of mast cells at the end of the experiment and their ability to respond to subsequent application of compound 48/80 indicates that the mast cells were viable across the time span of the experiment.

Mast cell activation and fluorescence

Mast cells were not activated by either SFRM-B (200  $\mu\text{g/ml}$ ), or the highest concentrations of DMSO (1%) and DL ( $10^{-4}$  M). In contrast, the peritoneal mast cells did respond to a low concentration of compound 48/80 (0.125  $\mu\text{g/ml}$ ) (Fig. 2A).

A bright-field plus fluorescent image showing six of seven mast cells that were activated by compound 48/80 (0.125  $\mu\text{g/ml}$  diluted in SFRM-B solution without DL pretreatment) is illustrated in Fig. 2B. The corresponding fluorescent image is presented in Fig. 2C. Note the degree of activation was variable, with the larger fluorescent area reflecting more intense activation.

Pretreatment with desloratadine inhibits compound 48/80-induced mast cell degranulation

Pretreatment of mast cells with desloratadine inhibited compound 48/80 induced degranulation in a concentration-dependent manner ( $F_{6,5,30} = 28.0, P < 0.0001$ , repeated measure analysis of variance; Fig. 3). There was a linear reduction of fluorescence induced by desloratadine at concentrations ranging from  $10^{-8}$  to  $10^{-5}$  M with complete suppression at  $10^{-4}$  M.

Desloratadine reduces the loss of fluorescence in mast cells previously activated by compound 48/80

Second exposure of mast cells to compound 48/80 without SFRM-B in the medium alters the fluorescent

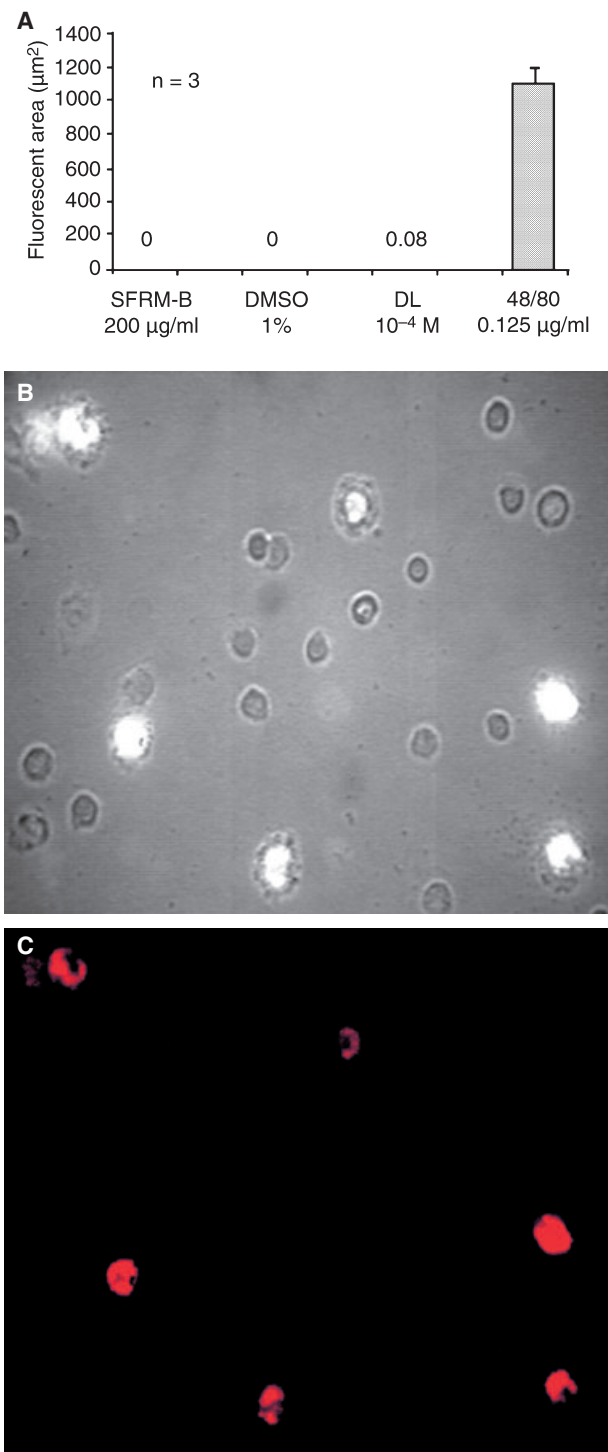


Figure 2. (A) A bar chart shows the result of three control experiments. PMCs were not activated by SFRM-B, DMSO (1%) and desloratadine ( $10^{-4}$  M), but did respond to compound 48/80 (0.125  $\mu\text{g/ml}$ ). (B) A bright-field plus fluorescent image of activated mast cells that were treated with 0.125  $\mu\text{g/ml}$  of compound 48/80 and 0.1% DMSO, 200  $\mu\text{g/ml}$  SFRM-B and 0 M desloratadine. The corresponding fluorescent image (pseudo-color presentation) is presented in (C).

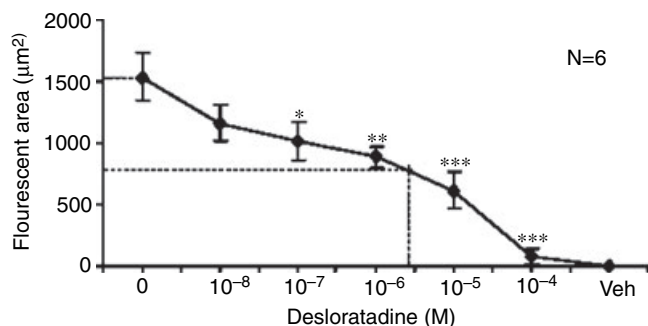


**Figure 3.** Desloratadine concentration-dependently inhibited the degranulation of mast cells exposed to 0.125 µg/ml of compound 48/80. The difference from control (0 M desloratadine) was statistically significant from  $10^{-7}$  M and higher. The  $ED_{50}$  for desloratadine is indicated by the dotted line. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

image of pre-activated mast cells (example shown in Fig. 4A). Because the source of fluorescence was the engulfed SFRM-B stored in the granules of the pre-activated mast cells, the fluorescent image disappeared when the mast cells were re-exposed to compound 48/80. Elimination of fluorescence is seen in three of eight mast cells within seconds of addition of compound 48/80 (Fig. 4B), while 10 s later, three of the five remaining mast cells were also affected (Fig. 4C).

Desloratadine ( $10^{-7}$ – $10^{-5}$  M) reduced significantly the loss of fluorescent images caused by re-exposure to compound 48/80 ( $F_{4,5,20} = 6.17$ ,  $P = 0.002$ , repeated measure analysis of variance; Fig. 5).

## Discussion

This study describes for the first time the use of this vital dye method to assess the impact of an antihistamine on compound 48/80-induced activation and re-activation of mast cells. We have previously validated this model for visualizing and recording changes in the static and dynamic images of the activity-dependent uptake and discharge of SFRM-B by compound 48/80-activated peritoneal mast cells (18). In the current study, we report a number of novel findings regarding the effects of desloratadine on the behaviour of peritoneal mast cells. In the experiments, the number of peritoneal mast cells was homogeneous among different samples, indicating that the estimated 5–6% of mast cells contained in rat peritoneal eluate (20, 21) is evenly distributed within the various dishes and the data collected from different dishes are comparable. In this preparation, peritoneal mast cells respond to a low concentration of compound 48/80, degranulate and remain viable in response to subsequent compound 48/80 activation. In contrast to compound 48/80, the other reagents, including  $10^{-4}$  M desloratadine, produced no mast cell degranulation at the concentrations used in this experiment. Pretreatment with

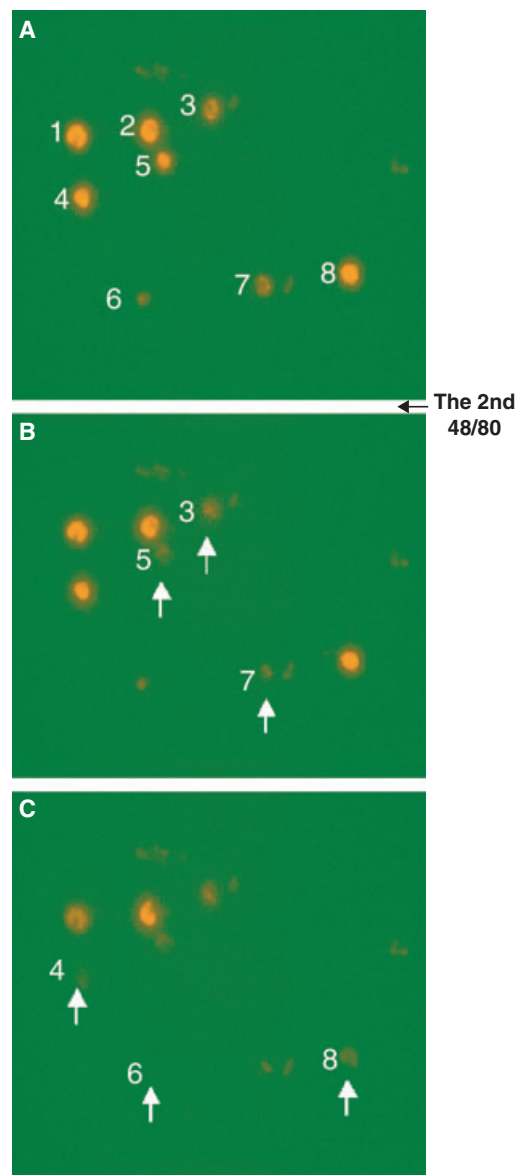


**Figure 4.** Examples of the loss of SFRM-B vital fluorescent dye due to reactivation with compound 48/80. (A) The fluorescent image of eight mast cells pre-exposed to compound 48/80 with SFRM-B. (B) The second compound 48/80 application without SFRM-B in the medium is indicated by an arrow. The fluorescent images of 3/8 mast cells diminished. (C) Later, the fluorescent images of 3/5 of the remaining cells were also eliminated.

desloratadine, across a concentration range of  $10^{-8}$ – $10^{-4}$  M, concentration dependently inhibited compound 48/80-induced activation of peritoneal mast cells by 34–94%. In addition, the present studies suggest that desloratadine treatment across the same concentration range inhibited the re-activation of peritoneal mast cells following re-exposure to compound 48/80. The magnitude of this effect compared with control (no desloratadine) was 83% at a desloratadine concentration of  $10^{-7}$  M.

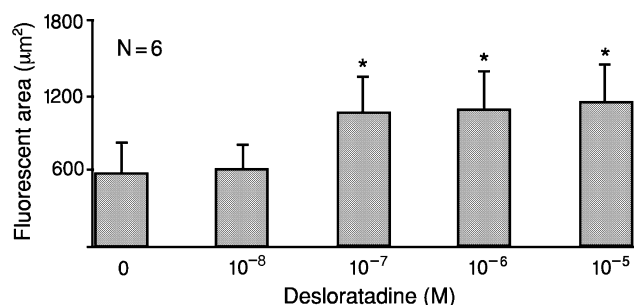


Figure 5. Desloratadine treatment significantly reduced the loss of fluorescent area caused by re-exposure to compound 48/80 induced degranulation. \* $P < 0.05$  vs control dish (0 M desloratadine).

Activation of mast cells can be achieved through immunologic (IgE, FcεRI and specific antibodies) and nonimmunologic (chemical, temperature, physical) stimuli. Compound 48/80 (methoxyphenylethyl-methyl-amine and formaldehyde), one of the most commonly used nonimmunologic chemical secretagogues, was discovered in 1949 when it was found to induce a hypotensive response following intravenous administration at low doses (22). This physiological response was established to involve peripheral histamine release (23), predominantly from mast cell degranulation (24). Compound 48/80 acts by selectively binding to the mast cell membrane (25), and more recently, the target of compound 48/80 has been identified as G<sub>i</sub> and G<sub>o</sub> heterotrimeric G-proteins (6). Mast cell degranulation by compound 48/80 is mediated by intracellular calcium fluxes and activation of protein kinase C and phospholipases C and D (6–8). Future studies using these models will provide useful information on the differences among immunological and nonimmunological stimuli of mast cell degranulation.

Previous studies on mast cell stabilization have measured the effects of stimuli and H<sub>1</sub>-antihistamines on degranulation indirectly by assaying secreted mediator concentrations in the incubation fluid. We felt that a direct visualization method to assess the effects of compound 48/80 on mast cell degranulation would provide relevant information to the status of mast cell activation related to histamine release. First, compound 48/80-induced mast cell degranulation has been shown to lead to histamine release in a compound 48/80-dependent manner (26, 27). Similarly, Slutsky et al. (28) demonstrated that compound 48/80-activated rat peritoneal mast cells have the ability to engulf and entrap molecules from the extracellular fluid, again in a compound 48/80 concentration-dependent manner. Finally, we have previously demonstrated that compound 48/80-activated rat peritoneal mast cells have the ability to engulf the fluorescent vital dye SFRM-B from their surrounding medium and to sequester the dye into granules (18). Activation of mast cells is associated with loss of granule

fluorescence as the mast cell degranulates and releases the dye into the extracellular space. In our experiments, we used rat peritoneal mast cells, which belong to a subgroup of connective tissue mast cells and are widely characterized in the literature. It has been shown previously that rat peritoneal mast cells endocytose vital dyes, such as, acridine orange (29). SFRM-B has been used in vital staining in immunology since the mid-1960's (30). We showed previously that SFRM-B at a concentration of 200 μg/ml had no cytotoxic effect on rat peritoneal mast cells and the afferent terminals of the greater splanchnic nerve (18). The main advantage of SFRM-B over acridine orange is that the fluorescent image of SFRM-B lasts during fluorescence excitation for more than 5 h, whereas the image from acridine orange staining fades within 45–60 s of stimulation.

Mast cells are key effector cells in hypersensitivity, allergic and inflammatory reactions, and are found in connective tissue, and close to epithelial and mucosal interfaces (1, 3, 9). The role of both histamine and the mast cell in chronic allergic inflammation has been re-evaluated recently (3, 9, 10). *In vitro* studies indicate that histamine can modulate Th1/Th2 type immune responses (11, 31) and induce the production of specific patterns of cytokines, chemokines and adhesion molecules, all of which are important for directing the trafficking of eosinophils and other chronic inflammatory cells (9). Mast cells are not simply a repository for preformed mediators, and have been shown to generate cytokines, such as, IL-4, IL-6, IL-13 and TNF-α (32, 33) and also modulate IgE production by B cells (34). Based on the growing evidence regarding the important roles of histamine and mast cells in mediating early and chronic allergic inflammation, research interest has focused on whether mast cell degranulation can be modulated pharmacologically. Early studies of H<sub>1</sub>-antihistamines demonstrated that *in vitro* release of histamine and other mediators from mast cells could be inhibited [for review see ref. (32)]. These original studies were performed at relatively high concentrations and showed that H<sub>1</sub>-antihistamines had heterogeneous effects on mast cell mediator release, with not all compounds demonstrating this so-called mast cell stabilization activity. Desloratadine is a newer, nonsedating H<sub>1</sub>-antihistamine that is used once daily in the treatment of seasonal and perennial allergic rhinitis and chronic idiopathic urticaria (35, 36). Previous *in vitro* studies of desloratadine have shown mast cell stabilizing effects, with reduced histamine and eicosanoid release by mast cells in response to FcεRI stimulation (15, 37). Desloratadine at low concentrations ( $\leq 10^{-7}$  M) has been shown to have significant effects on allergic inflammation *in vitro* (38), and anti-allergic effects in *in vivo* animal studies (39). Lippert et al. (16, 17) showed that low concentrations ( $10^{-9}$  M) of desloratadine reduced allergic inflammatory mediator (IL-3, IL-6, IL-8 and TNF-α) release by HMC-1 mast cells in response to nonIgE mediated stimuli (calcium ionophore

or phorbol myristate acetate). At equimolar concentrations, desloratadine was more effective at blocking cytokine release than cetirizine in this model. However, clinical correlates of these interesting preclinical and animal studies remain to be demonstrated in allergic patients receiving therapeutic doses of desloratadine. Our current data indicate that desloratadine exerts a concentration-related inhibitory action on mast cell degranulation induced by compound 48/80, while having no prodegranulating action itself. Furthermore, using this vital dye fluorescent method, it was possible to demonstrate that mast cells remained intact after initial exposure to compound 48/80, and that desloratadine inhibited degranulation of these activated mast cells following a

second exposure to compound 48/80. These *in vitro* findings may have biological relevance. As mast cells remain viable after IgE-mediated stimulation by allergen, and can continue to release mediators following repeated allergen exposure, relevant mast cell stabilizing effects should remain apparent after multiple activations of the mast cell degranulation process.

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