Antihistamines, nasal decongestants, mast cell stabilizers, and immunotherapy (allergy shots) represent the current remedies available for the one-in-three Americans who suffer from allergies. Allergies, which are caused by the release of histamine through the degranulation of mast cells, can manifest into a life-threatening condition called anaphylaxis, triggering a severe, whole-body allergic reaction.

Antihistamines work as H1 histamine receptor antagonists, blocking the primary receptors involved with allergic symptoms. Nasal decongestants reduce nasal congestion by vasoconstriction. However, these cause several adverse effects to the nervous system and should be avoided in hypertensive patients. Mast cell stabilizers block calcium channels inside mast cells responsible for releasing histamine [1], but take two to six weeks to take effect. Allergy shots will familiarize the body to an allergen, but the cost and duration of treatments are taxing [2].

With these drawbacks in mind, this study investigated curcumin, a potent anti-inflammatory, and common golden spice found in many Asian cuisines, as a possible treatment for allergy therapy [3,4]. In this study, rat basophilic leukemia (RBL-2H3) cells were treated with either allergen, anti-rat immunoglobulin E (IgE) which stimulates degranulation (the release of histamine), or both curcumin and IgE. Degranulation is, in part, caused by the translocation of polymerized microtubules in a calcium-independent fashion and F-actin ring disassembly in a calcium-dependent fashion. Fluorescent and electron microscopy techniques were used to determine the mechanism by which curcumin inhibits degranulation and the release of histamine.

Materials & Methods

Fluorescent Microscopy and Assays

Degranulation-Stimulated RBL-2H3 cells were stimulated to degranulate using Anti-Rat IgE (MAB-1) bovine monoclonal antibodies. Cells were given 1 µg/mL of a 1:10 dilution of IgE antibody in PBS, which had no statistically significant effect on cell viability (data not shown). These monoclonal antibodies were used to bind to the FcεRI receptors on the cells to stimulate degranulation. Cells were given Anti-Rat IgE 30-45 minutes prior to any assay.

Staining of Microtubules. Aliquots of cells suspended in media were placed on chamber slides and incubated at 37 °C (5% CO2). After 24 hours, the cells were either exposed to 1 µL of ethidium (soxicient control), 1 µg/mL curcumin, 1 µg/mL of 1:3 anti-actin IgG, or both 1 µg/mL curcumin and 1 µg/mL IgG. The cells were then incubated for an additional 24 hours. Cells were stained using 1 µL labeled Pacifico Oregon Green® 488 conjugate in PBS (0.2 mM PHEs Buffer, 2 mM EDTA, and MgCl2). The cells were incubated for 30 minutes and washed 3 times with 2% Bovine serum albumin in PBS at room temperature. Cells were imaged with a Nikon Eclipse IX81 fluorescence microscope using FITC fiber cube.

Staining of F-actin rings. Aliquots of cells suspended in media were placed on chamber slides and incubated at 37 °C with 5% CO2. After 24 hours, the cells were either exposed to 1 µL of ethidium (soxicient control), 1 µg/mL curcumin, 1 µg/mL of 1:3 anti-actin IgG, or both 1 µg/mL curcumin and 1 µg/mL IgG. The cells were then incubated for an additional 24 hours. Cells were stained with Alexa Fluor® 488 phalloidin using the manufacturer’s protocol (nuclease) and imaged with the FITC fiber cube.

Electron Microscopy

After being stimulated for degranulation (see above), note control ethidium treated cells were substituted with untreated cells), the cells were prepared for imaging by transmission electron microscopy (TEM). Cells were fixed with 4% glutaraldehyde/2% formaldehyde in 0.2M sodium cacodylate buffer, pH 7.4, stained with the wash with Tiscon and gently spun down to form a pellet. The pellet was post-fixed in 2% osmium tetroxide, dehydrated in a graded series of acetone, and infiltrated (1:1) Epon/1,2,3 Epon resin. 100nm sections were collected onto 200 mesh copper grids and post-stained with 4% uranyl acetate and 0.5% lead citrate.

Results & Discussion

ELISA demonstrated that treating RBL-2H3 cells with curcumin (1 µg/mL) and stimulating degranulation significantly decreased the release of histamine as compared to control, control cells (data not presented here). Fluorescent microscopy was used to analyze the effect of curcumin on microtubule structure and F-actin ring disassembly to determine which mechanism of degranulation-curcumin acts on.

In degranulating cells (treated with IgE), the microtubules were located around the periphery due to the translocation of vesicles to the outer membrane for the release of histamine (Figure 1A). When degranulating cells were treated with curcumin, bright microtubule aggregates formed at the center of the cells (Figure 1B). Curcumin caused a significant increase in microtubule aggregates as compared to controls and cells treated with IgE (Figure 1C). These findings suggest that curcumin inhibits the microtubule dynamics necessary for degranulation, causing microtubules to congregate instead of translocating the granules to the periphery of the cells. F-actin rings in degranulating cells treated with IgE and curcumin were defined as in cells treated with only IgE (Figure 1D). Curcumin significantly increased the disassembly of F-actin rings (Figure 2) which should result in an allergic response (increased release of histamine). However, the compromised microtubule caused by curcumin treatment (as seen in Figure 3) prevented translocation of the granules and thus inhibited the release of histamine.

To discern any morphological alterations caused by curcumin treatment, cells were imaged by transmission electron microscopy (TEM). Untreated, control cells and cells treated with curcumin both appeared compact and well defined, indicating that curcumin treatment was not degrading the cells (Figure 5A &B). However, in cells treated with IgE, the cell membranes appeared discontinuous, most likely because of the large amount of exocytosis occurring due to degranulation (Figure 5C). The cells treated with both IgE and curcumin had morphologies more similar to the control than the IgE treated cells (Figure 5D). The TEM micrographs show that curcumin inhibits the release of histamine in these cells by the presence of multiple granules and the lack of apparent exocytosis.

The data presented in this study show that curcumin could prove to be useful as an allergic medication, and that microtubules may be a novel intracellular target for anti-allergic medications. Future experiments would involve immunological labeling of curcumin and histamine for TEM visualization to more accurately determine the location of curcumin within the cell.

References


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