Dymista Proposal

Rationale: Dymista provides superior clinical efficacy to both fluticasone propionate and azelastine hydrochloride in the treatment of seasonal allergic rhinitis. The superiority of efficacy not only occurs at the initiation of treatment, but persists for its duration. The mechanism underlying the superior efficacy of Dymista is not known. One can postulate many potential interactions between the components of Dymista overall as well as different effects of these interactions occurring during the course of treatment. This proposal focuses on examining the effects of Dymista on the dynamics of the allergic response in man using nasal provocation with antigen. We will study the relationship between symptoms, physiology, cells and mediators.

Hypothesis: We hypothesize that Dymista affects multiple phases of the allergic response, which in sum are greater than the effects of fluticasone propionate or azelastine hydrochloride alone.

Specific aims/objective: Our objectives for this study are to demonstrate:

1. that the induction of allergic inflammation by nasal provocation with antigen causes a cellular infiltration, with subsequent release of inflammatory biomarkers that cause augmented responses to subsequent exposure to antigens.
2. that fluticasone prevents allergic inflammation from developing after antigen challenge and subsequently prevents the augmentation of the nasal response to nasal challenge with antigen.
3. that the azelastine in Dymista reduces the effects of released histamine

Methods (including study design): We propose to perform a 3-way, randomized, placebo-controlled, and crossover trial. We will recruit 20 asymptomatic seasonal allergic rhinitis patients outside of the relevant season. The subjects will receive placebo, fluticasone propionate and Dymista. The nasal provocations will be separated by 2 weeks. Treatment will begin 15 minutes before nasal provocation with ragweed or grass antigen and the treatment will continue twice a day for 3 days. Nasal provocation will occur daily for three days to evaluate for priming (increased sensitization with repeated antigen exposure, which mimics seasonal disease where antigen exposure occurs in the setting of continued allergic inflammation). For outcome measures, we will monitor both nasal symptoms after nasal provocation as well as collect nasal lavage to evaluate effects on eosinophils and biomarkers of the immune response. In the nasal lavage, we will quantify the number of eosinophils (a marker of cellular recruitment) and measure the levels of histamine (a marker of basophil and mast cell activation), tryptase (a marker of mast cell activation), albumin (a marker of vascular permeability), lactoferrin (a marker of glandular activation) and ECP (a marker of eosinophil activation). Thus we expect to generate information on both clinical effects and physiologic differences between the treatments.

We will recruit normal healthy volunteers with grass and/or ragweed allergic rhinitis. Based on previous experience, we anticipate screening about 55 subjects to have 20 complete this study. After preliminary telephone contact, subjects will come to the nasal physiology laboratory for a screening visit. At this visit, after signing consent, they will answer a nasal questionnaire, and undergo skin testing and nasal challenge with antigen. Depending on skin test results, eligible subjects will then undergo nasal challenge with either grass or ragweed allergen outside their allergy season. Female subjects will be asked to take a urine pregnancy test prior to enrollment. Twenty subjects with a positive response, defined as 2 or more sneezes and an increase in symptom score after allergen challenge, will be recruited.

Inclusion Criteria

1. Males and females between 18 and 55 years of age.
2. History of grass and/or ragweed allergic rhinitis.
3. Positive skin test to grass and/or ragweed antigen.
4. Positive response to screening nasal challenge.

5. Off all anti-allergic medications for a minimum of 2 weeks.

Exclusion Criteria

1. Physical signs or symptoms suggestive of renal, hepatic or cardiovascular disease.
2. Pregnant or lactating women.
3. Upper respiratory infection within 14 days of study start.

Symptom Scores: Symptom scores are recorded by subjects after each nasal challenge. Subjects report four symptoms: sneezing, rhinorrhea, nasal congestion, and nasal itching on a scale from 0 to 3 (0= no symptoms, 1= mild symptoms, 2= moderate symptoms, 3= severe symptoms). They will also count their sneezes and record them after each challenge.

Subjects will come to the nasal physiology laboratory for antigen challenge after 2 weeks of washout. On the day of experiment, the subjects will present to the laboratory and be allowed to rest for 15 minutes so that equilibration of the nasal mucosa with the environmental condition of the laboratory is achieved. Symptom score evaluation will be performed first as a baseline. Then, 4 nasal lavages are performed to bring mediator levels to a stable baseline and combined for measurement of eosinophils. After that, another nasal lavage (B1) is performed and kept as a baseline. A nasal lavage involves instilling 2.5 ml of warmed (37°C) lactated Ringer's solution into each nostril and, after 10 seconds, the secretions are expelled into a plastic collection basin. The returned volume ranges between 75%-85% of that instilled. All samples are vigorously shaken to homogenize the mixture of sol and gel phase and are stored on ice in plastic tubes until the experiment is done. Next, oxymetazoline is applied topically to the nasal mucosa to prevent mucosal congestion that interferes with lavage. Prior experiments have shown that oxymetazoline does not interfere with sneezing or histamine release. After a 10-minute wait, the subjects will receive their first treatment. Ten minutes later, a control challenge with the diluent used for the allergen extract (4% phenol buffer saline) will be performed by spray method. Ten minutes later, symptom score evaluation and nasal lavage are repeated. Then, antigen challenge with two increasing doses of antigen (The concentrations used for ragweed will be 1:200 w/v, and 1:66 w/v and for grass 3333, and 6666 bioequivalent allergy unit BAU/mL). The total amount delivered per challenge will be 2 puffs (0.18 mL), 12 minutes apart, will be performed by the same method. Ten minutes after each challenge, symptom score evaluation and nasal lavage are repeated in the same way as after diluent challenge.

After the lavage that follows the last antigen challenge, the subjects will be discharged from the laboratory and instructed to return the following day. They will also be instructed to use their spray at night. When they arrive the next day, a symptom score evaluation and a lavage will be performed to evaluate for the late phase response. They will receive the morning dose of the medication during the nasal challenge as on the first day of the nasal challenge. This will be followed by a second challenge as on day 1. The same will be repeated on the third day while the subjects are still receiving study medication. The baseline lavage will be evaluated for eosinophils and the level of ECP, and all subsequent lavages are assayed for histamine, tryptase, albumin, and lactoferrin.

The total number of cells in nasal lavage fluid will be counted using a hemocytometer. The lavage is then placed on ice until centrifugation at 5,000 rpm for 15 minutes at 4°C. The cell pellet is resuspended in an adequate volume of buffer and cytospun onto one or more slides. The cells will then be dried, fixed and stained with Diff-Quick® Stain to allow enumeration of different types of cells. The percentage of eosinophils is counted and the total number of eosinophils is calculated by multiplying that percentage by the total cell count. Two hundred cells will be counted. If the number of countable cells is more than 50, but there are no eosinophils, the total number of eosinophils will be assigned a number equivalent to the lowest number of eosinophils obtained from counting an acceptable and technically adequate slide. This

number usually varies between 25-50 total eosinophils. The supernatant is stored at - 80°C until assayed for albumin, eosinophil cationic protein (ECP), histamine, tryptase and lactoferrin.

Levels of human serum albumin (HSA) will be assayed in nasal lavage fluid to evaluate plasma leakage. HSA is measured by an enzyme-linked immunosorbent assay (ELISA) sensitive to 1 ng/ml of albumin. Levels of histamine and tryptase will also be assayed in nasal lavage fluid to evaluate mast cell activation. Histamine is determined using a radioimmunoassay sensitive to 20 ng/ml. Tryptase is measured by fluoroenzyme immunoassay sensitive to 1 ng/ ml. ECP will be assayed using radioimmunoassay sensitivity to 2 µg/ml to indicate eosinophil activation. An ELISA sensitive to 0.73 ng/ml will measure lactoferrin.

We anticipate that in the placebo arm symptoms will increase each day in response to the nasal provocation. Similarly, the levels of histamine, albumin, ECP and lactoferrin, and the number of eosinophils will increase on the second and third day of challenge. The level of tryptase should remain constant. At a minimum, the azelastine hydrochloride in Dymista should reduce symptoms and the levels of albumin and lactoferrin on all days of nasal challenge. The model will also allow us to determine if azelastine operates in addition to its role as an antihistamine by reducing mast cell activation. Furthermore we will evaluate whether the cellular influx caused by the nasal provocations is reduced more by Dymista compared to fluticasone. We anticipate that fluticasone propionate will have no effect on the first day of challenge, but will prevent priming, eosinophil influx, and the increases in level of biomarkers associated with priming on subsequent days. We do not anticipate that 2 days of fluticasone will affect mast cell activation. Lastly, we anticipate, at a minimum, that Dymista will reduce symptoms beginning on day 1 of nasal challenge and cellular influx and its consequences on days 2 and 3 of challenge.

Stats: The primary endpoint will be the daily change in albumin levels at visit 1. To determine the sample size, we made some assumptions. Based on a study of patients receiving 60 mg fexofenadine the mean albumin changed from 294 to 64 after treatment. We assumed azelastine would reduce albumin by 75% with a standard deviation of 300. Using a significance level α=0.05, then 20 completed subjects per treatment group are needed for at least 80% power to detect a difference from placebo and azelastine during the acute reaction. The same size sample would show a reduction for Dymista on all visits and the eosinophil influx compared to placebo. The sample size is probably insufficient to show a significant reduction of eosinophils after Dymista compared to fluticasone, though a trend could be noted.

All statistical analyses will test the null hypothesis of no treatment difference between active treatment and placebo versus the alternative hypothesis that there is a difference between treatments. All statistical tests will be two-sided and will be conducted at a significance level of α=0.05.

Secondary efficacy measures include the mean changes from baseline in sneezes, total nasal symptom scores, number of eosinophils and the levels of ECP, lactoferrin, tryptase and histamine.

Schedule of event: see attached

Detailed study budget: see attached

We would need a 3 day supply of fluticasone propionate, Dymista and placebo for each of 20 subjects.

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