CHAPTER 2
METERIALS AND METHODS

2.1 STUDY DESIGN

The study was a randomized, open-label and parallel group study. One hundred forty patients considered eligible for the study were recruited and randomized into 2 groups receiving either MF or FF for 4 weeks.

2.2 PATIENT SELECTION

2.2.1 Number of patients

The number of patients required was calculated based on the following assumption.

a) The percentage of responders in each group was the primary efficacy outcome.

b) The responder was characterized by exhibiting reduced total nasal symptom scores (TNSSs) by 50% after treatment.

c) Responder rate was estimated to be 80% in each group. The efficacy of both treatments was assumed comparable if the difference in response rate between the 2 groups is not more than 15%

d) $\alpha = 0.1$ and $\beta = 0.20$

The number of patients required was calculated based on the following formula:

$$N = \frac{(Z\alpha\sqrt{2PQ} + Z\beta\sqrt{PcQc+PtQt})^2}{(|Pt-Pc| - \delta)^2}$$

δ = responder rate difference between 2 groups (%)

$P = (Pc+\lambda Pt) / (1+\lambda)$; $\lambda = 1$ (if both of N in 2 groups is equal)

$Q = 1- P$

$Qc = 1-Pc$

Qt = 1-Pt

Pc = responder rate in group 1 (MF)

Pt = responder rate in group 2 (FF)

e) Drop-out rate of 10%

According to these assumptions, the number of patients needed was 64 per treatment group. With a projected drop-out rate of 10%, 6 patients were added to each group and 70 patients per treatment group were needed.

2.2.2 Inclusion criteria

1. Male and female outpatients at Maharaj Nakohn Chiang Mai Hospital, 18 - 60 years of age.
2. Patients with a minimum of a 6-month history of PER according to ARIA guideline.
3. Patients with positive skin prick test (SPT) response to 1 or more allergen.
4. Patients must have nasal symptoms of TNSSs $\geq$ 6 with or without total ocular symptom scores (TOSSs) of $\geq$ 4 at baseline in the 1 previous week.
5. All patients must be explained and understood the detail of the research and gave their written informed consent before entering the study.

2.2.3 Exclusion criteria

1. Patients with a history of hypersensitivity to corticosteroids, rhinitis medicamentosa, asthma and sinusitis.
2. Patients who used antibiotics and systemic or INCs within 4 weeks, used immunotherapy during the preceding 1 year before entering the study.
3. Patients who had acute or chronic upper respiratory infections within the last 4 weeks before the study.
4. Patients with structural abnormalities of the nose such as nasal septum deviation more than 50% and nasal polyp.
5. Patients who had nasal surgery within the last 4 weeks.

6. Patients who had kidney or liver disease, hypertension, diabetes mellitus, glaucoma or severe chronic illness.

7. Pregnant or nursing women

2.2.4 Withdrawal criteria

1. Subject who experienced serious adverse drug reactions during the study.

2. Subject who could not comply with the study protocol or voluntarily withdrew from the study.

3. Subject who required other medication during the study period that possibly interfering with the evaluation of the AR symptoms.

4. Subject who experienced infection that interfering with the evaluation of the efficacy outcome.

5. Subject who used rescue treatment such as pseudoephedrine hydrochloride (60 mg/tablet) more than 3 tablets per day, or normal saline irrigation more than 3 times per day during the first week of the study period.

2.3 TREATMENT PROCEDURES

Drugs and chemicals

1. MF (Nasonax^{TM}, 50 µg/spray) was purchased from Merck Thailand.

2. FF (Avamys^{TM}, 27.5 µg/spray) was purchased from Glaxosmithkline Pharmaceuticals Limited.

3. Pseudoephedrine hydrochloride (60 mg/tablet) was purchased from Maharaj Makhon Chiang Mai Hospital.

4. Normal saline irrigate (1000 mL) was purchased from General Hospital Product Public Co., Ltd.

5. Histamine base 1 mg/mL, allergenic extract of standardized mite *Dermatophagoides farinae* (10,000 AU/mL), *D. pteronyssinus* (10,000 AU/mL), house dust (10,000 PNU/mL) and American cockroach (1:20 W/V) were purchased from Allertechcompany, produced by ALK Abello Company.

2.4 PROTOCOL OUTLINE

All patients entered a 1-week run-in period without any medication. Then patients were randomized into 2 groups to receive once daily MF 200 µg or FF 110 µg, 2 sprays per nostril in the morning for 4 weeks and were instructed to record 24-h reflective symptom evaluations during 1-week run-in period and throughout the study. Randomization was performed according to computer-generated schedule as described in www.randomization.com. Compliance regarding the use of all study medications was monitored throughout the study by examination of the content remained in spray bottles and by a thorough review of the daily card recording.

In addition to study medications, patients were provided with pseudoephedrine hydrochloride 60 mg tablet (one tablet as needed) and normal saline (NSS) for nasal wash (as needed except 24 h before the visit) as rescue treatments if nasal symptoms become intolerable. Other medications that possibly interfering with the evaluation of the AR symptoms were not allowed during the study.

Patients were required to visit the study center 3 times throughout the study: at baseline and after week 2 and week 4 of treatment.

Safety was assessed at each visit by monitoring adverse events (AEs) and by nasal examination. Vital signs (pulse and blood pressure) were recorded at each visit (Figure 4).

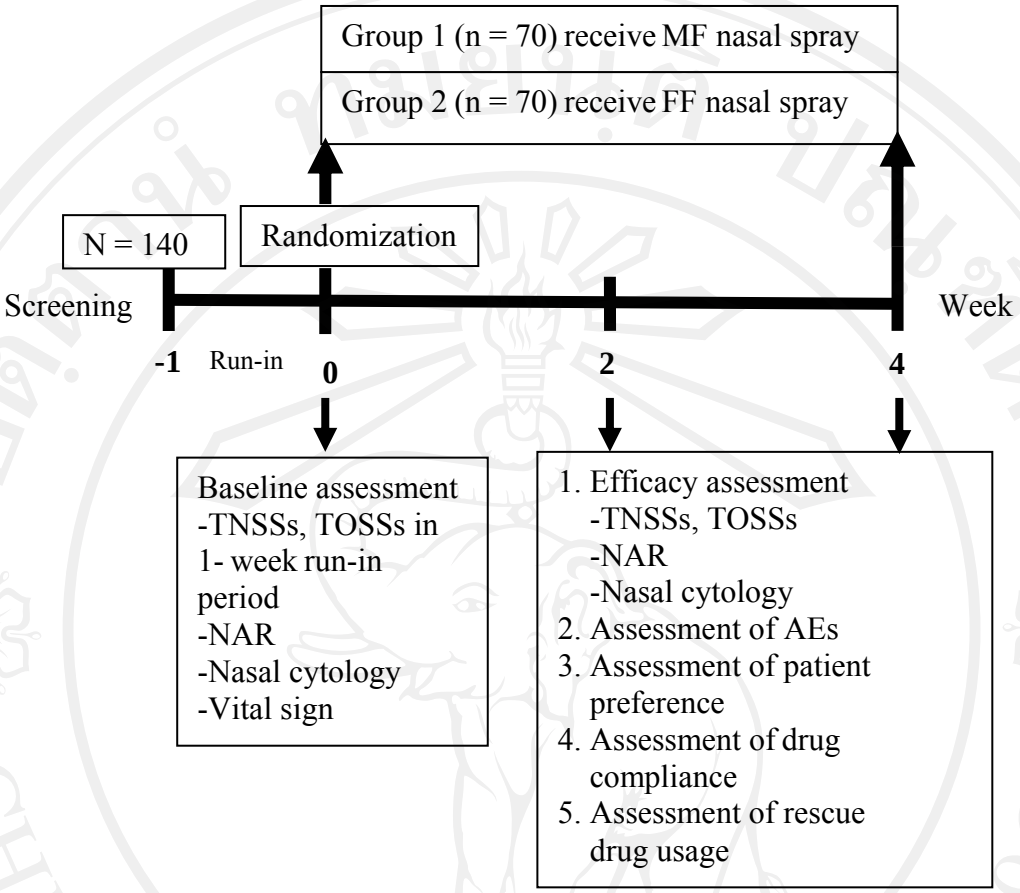

Figure 4 Diagram illustrates the protocol procedure of the study.

Run-in period: Screening

A nasal examination was performed at otolaryngology outpatient clinic. Subsequently, patients were given a skin prick test with a panel of 4 inhalant allergens: house dust mites (*D. farinae* and *D. pteronyssinus*), house dust and American cockroach. Histamine solution in distilled water (10 mg/mL) was used as positive control. Skin tests were performed on the forearm using 1 mm prick lancets. The skin reactions usually occurred within 15 min after which the results could be assessed. Patients were considered allergic if at least one allergen caused a wheal diameter equal to or greater than 3 mm.

Qualified patients were given a daily diary card to record symptom severity upon awakening in the morning 1-week before the study for baseline TNSSs and TOSSs.

TNSSs of 4 nasal symptoms (i.e., rhinorrhea, itching, sneezing and nasal congestion) and TOSSs of 3 symptoms (i.e., eye itching, eye tearing and eye redness) were recorded according to the following 4-point scale

0 = no symptom

1 = mild symptoms (present but not troublesome)

2 = moderate symptoms (frequently troublesome, but not sufficient to interfere with normal daily activity or night-time sleep)

3 = severe symptoms (sufficiently troublesome to interfere with normal daily activity or night-time sleep)

TNSSs and TOSSs were the sum of each individual symptom.

Visit 1: Randomization and evaluation at base line

Patients eligible to enroll in the study were randomized into two groups to receive either MF or FF. Then evaluation at base line was performed.

1. Vital signs (pulse and blood pressure)
2. TNSSs and TOSSs 1-week before the study from daily diary card recording.
3. NAR was measured by active anterior RMM technique.
4. Nasal mucosal specimens were obtained by scraping the middle one-third of interior turbinate with rhino probes and then the specimens were evaluated by nasal cytology.

Every patient was given a new diary card to record symptom severity daily upon awakening, which reflects the previous 24 h. Then these data were calculated for the determination of the onset of action of the study drugs. Time to onset of action was defined as the time to attain a statistically significant difference relative to base line.

Visit 2 and 3: Follow-up

After 2 and 4 weeks of treatment, patients returned to the study center for

1. Vital signs (pulse and blood pressure) examination
2. TNSSs and TOSSs measurement
3. NAR measurement

4. Nasal cytology examination
5. Assessment of AEs
6. Assessment of patient preference of test drugs such as scent, taste, and ease of use.
7. Assessment of drug compliance
8. Assessment of rescue drug usage

Anterior RMM

NAR was measured bilaterally by anterior RMM using the ATMOS rhinomanometer 300 (Lenzkirch, Germany) (Figure 5). The researcher informed the patient briefly about the procedure. Every patient was asked to blow his/her nose before starting the test. Measurements were made with the patient sitting upright in a comfortable chair (Figure 6) after resting for at least 30 min. One nasal cavity was occluded while the cavity whose resistance to be measured was left opened for flow measurements to be carried out. A nasal adapter or nozzle of suitable size for each patient was inserted just inside the nostril. A tight-fitting without blocking facemask was pressed onto the face to measure the flow. A small bore silastic tube for measuring choanal pressure was connected to an electromechamical pressure transducer, passed through the nasal adaptor to just inside the external nares to measured pressure difference and flow. The patient had to breathe in and out with the mouth closed. The subject was asked to breathe quietly through the mask for 16 sec or 3 cycles continuously. The left nostril was always assessed first and followed by the right. Nasal airflow was reported as the sum of the recorded airflow through the right and left nostrils at a transnasal pressure of 75 and 150 Pa. This measurement was performed in air-conditioned room at the Clinical Pharmacology Unit of the Department of Pharmacology, Faculty of Medicine, Chiang Mai University.

Figure 5 ATMOS rhinomanometer PC300.

Figure 6 Position of patient for active anterior RMM.

Nasal cytology examination

Nasal mucosal specimen was obtained by scraping 2-3 mm of mucosal surface of the mid-third inferior portion of inferior turbinate with disposable plastic scoop (Rhinoprobe, Allertech Corp., Thailand). The procedure was repeated 2-3 times. The specimen was spreaded onto a plain slide and was fixed with 95% alcohol fixative, stained with modified Wright-Giemsa stain for 3 min and phosphate buffer for 5 min. The slide was drained of excess fluid between procedures. After air drying, the cytogram was examined under light microscope, using x1000 magnification (oil-immersion lens). Twenty random fields were analyzed and the total number of cells in each microscopic field was counted. Cells count categorized as eosinophils, basophils, neutrophils, macrophages, lymphocytes and epithelium cells. The individual inflammatory cell per total cells counting of nasal specimen was used in the statistical analysis.

2.5 EFFICACY ASSESSMENT

Clinical efficacy of treatment was estimated at the end of study (week 4) that consisted of

1. TNSSs of rhinorrhea, itching, sneezing and nasal congestion
2. TOSSs of eye itching, eye tearing and eye redness.
3. The mean of unilateral and total NAR
4. The percentage of inflammatory cells: eosinophils, basophils, neutrophils, macrophages, and lymphocytes.
5. The number of responders who exhibiting reduced TNSSs by 50%, 75% and 100% from baseline.

2.6 SAFETY ASSESSMENT

Non-directive questioning for AEs was performed at each visit for safety assessment during the study. Nasal examination was evaluated for clinical evidence of candidiasis and vital signs were recorded at each visit. Patients were asked to report to the researcher whenever serious AEs arase.

2.7 ASSESSMENT OF PATIENT PREFERENCE OF TEST DRUGS

Patient-rated preference of test drugs was assessed on 9 questionnaires using a 5-point Likert scale (5: very satisfied/very strong, 4: satisfied/strong, 3: neither satisfied nor dissatisfied/neither strong nor weak, 2: dissatisfied/weak, or 1: very dissatisfied/very weak) for rating of odor, taste and ease of use. The questions of assessment of patient preference were listed in Table 1.

Table 1 Assessment of patient preference questionnaire items

Question	Rating scores
1. Did this product have any odor?	very satisfied (5) - very dissatisfied (1)
2. If this product has an odor, are you satisfied with the odor of the product?	very satisfied (5) - very dissatisfied (1)
3. Did this product have any taste?	very strong (5)-very weak (1)
4. If this product has a taste, are you satisfied with the taste of the product?	very strong (5)-very weak (1)
5. Did you feel this product is comfortable and easy to use?	very strong (5)-very weak (1)
6. Did medicine run out of your noses?	very strong (5)-very weak (1)
7. Did you feel the nose tip of product is invasive and cause of pain in your nostrils?	very strong (5)-very weak (1)
8. Overall, did this product improve your symptoms?	very strong (5)-very weak (1)
9. Overall, how satisfied are you with this product?	very satisfied (5) - very dissatisfied (1)

2.8 STATISTICAL ANALYSIS

Within Treatment

Changes in TNSSs, NAR, and percentage of inflammatory cells from base line within each treatment were analyzed using an analysis of variances (ANOVA) with repeated measurement and a post hoc Bonferroni analysis was used to identify differences between time points. A non-parametric Wilcoxon's signed-rank test was used to compare TOSSs between base-line and the following weeks.

Between Treatment

Unpaired *t*-test was used to determine differences in TNSSs, NAR, percentage of inflammatory cells, and the mean of individual preference ratings between the two treatments. TOSSs between treatment groups were compared using the Mann-Whitney U test. Differences among the treatment groups in the number of patients considered being responders, the percentage of patient preference ratings, the use of rescue treatment and incidences of AEs were evaluated by chi-square test.

The statistical software use to process the data was SPSS 16.0. All comparisons were performed as two-sided tests, *p* values of less than 0.05 were considered significant.