Research Report: Therapy of Nasal Cavity Congestion

Study Title: Acoustic Rhinometry and Pathologic Analysis on Ovalbumin-Sensitized Guinea Pigs Treated with Calcium Glycerophosphate

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1.0 ABSTRACT

The goal of this five day study was to determine whether twice daily dosing of intranasal calcium glycerophosphate improves nasal congestion as measured by acoustic rhinometry, and to determine any histopathologic effects to brain, lungs and/or any organs or cells. Adult guinea pigs were sensitized by repeated exposure to chicken ovalbumin, then challenged on day 1 of the study to induce acute, allergic rhinitis. On each of days 1-5, human-dose equivalents were given twice daily of either calcium glycerophosphate, known OTC anti-rhinitis drug, or placebo. The largest congestion-associated effect with calcium glycerophosphate treatment occurred on day 1 with significant improvement of nasal cavity volume over baseline ($p<0.05$). Days 2-5 showed definite trends toward improvement in nasal cavity volume in a dose-dependent manner that did not reach statistical significance ($p>0.05$). Exceptions to the trending observation existed on day 2 where the 1.875% CGP group (teal) performed as well as the 7.5% CGP group (green), on day 3 where the 1.875% and 3.75% CGP groups (teal and violet, respectively) outperformed the 7.5% CGP group (green), and on day 5 where the 3.75% CGP group (violet) outperformed the 7.5% CGP group (green). The 7.5% CGP group (green) trend either equaled or outperformed the known OTC anti-rhinitis drug on 4 of the 5 study days. Treatment with calcium glycerophosphate was well tolerated by the animals and no visible adverse clinical signs were observed including very low nose rub and sneeze counts during post-dosing observation. Blood counts and serum clinical chemistries showed only minor changes due to sensitization, and elevated serum IgE confirmed all treated animals had acute rhinitis. Tests of nasal lavage fluid including cell counts and inflammatory mediators showed no adverse effects of treatments. Tissue histopathologic analysis showed lesions confined solely to the nasal mucosa and were associated with experimental rhinitis. These representative lesions were not considered to be related to the test articles. Overall, the responses using calcium glycerophosphate treatment demonstrated improved nasal congestion in the guinea pig model of acute, allergic rhinitis and treatments were well tolerated by the animals.

2.0 TEXAS TECH UNIVERSITY IACUC APPROVAL

The study protocol was submitted by Dr. Brackee for review at the April 19, 2011 meeting of the TTUHSC institutional animal care and use committee (IACUC). The protocol was then amended to include six additional animals, change the saline control group to sterile water control group, change the intranasal dosing from once daily to twice daily, change the vehicle of the ovalbumin solution from saline to sterile water, and to add brain slice to the tissues harvested at necropsy.

All procedures in this study were approved by the Texas Tech University HSC IACUC on May 13, 2011 (Appendix 1).

3.0 KEY STUDY PERSONNEL

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4.0 TEST ARTICLE

Identity: calcium glycerophosphate

Supplier: AKPharma, Inc.

Formulation: Purple/Violet - #051211A – CGP Nasal Spray Formulation (3.75% CGP)
Aqua Blue (Teal) - #051211B - CGP Nasal Spray Formulation (1.875% CGP)
Green - #051211C – CGP Nasal Spray Formulation (7.5% CGP)
Red - #051211D – water/placebo
Yellow - #051211E – Afrin OTC

5.0 TEST SYSTEM

Three to four week old, female, guinea pigs (300-350 grams body weight, approximately 4 weeks old) were acquired (Charles River, Hartley Guinea Pig, Strain Code 051) and acclimated for 4 days prior to study.

Number of guinea pigs at study start:

The number of guinea pigs were calculated by testing 6 guinea pigs per group in each of 6 groups (6x6=36).

Summary of the groups (n=6) are:

1. Non-sensitized, non-treated (Grp 1)
2. Sensitized, water treated (group Red)
3. Known anti-rhinitis drug (Afrin) treated (group Yellow)
4. CGP article nasal droplet (1.875%) treated (group Teal)
5. CGP article nasal droplet (3.75%) treated (group Violet)
6. CGP article nasal droplet (7.5%) treated (group Green)

Body weight (grams) were taken at study start, and group average body weight was calculated.

6.0 ANIMAL HUSBANDRY

Housing: USDA-approved, dedicated guinea pig housing (Allentown, Inc.) providing 618 sq. inches floor space for each cages. Up to three guinea pigs were housed per cage.

Acclimation Period: 4 days prior to start of sensitization protocol

Feed: 5P18 Purina Prolab Guinea Pig Diet (LabDiet, St. Louis, Mo), provided ad libitum.

Water: House RO water with ultraviolet light polishing. Unlimited access provided by 450 ml standard water bottles.

Water is analyzed annually by the City of Lubbock Water Department for heavy metals, chlorinated hydrocarbons, organophosphates, nitrates, nitrites, coliforms, total trihalomethanes, and dissolved minerals (hardness).

Water provided to animals is tested by the LARC using the Charms Watergiene swabs. A reading of zero (ATP count count measured in reflected light units, RLU) is required for passing.

The Study Director reviewed the water analysis documentation.

Environmental Conditions:

The targeted indoor conditions for temperature and photoperiod were as follows:
Temperature: 18–29 °C
Relative Humidity: 30-70%
Light Cycle: 12-h

No excursions.

Morbidity and Mortality:

Animals were observed at least twice daily for any adverse health conditions. Prior to the onset of the study, one guinea pig was dropped by the animal care technician during routine handling and immediately died. During sensitization treatment with 1% Ovalbumin in sterile water, the 20 microliter nasal instillation caused the guinea pig to become hypoxic and died within 2 minutes. The clinical veterinarian attended this animal, however was unsuccessfully resuscitated. None of the other animals exhibited any adverse health conditions.

Health Status:

The clinical veterinarian visually examined the animals before assignment to study and during the entire study period. All animals were visually healthy prior to the study and during the study period.

7.0 EXPERIMENTAL DESIGN

7.1 Allergen Sensitization Protocol

On day 0 and day 7 of the study each guinea pig from groups 2-6 (x30) was briefly anesthetized with Isoflurane inhalant and have AR measurement of the nasal cavity and 10 minutes delivery of 1% Ovalbumin (A-5503, Grade V, Lot #73H7020, Sigma, Inc) in sterile water vehicle (Lot #091105A3, Vedco Inc.) via facemask nebulizer (VixOne Nebulizer, Invacare, Elyria, OH). During the nebulizer treatment guinea pigs were allowed to recover from anesthesia.

On days 14, 15, 16, and 21 of the study each guinea pig from groups 2-6 (x30) had AR measurement of the nasal cavity, then briefly anesthetized with Isoflurane inhalant and 20 microliter delivery of 1% Ovalbumin in sterile water vehicle via pipette installation in each nostril. Guinea pigs were held upright to allow the liquid installation to permeate the entire nasal cavity while they are recovering from anesthesia (approximately 1-2 minutes).

7.2 Allergen Challenge and 5-day Study Protocol

7.2.1 TREATMENT DAY ONE

On Day 1 of the study (4 days after sensitization protocol ends) each guinea pig from groups 1-6 (x 36) were briefly anesthetized with Isoflurane inhalant and had AR measurement of the nasal cavity, then groups 2-6 (x 30) were challenged with 20 microliter delivery of 2% Ovalbumin in sterile water vehicle via pipette installation in each nostril (Time=0 minutes). Guinea pigs were held upright to allow the liquid installation to permeate the entire nasal cavity while they were recovering from anesthesia (approximately 1-2 minutes). At Time=60 minutes, each guinea pig from groups 1-6 (x 36) were briefly anesthetized with Isoflurane inhalant and had AR measurement of the nasal cavity (Pre-treatment AR), then guinea pigs from groups 2-6 (x 30) had nasal droplet delivery of 10 microliter (5 microliter per nostril) of either placebo, Afrin, 1.875%, 3.75%, or 7.5% CGP test article depending on the experimental group. The graders were blinded as to the nasal article composition until the end of the study. For 30 minutes after dosing, each guinea pig was monitored and the number of nose rubs (perinasal scratch with the forelimb) and sneezes (explosive expiration after deep inspiration) was recorded. At time=120 each guinea pig from groups 1-6 (x 36) was briefly anesthetized with Isoflurane inhalant and had AR measurement of the nasal cavity only. At time=300 minutes (5 hours from initial dose), each guinea pig from groups 1-6 (x 36) was briefly anesthetized with Isoflurane inhalant and had AR measurement of the nasal cavity, then guinea pigs from groups 2, 4, 5, 6 (24) had nasal droplet delivery of 10 microliter (5 microliter per nostril) of either placebo, 1.875%, 3.75%, or 7.5% CGP test article depending on the experimental group (excluding the Afrin group 3). The administrator was no longer blinded to the Afrin study group (#3 yellow).

7.2.2 TREATMENT DAYS TWO THROUGH FIVE

On each of study days 2, 3, 4, and 5, the day 1 protocol was generally repeated, except 2% Ovalbumin challenge was not given. At Time=0 minutes, each guinea pig from groups 1-6 (x 36) was briefly anesthetized with Isoflurane inhalant and had AR measurement of the nasal cavity (Pre-treatment AR), then groups 2-6 (x 30) had nasal droplet delivery of either placebo, Afrin, 1.875%, 3.75%, or 7.5% CGP test article depending on the experimental group. Groups 2 (sterile water placebo), 4 (test article 1), 5 (test article 2), and 6 (test article 3), administered as droplets twice daily, were 2.5 microliters per nostril (equivalent to 400 mg human dose per nostril), for a total daily dosage of 10 microliters. The nasal dosage for the animals in Group 3 (Afrin OTC), administered as droplets twice daily, was 1.25 microliters per nostril (equivalent to 200 mg human dose per nostril), for a total daily dosage of 5 microliters. From time=0 to 30 minutes, each guinea pig was monitored and the number of nose rubs (perinasal scratch with the forelimb) and sneezes (explosive expiration after deep inspiration) was recorded. At time=60 and 120 minutes each guinea pig from groups 1-6 (x 36) had AR measurement of the nasal cavity. The nasal droplet delivery was repeated 4-6 hours after the initial dose to accomplish twice daily dosing. Study group 1 (non-sensitized controls) had AR measurements only. For Treatment Day 2 through 5, the nasal dosage for the animals in Groups 2 (sterile water placebo), 4 (test article 1), 5 (test article 2), and 6 (test article 3), administered as droplets twice daily, was 2.5 microliters per nostril (equivalent to 400 mg human dose per nostril), for a total daily dosage of 10 microliters. The nasal dosage for the animals in Group 3 (Afrin OTC), administered as droplets twice daily, was 1.25 microliters per nostril (equivalent to 200 mg human dose per nostril), for a total daily dosage of 5 microliters. All animals in Groups 2-6 will start with a double-dose (i.e. loading dose) on Treatment Day 1 only.

7.3 Summary of Dosing Schedule:

For treatment day 1, twice (2X) the human dose equivalent was administered. At time=60 minutes, each guinea pig in groups 2-6 received 10 microliters (5 microliters per nostril) of test article, sterile water placebo, and Afrin OTC. At time=300 minutes, each guinea pig in groups 2, 3, 4 , 5 only were redosed with another 10 microliters (5 microliters per nostril) of test article, sterile water placebo. For 20 microliters total daily dose (equivalent to 2x total daily dose). The Afrin OTC group #6 was only dosed in the AM to equal 2x the total daily dose. Treatment Day 2 through 5, the nasal dosage for the animals in Groups 2 (sterile water placebo), 4 (test article 1), 5 (test article 2), and 6 (test article 3), administered as droplets twice daily, was 2.5 microliters per nostril (equivalent to 400 mg human dose per nostril), for a total daily dosage of 10 microliters. The nasal dosage for the animals in Group 3 (Afrin OTC), administered as droplets twice daily, was 1.25 microliters per nostril (equivalent to 200 mg human dose per nostril), for a total daily dosage of 5 microliters.

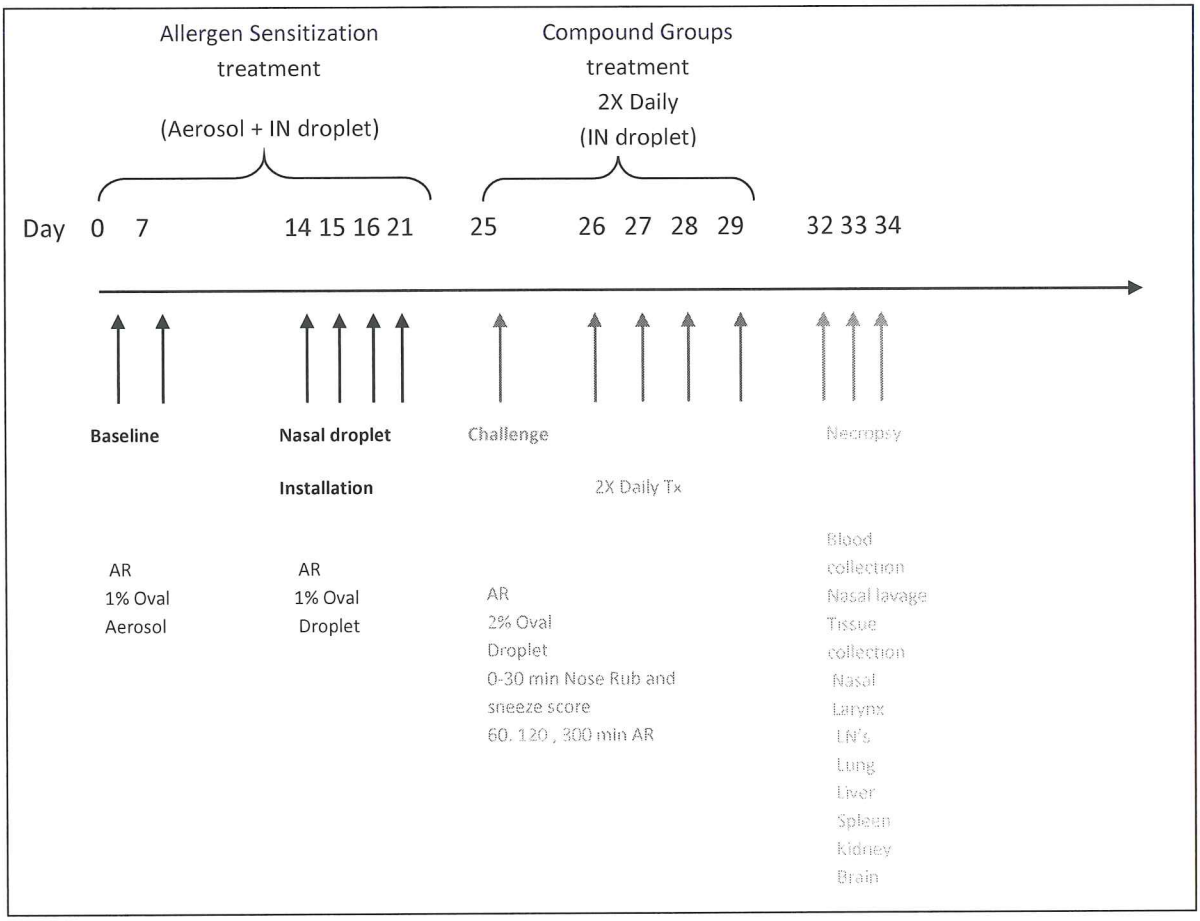

Figure 1: Summary of the study design.

7.4 Anesthesia Delivery

Guinea pigs were lightly anesthetized with isoflurane gas anesthesia to facilitate the installation of nasal droplets. This allowed the liquid installation to permeate the entire nasal cavity without struggle and rejection of the material by breath holding, sneezing, etc. Isoflurane gas anesthesia is delivered via the respiratory route so is very rapid acting with no carry-over to the next anesthesia event.

7.5 Acoustic Rhinometry Measurement of the Nasal Cavity:

The acoustic rhinometry (AR) instrument (Model #A1, Research Rhinometer, GM Instruments, Kilwinning, UK) is a non-invasive instrument that uses sound waves reflection to measure the nasal cavity. Briefly, the small sound tube is placed within the nasal opening while the operator

holds the skin to seal around the tube. The instrument produces audible clicks and reflected sound is measured by the microphone within the instrument. The sound is set to penetrate 2 cm within the nasal cavity and distance/volume algorithm calculates the measured nasal cavity volume, first minimal cross-sectional area (MCA1), and the distance of the MCA1 from the nasal opening. Results are downloaded to a computer for analysis and printing.

7.6 Acoustic Rhinometry Data Analysis

Data was collected from the AR instrument and the following criteria were used for AR data inclusion in the study.

- Repeat testing with three waveforms to verify reproducibility.
- Wide, erratic, and bizarre wave forms indicating movement artifact, were eliminated.
- Graphical evidence that sound traveling in the pre-patient sound tube is as a regular sinusoidal wave corresponding to the sound tube diameter.
- Patient sound waves attenuate between 0.004 and 0.300 square centimeters to indicate proper positioning of the patient.
- Patient sound waves that attenuate towards zero indicate blockage/breath holding and were eliminated.
- Patient sound waves that attenuate towards infinity indicate leakage and were eliminated.
- Variable patient sound waveforms meeting the above criteria were averaged to produce one reproducible waveform.

Data for each day were normalized to the time 0 value, and analyzed by ANOVA for repeated measures using the PRISM 5.0b software package. If the probability of randomly observing the treatment effect was 5% or less, the results were considered significant.

7.7 Necropsy and Tissue Collection

After completion of the 5-day study, guinea pigs in groups 1 through 6 were humanely euthanized by CO2 inhalant. Two groups were done per sequential day to allow time for sample handling and tissue processing. Twice daily dosing of the nasal cavity was continued over the weekend until all guinea pigs were processed.

Blood was collected by cardiac puncture (14 cc) for CBC and differential counts, serum clinical chemistry and one-half frozen for IgE levels. The larynx side of the nasal passage was opened and 2 cc saline infused in the right nasal cavity towards the nose and the first 1 cc collected at the nares. The sample was divided for cell counts and differential and the other half frozen for mediator testing (histamine, prostaglandin E2 and D2, and leukotrienes). The nasal passage, larynx, neck lymph nodes, lung, liver, spleen, kidney, and brain were sampled and placed into formalin fixative for routine histopathology.

7.8 Blood Analysis

Complete blood count analysis, clinical serum chemistry, and nasal lavage count analysis was done on each guinea pig by the Texas Tech University Health Sciences Center Clinical Laboratory (TTUHSC, Lubbock, TX) using routine laboratory methods. Serum anti-Ovalbumin (OVA) IgE antibody quantification was done by enzyme-linked, immunosorbent assay (Catalog #3010, Chondrex, Inc., Redmond, WA) following manufacturers protocol.

7.9 Nasal Lavage Fluid Analysis

The nasal lavage sample was divided and cellular counts were done on each guinea pig by the Texas Tech University Health Sciences Center Clinical Laboratory (TTUHSC, Lubbock, TX) using routine laboratory methods. Histamine analysis was done by enzyme immunoassay (EIA) using the histamine EIA kit developed and validated by SPI-BIO (Catalog #589651, Cayman Chemical Co, Ann Arbor, MI) following manufacturers protocol. Prostaglandin D2 analysis was done by EIA kit (Catalog #512031, Cayman Chemical Co, Ann Arbor, MI) following manufacturers protocol. Prostaglandin E2 analysis was done by EIA kit (Catalog #514010, Cayman Chemical Co, Ann Arbor, MI) following manufacturers protocol. Leukotriene C4/D4/E4 analysis was done by EIA kit (Catalog #EA39, Oxford Biomedical Research, Oxford, MI) following manufacturers protocol.

7.10 Tissue Histopathology

Tissues were collected during necropsy and fixed in neutral-buffered formalin for routine histopathology. After fixation, tissues were mounted in wax and thin sectioned (5 micron) in preparation for routine hematoxylin and eosin (H&E) staining. Nasal passages containing nasal bone were decalcified prior to thin section and H&E staining. All tissues were processed at Texas Tech University Health Sciences Center Clinical Pathology Laboratory (TTUHSC, Lubbock, TX) using routine laboratory methods. At TTUHSC, a board-certified pathologist (Dr. Bradley Miller) provided the interpretations and lesion scoring while blinded to the individual animals within each treatment vs. control groups.

8.0 RESULTS AND DISCUSSION

8.1 Group Body Weights

On the first day of the study (day 1, time 0) the individual body weight was taken for each guinea pig. Groups 1, red, yellow and violet had n=6, and group teal and green had n=5. The group averages were consistent amongst each group.

Grp 1	Grp Green	Grp Red	Grp Teal	Grp Violet	Grp Yellow
495	480	538	451	472	437
540	463	500	492	423	462
457	*	500	476	479	455
453	441	478	452	489	464
490	423	532	425	450	455
453	505	532	*	458	452
Grp Ave: 481	Grp Ave: 462	Grp Ave: 513	Grp Ave: 459	Grp Ave: 462	Grp Ave: 452

Table 1: Body weight (grams) at study start and group average. *These individual animals died prior to onset of the study.

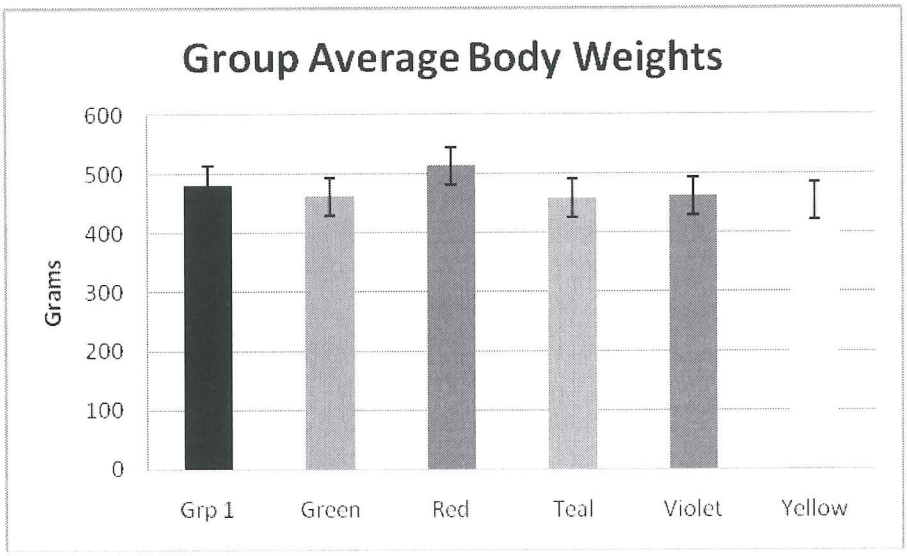

Figure 2: Group average body weights at the start of the study. Group ± σ.

8.2 Nose Rubs and Sneeze Scores

For 30 minutes after dosing, each guinea pig was monitored and the number of nose rubs (perinasal scratch with the forelimb) and sneezes (explosive expiration after deep inspiration) recorded (table 1). The numbers of nose rubs and sneezes were extremely low on each day of the product dosing indicating the materials used were clinically non-irritating to the animals.

	Day 1		Day 2		Day 3		Day 4		Day 5	
Group:	Nose Rub:	Sneeze:	Nose Rub:	Sneeze:	Nose Rub:	Sneeze:	Nose Rub:	Sneeze:	Nose Rub:	Sneeze:
Green	23	4	14	6	15	4	26	4	18	4
Red	11	3	22	5	17	3	22	4	17	2
Teal	11	3	14	4	21	3	18	6	20	3
Violet	14	1	30	6	18	0	21	6	18	3
Yellow	15	0	34	7	18	0	28	8	22	1

Table 2: Group count of nose rubs and sneezes for 30 minutes post-morning dose.

8.3 Nasal Congestion as Measured by Acoustic Rhinometry

Each group had four AR measurements on each day of the study at times=0, 60, 120, and 300. The daily time=0 data set was used as the daily baseline for each individual guinea pig within each group since changes from baseline from each individual animal is a more accurate measure than changes in the group average.

Since day 1 included sensitization challenge at time=0 and first treatment at time=60, the time=60 and 120 data sets were actually time=0 and 60 post-treatment that corresponds to the treatment schedule for study days 2-5. Hence, on day 1 only, the data set corresponding to time=120 post-treatment is not available.

AR measurements and total nasal cavity volumes are shown in Appendix 2 (tables). Changes from baseline in each study group on each study day are shown in Figure 1 below. On day 1 of the study each group either improved total nasal cavity volume (sum of left and right nasal cavity measurements), or fluctuated around baseline. The groups that improved nasal cavity volume include the 7.5% calcium glycerophosphate (CGP) and Afrin (OTC) treatment, however only the 7.5% CGP group (green) was statistically significant ($p<0.05$). As expected, the non-sensitized, non-treated control group (group 1) was also significantly elevated over baseline on study day 1. Subsequently, on days 2-5 total nasal cavity volume showed baseline readings in all groups that did not reach statistical significance, however the treated groups demonstrated marked trends toward improvement in nasal cavity volume in a dose-dependent manner. Exceptions to the trending observation exist on day 2 where the 1.875% CGP group (teal) performed as well as the 7.5% CGP group (green), on day 3 where the 1.875% and 3.75% CGP

groups (teal and violet, respectfully) outperformed the 7.5% CGP group (green), and on day 5 where the 3.75% CGP group (violet) outperformed the 7.5% CGP group (green).

On each day of the study, the 7.5% CGP group (green) trend either outperformed the Afrin OTC group (yellow, days 1 and 2), under performed (day 3), or were about equal (days 4 and 5) in performance. Although this data did not reach statistical significance ($p>0.05$), this trend may indicate that the highest dose CGP group was equal, if not better, than the Afrin OTC product at improving the nasal cavity volume in the experimentally congested, guinea pig model.

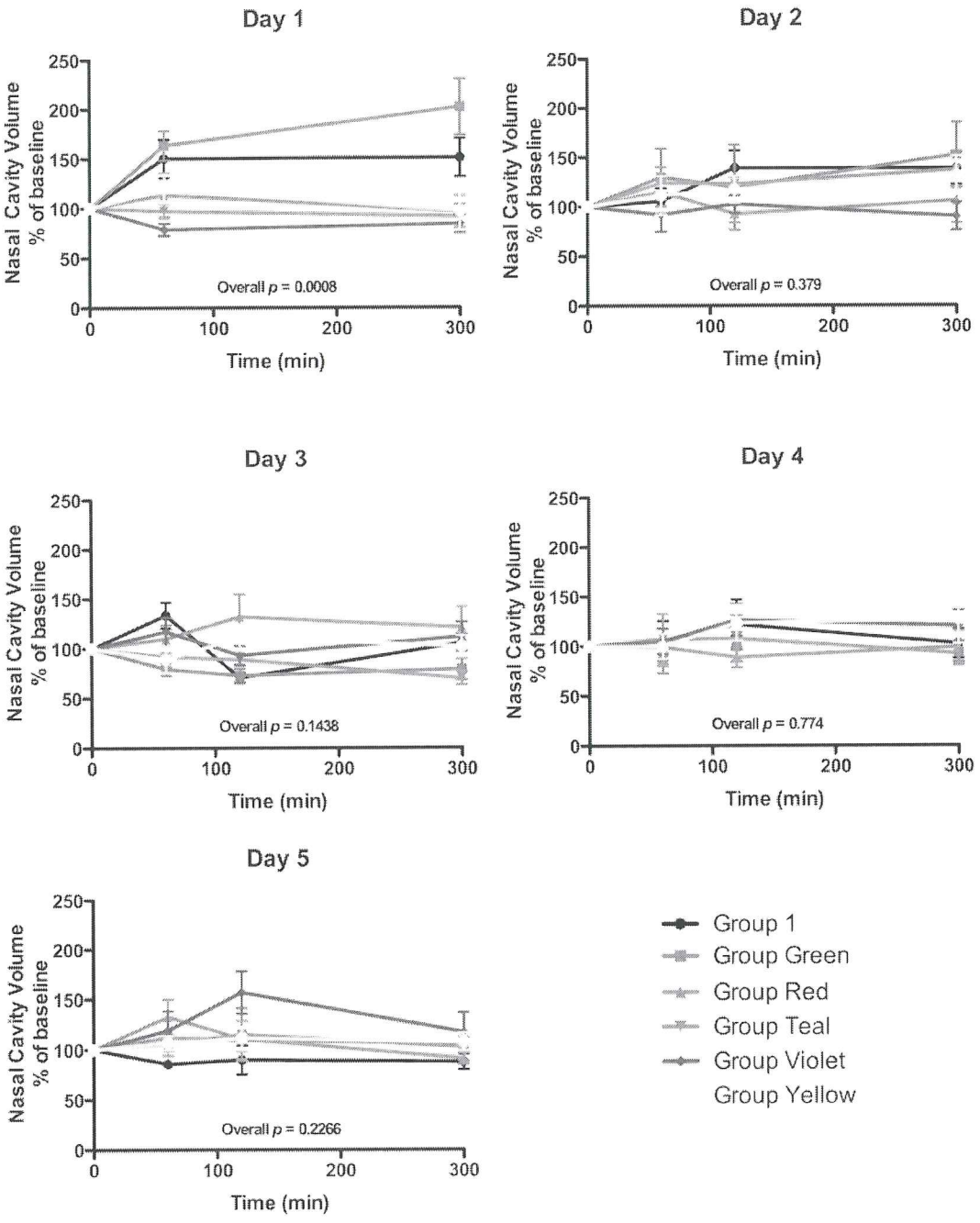

Figure 3: Group averages (± SE) of total nasal cavity AR measurements (sum of left and right measurement) and overall statistical significance on each study day. Group treatments were done twice daily and AR measurements taken after the morning dose (time=0). The group data for each day was normalized to the time 0 value, and analyzed by ANOVA for repeated measures using the PRISM 5.0b software package.

8.4 AR Calculation of MCA1 and Distance within the Nasal Cavity

Another measure of nasal congestion is the determination of the location and size of the first constriction inside the nasal cavity. The first minimal cross sectional area (MCA1) at a finite distance within the nasal cavity is an anatomical feature that will likely improve as swollen nasal tissues are reduced with topical nasal therapy. The distance from the nasal opening to the MCA1 may likely change since the change in cross sectional area will be at a new distance given the folding array of the nasal mucosa and supporting bone conchae.

The MCA1 and distance calculations from AR measurements (both left and right nasal cavities) are shown in Appendix 2 (tables). Figures 4 - 7 show the left and right nasal cavity change from baseline in each study group on each study day for first minimal cross sectional area (MCA1) at the corresponding distance, respectfully.

Although there were no statistically significant results in the right and left MCA1 calculation ($p>0.05$), the data shows the highly variant nasal cavity anatomy of the guinea pig. Of particular interest is the change from baseline data on day 1 and 2 mimic the trend in the day 1 and 2 total nasal cavity volume data (Figures 4 and 5 vs. Figure 3), while days 3-5 show similar results as fluctuation around baseline. This trend may demonstrate additional evidence that the 7.5% CGP group (green) has likely reduced nasal mucosa swelling (i.e. increased MCA1) and has outperformed the Afrin (yellow) group on these days.

The change from baseline of the distance calculation from left and right AR measurements is shown in Figures 6 and 7. The results indicate overall statistical significance on each day of the study (i.e. left and right combined). However, this finding is likely a direct result of the anatomical features of the guinea pig nasal cavity as new MCA1 locations are established. This finding likely provides further evidence that the nasal mucosa has been changed with therapy.

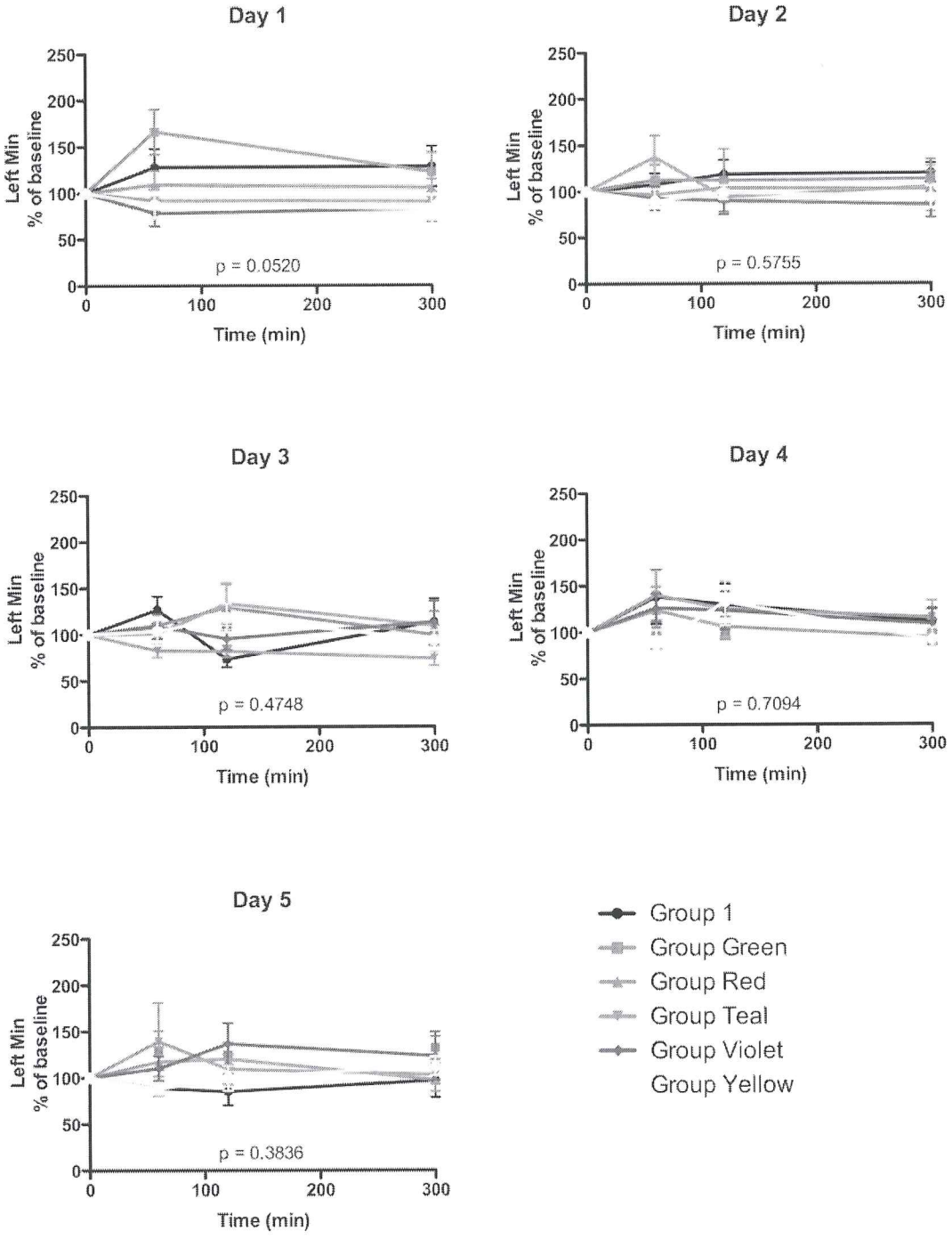

Figure 4: Change from daily baseline of the first minimal cross-sectional area (MCA1) calculated from AR measurement of the left nasal cavity.

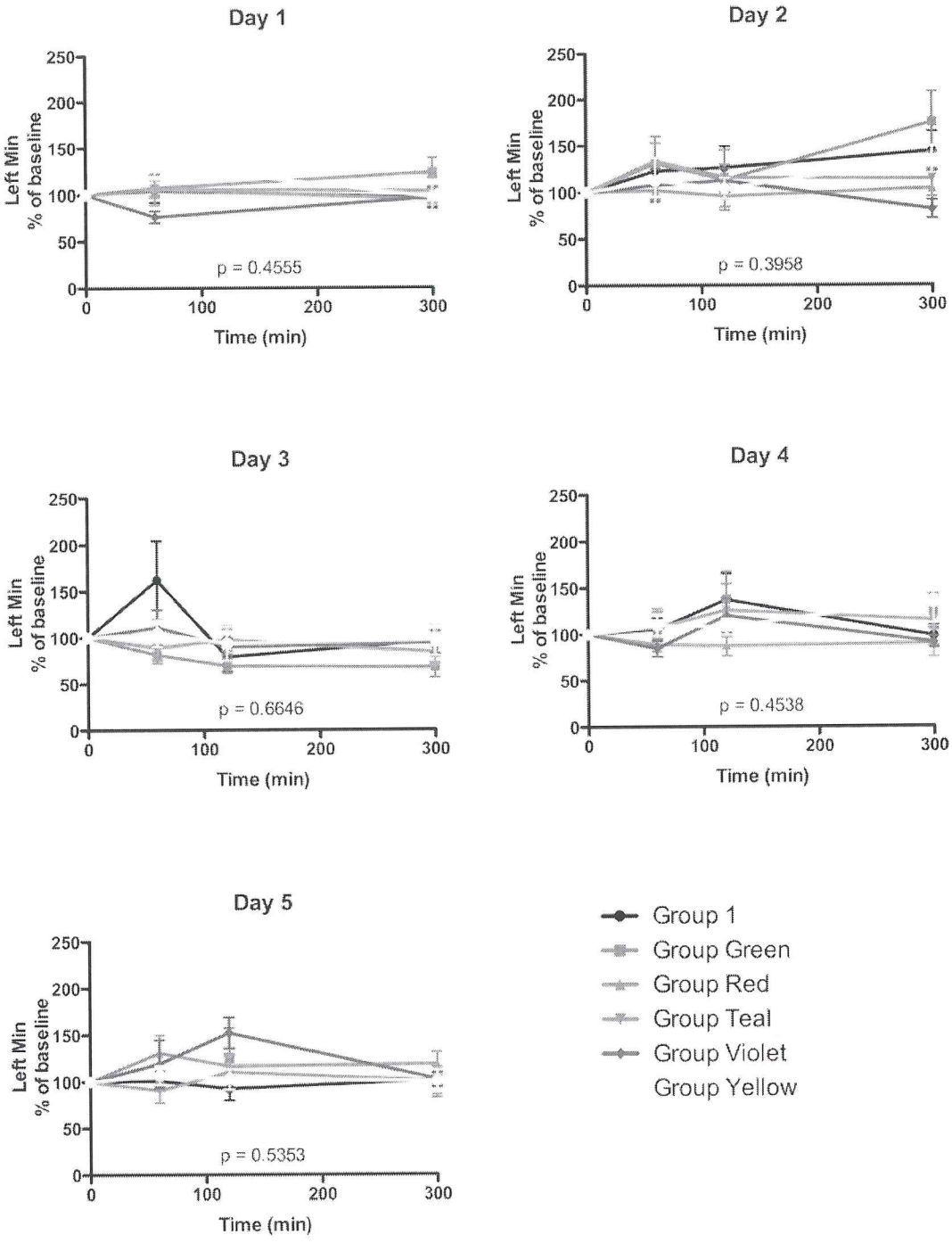

Figure 5: Change from daily baseline of the first minimal cross-sectional area (MCA1) calculated from AR measurement of the right nasal cavity.

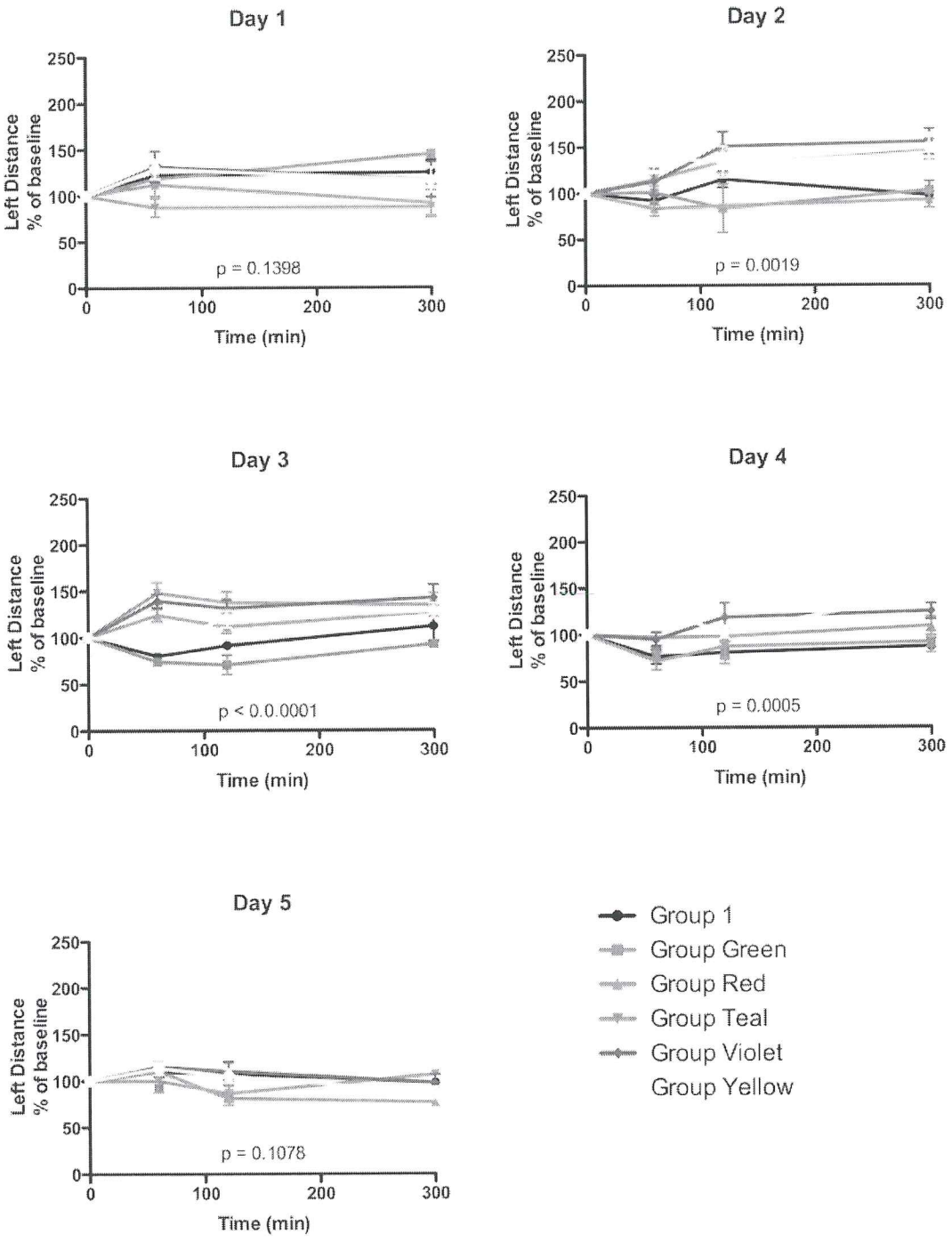

Figure 6: Change from daily baseline of the corresponding distance of the first minimal cross-sectional area (MCA1) calculated from AR measurement of the left nasal cavity.

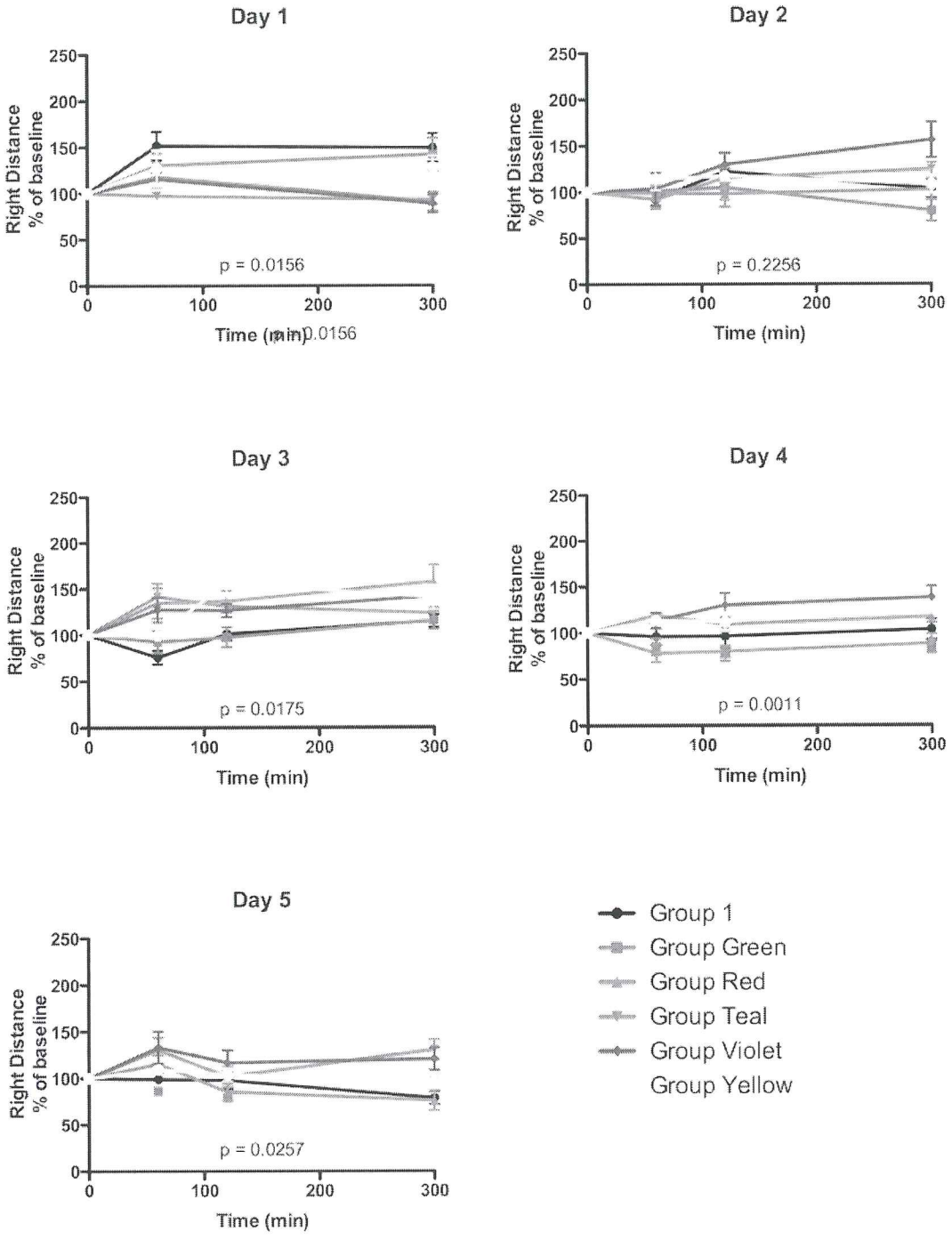

Figure 7: Change from daily baseline of the corresponding distance of the first minimal cross-sectional area (MCA1) calculated from AR measurement of the right nasal cavity.

8.5 Blood CBC, Clinical Chemistry, Serum IgE Analysis

The blood complete blood count (CBC) analysis is shown in Appendix 4. The white blood cell count trend was depressed in treatment groups (excluding Afrin OTC yellow group) compared to non-sensitized controls (group 1), however only the red (placebo control) and violet (3.75% CGP) groups reached statistical significance ($p<0.05$). This trend is unknown, however likely due to the reduction in nasal cavity inflammation in the treated groups.

The red blood cell count (RBC), hemoglobin, and hematocrit analysis was similar in all groups, however the green (7.5% CGP) formulation was reduced in each category when compared to the other groups ($p<0.05$). This finding is consistent with the increase observed in the mean corpuscular hemaglobin concentration (MCHC) for the green (7.5% CGP) formulation and Afrin OTC groups and likely due to the reduction in nasal cavity inflammation leading to reduced RBC recruitment and utilization.

The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell width (RDW), platelet count, and mean platelet volume (MPV) were similar when compared to all groups ($p>0.05$).

Serum clinical chemistry analysis is shown in Appendix 5. The alkaline phosphatase analyte was found to differ in treatment groups from the non-sensitized group (group 1). The red (placebo control), green (7.5% CGP), and violet (3.75% CGP) reached significance ($p<0.05$) however all groups tended to be reduced when compared to group 1. Similarly, total bilirubin tended to be reduced over group 1, however did not reach statistical significance ($p>0.05$).

The following serum clinical chemistry analytes' were similar when compared to all groups ($p>0.05$): Sodium, potassium, chloride, carbon dioxide, anion gap, glucose, blood urea nitrogen, creatinine, calcium, phosphorus, total serum protein, serum albumin, total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST).

Serum anti-ovalbumin (OVA) immunoglobulin E (IgE) analysis (Figure 8) showed as marked trend towards elevated values in OVA-sensitized groups (groups 2-6) when compared to the non-sensitized controls (group 1). Since OVA antigen exposure was via the nasal mucosa, serum IgE against OVA was detectable, however not robust enough to produce highly elevated IgE levels. The level attained was reaching the limits of the kit assay procedure and since individual assays were highly variable, they did not reach significance ($p=0.096$).

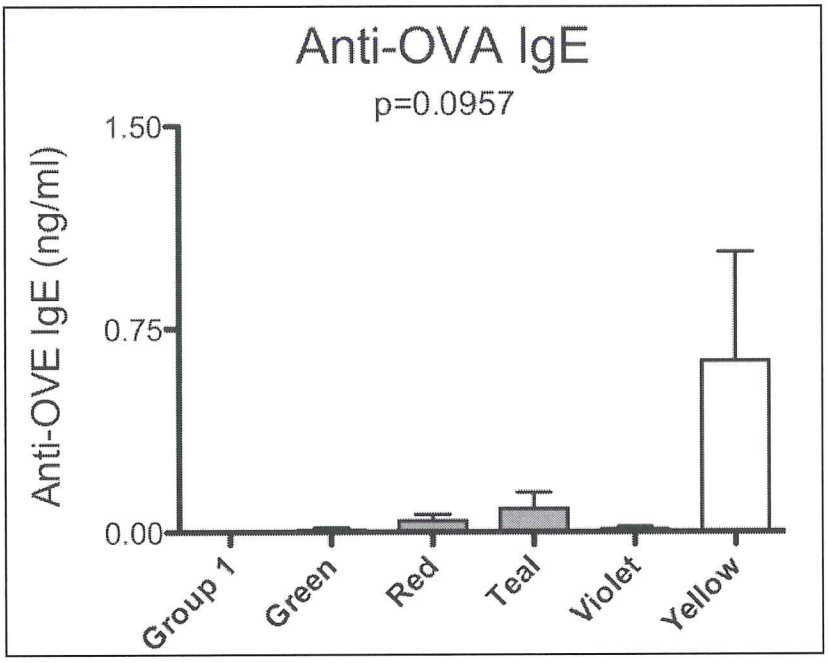

Figure 8: Average group concentrations of specific anti-ovalbumin (OVA) IgE in gunea pig serum. Group 1 was not exposed to OVA sensitization via the nasal mucosa and groups green, red, teal, violet, and yellow were exposed to OVA aerosal and droplet instillation over a 21-day sensitization protocol. The only detectable anti-OVA antibody was found in the OVA sensitized groups, however due to high variation all groups did not reach significance (p=0.0957).

8.6 Nasal Lavage Fluid Cellular Analysis

The nasal lavage fluid cellular analysis is shown in Appendix 6. The inflammatory cells were increased in the OVA sensitized groups due to the inflammation present within the nasal cavity. In particular, the eosinophil counts tended to be elevated in all OVA sensitized groups when compared to the non-sensitized group (group 1) however this result did not reach significance ($p>0.05$). The group showing highest levels of total white blood cells and basophils was the red (placebo control) suggesting the inflammation caused by OVA sensitization and left untreated by the placebo. Also of interest, the green (7.5% CGP) group showed the highest levels of neutrophils and monocytes amongst the treated groups ($p>0.05$), however was lowest in lymphocyte count ($p<0.05$). These variations are likely due to character of the inflammation under treatment by nasal dosing with CGP. Red blood cells present in nasal lavage fluid are a common contaminate and likely due either to collection method as a necropsy technique or fragility of mucus membranes due to acute inflammation. The nasal mucosa lining cells were similar in all groups ($p>0.05$) and commonly present in nasal lavage fluid.

8.7 Nasal Lavage Fluid Histamine Analysis

Nasal lavage fluid histamine analysis (Figure 9) showed marked levels in all groups that all tended to be similar, however variation amongst samples precluded groups from reaching significance (p=0.595). This finding likely represents the nasal cavity histamine level present in the guinea pig model.

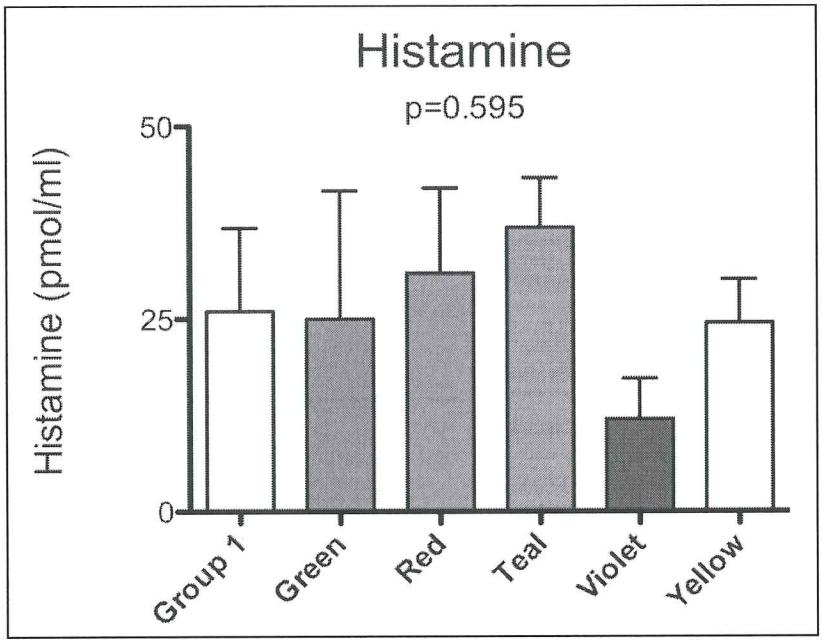

Figure 9: Average concentration of histamine measured in nasal lavage samples in each group. Due to high variation all groups did not reach significance (p=0.595).

8.8 Nasal Lavage Fluid Prostaglandin D2 and E2 Analysis

Nasal lavage fluid prostaglandin D2 (PgD2) analysis (Figure 10) showed a trend for decreased PGD2 in the OVA sensitized groups when compared to the non-sensitized group (group 1). This trend is also found for prostaglandin E2 analysis (Figure 11). These trends may likely demonstrate that the nasal cavity under treatment may likely respond with less release of inflammatory mediators, however these results did not reach significance (p=0.241).

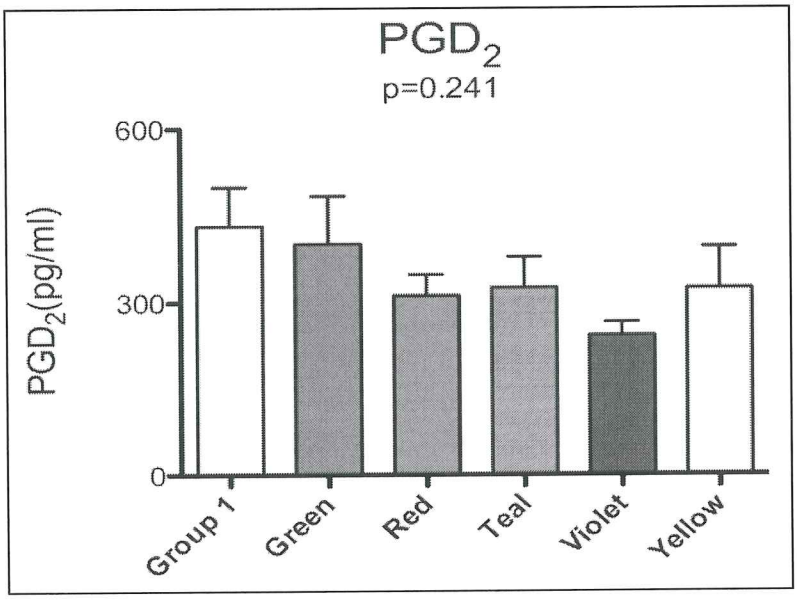

Figure 10: Average concentration of prostaglandin D2 (PgD2) in nasal lavage samples for each group. All groups showed strong levels of PgD2 with less in the treated groups (group 1 vs. green, red, teal, violet, and yellow), however this trend did not reach significance (p=0.241).

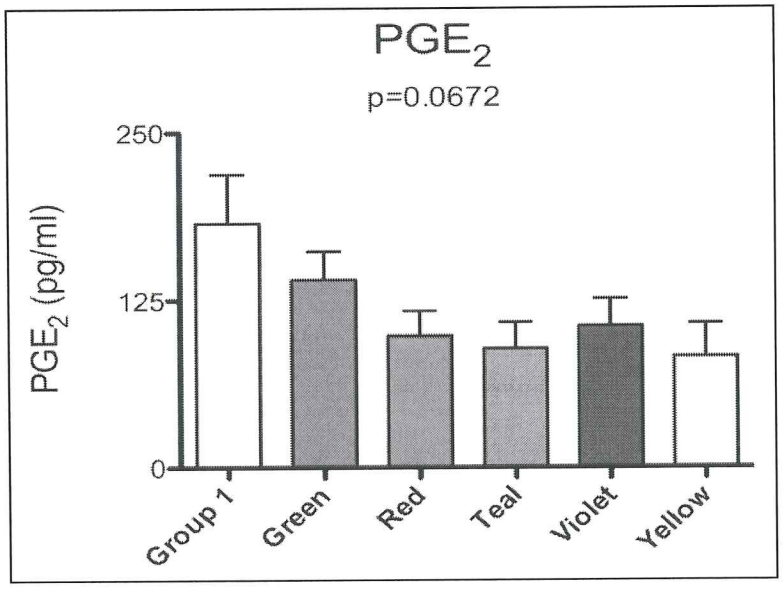

Figure 11: Average concentration of prostaglandin E2 (PgE2) in nasal lavage samples for each group. All groups showed strong levels of PgE2 with less in the treated groups (group 1 vs. green, red, teal, violet, and yellow), however this trend did not reach significance (p=0.0672).

8.9 Nasal Lavage Fluid Leukotriene C4/D4/E4 Analysis

Nasal lavage fluid leukotriene (LT) C4/D4/E4 analysis (Figure 12) showed highest levels in untreated and lowest dose treated groups that included non-sensitized (group 1), placebo-treated (red), and 1.875% CGP treated (teal). However, the treated groups of 7.5% CGP (green), 3.75% CGP (violet), and Afrin OTC reduced LTC4/D4/E4 production by the nasal cavity. However, this trend between treated and non-treated groups did not reach significance (p=0.2013). Similar to the results of the other inflammatory mediators, the trend for reduction of LT within the nasal cavity indicates reduction and control of inflammation amongst treated groups.

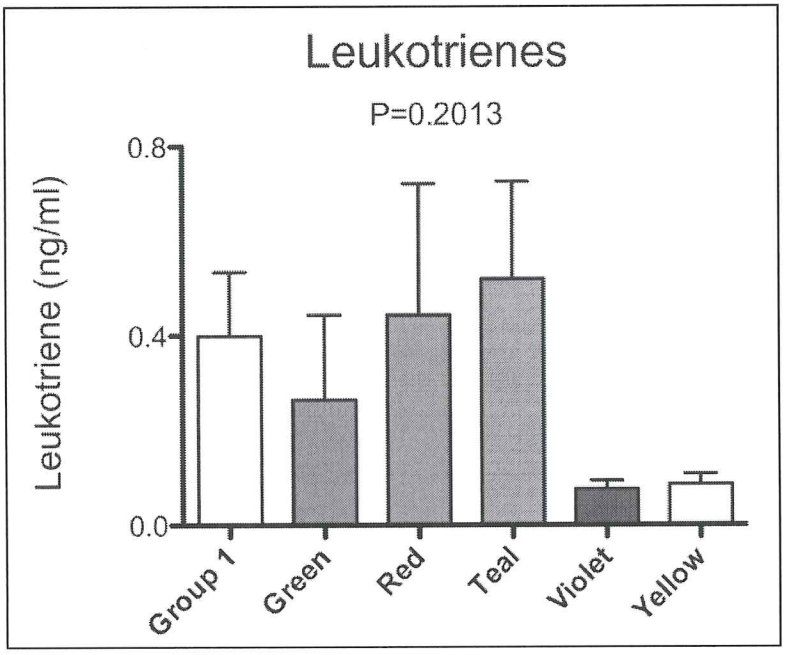

Figure 12: Average concentration of LTC4/D4/E4 measured in nasal lavage samples in each group. Treated groups showed a decrease in LT production within the nasal cavity, however this trend did not reach significance (p=0.2013).

8.10 Tissue Histopathologic Analysis

The tissues of the nasal mucosa showed marked lesions associated with inflammation due to OVA sensitization (Table 3) by histopathologic analysis. As expected, sensitization with OVA antigen of the nasal cavity had marked development of inflammatory lesions characterized by sub-epithelial foci of eosinophils, multifocal pan-eosinophilia with multiple foci of lymphocytosis,

mucosal eosinophilia, mucosal thickening due to edema and cellular infiltrate, and generalized epithelial and submucosal edema. As noted in Table 3, some of these inflammatory lesions are commonly reported in OVA non-sensitized guinea pigs (group 1) who are likely reacting to common allergens within their microenvironment. Similarily, the guinea pig, as an animal model of acute respiratory allergy, contains an overabundance of inflammatory and mast cells within the respiratory tract. However, incidence of these lesions are very low compared to OVA sensitized groups. The lesions found in the nasal mucosa were only the result of OVA antigen sensitization and were not considered to be related to the nasal cavity treatments of either test article (CGP), Afrin OTC, or placebo.

Multiple tissues including larynx, lymph nodes of the neck, lung, liver, spleen, kidney, and brain had no significant lesions and represented normal guinea pig.

Histopathologic Lesions of the Nasal Mucosa and Group Score: Groups Green, Red, Teal, Violet, Yellow were OVA sensitized Scored [0-1-2-3] none-mild-moderate-severe	Group 1 (n=6) OVA Non-Sensitized Control	Green (n=5) 7.5% CGP	Red (n=5) Placebo Control	Teal (n=5) 1.875% CGP	Violet (n=6) 3.75% CGP	Yellow (n=6) Afrin OTC
Eosinophils in Subepithelium	0.3	2.0	1.6	2.0	1.7	1.5
Eosinophils in Subepithelial Band	0.8	2.6	1.4	2.0	2.2	2.0
Eosinophils in Epithelium	0.5	1.8	1.0	1.4	1.2	1.2
Mucosal Thickening and Lymphocytosis	0.7	0.6	1.2	1.6	0.7	0.8
Edema	0.5	0.2	1.0	0.6	0.3	0.8
Neutrophils	0.0	0.0	0.0	0.0	0.0	0.0
Generalized Inflammation Score [0-10] none-severe	1.67	6.4	4.0	5.4	5.0	4.7

Table 3: Summary of the histopathologic lesions found in the guinea pig nasal mucosa in each group. The lesions represent those of inflammation and were not considered to be related to nasal cavity treatment.

References:

Pastor LM, Amores AE, Villaverde R, Calvo A, Sprekelsen C. Morphological study of the nasal conchae of the guinea pig. Acta Anat (Basel). 1990;139(3):254-64.

Pedersen OF, Yamagiwa M, Miyahara Y, Sakakura Y. Nasal Cavity Dimensions in Guinea Pigs Measured by Acoustic Reflections. Am J Rhinology. 1994;8(6):299-304.

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A1 Acoustic Software Manual, v9A, 01/01/11. GM Instruments, Kilwinning, Scotland, UK.

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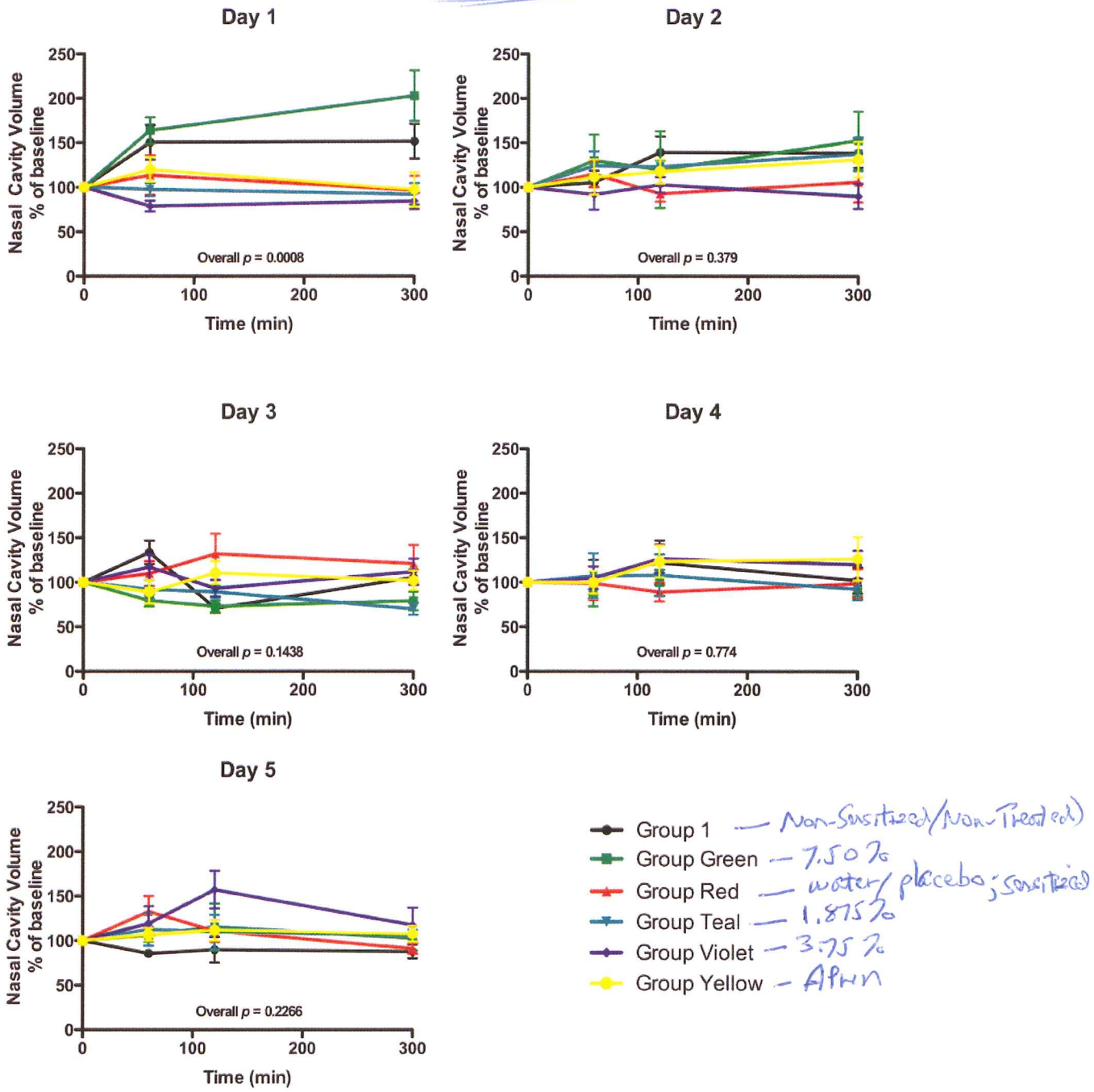

Figure 3: Group averages (± SE) of total nasal cavity AR measurements (sum of left and right measurement) and overall statistical significance on each study day. Group treatments were done twice daily and AR measurements taken after the morning dose (time=0). The group data for each day was normalized to the time 0 value, and analyzed by ANOVA for repeated measures using the PRISM 5.0b software package.

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holds the skin to seal around the tube. The instrument produces audible clicks and reflected sound is measured by the microphone within the instrument. The sound is set to penetrate 2 cm within the nasal cavity and distance/volume algorithm calculates the measured nasal cavity volume, first minimal cross-sectional area (MCA1), and the distance of the MCA1 from the nasal opening. Results are downloaded to a computer for analysis and printing.

7.6 Acoustic Rhinometry Data Analysis

Data was collected from the AR instrument and the following criteria were used for AR data inclusion in the study.

- Repeat testing with three waveforms to verify reproducibility.
- Wide, erratic, and bizarre wave forms indicating movement artifact, were eliminated.
- Graphical evidence that sound traveling in the pre-patient sound tube is as a regular sinusoidal wave corresponding to the sound tube diameter.
- Patient sound waves attenuate between 0.004 and 0.300 square centimeters to indicate proper positioning of the patient.
- Patient sound waves that attenuate towards zero indicate blockage/breath holding and were eliminated.
- Patient sound waves that attenuate towards infinity indicate leakage and were eliminated.
- Variable patient sound waveforms meeting the above criteria were averaged to produce one reproducible waveform.

Data for each day were normalized to the time 0 value, and analyzed by ANOVA for repeated measures using the PRISM 5.0b software package. If the probability of randomly observing the treatment effect was 5% or less, the results were considered significant.

7.7 Necropsy and Tissue Collection

After completion of the 5-day study, guinea pigs in groups 1 through 6 were humanely euthanized by CO2 inhalant. Two groups were done per sequential day to allow time for sample handling and tissue processing. Twice daily dosing of the nasal cavity was continued over the weekend until all guinea pigs were processed.

Blood was collected by cardiac puncture (14 cc) for CBC and differential counts, serum clinical chemistry and one-half frozen for IgE levels. The larynx side of the nasal passage was opened

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AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0					
Grp 1	0.102	0.130	0.160	0.156	0.150
Green	0.102	0.128	0.188	0.129	0.135
Red	0.132	0.168	0.127	0.167	0.138
Teal	0.145	0.115	0.162	0.172	0.125
Violet	0.167	0.170	0.140	0.123	0.128
Yellow	0.158	0.132	0.145	0.129	0.125
TIME 60					
Grp 1	0.151	0.134	0.214	0.154	0.128
Green	0.171	0.162	0.144	0.133	0.141
Red	0.157	0.186	0.128	0.150	0.177
Teal	0.140	0.141	0.147	0.162	0.138
Violet	0.128	0.145	0.155	0.124	0.138
Yellow	0.181	0.134	0.132	0.122	0.133
TIME 120					
Grp 1		0.175	0.113	0.174	0.129
Green		0.178	0.156	0.152	0.149
Red		0.151	0.154	0.140	0.151
Teal		0.140	0.141	0.166	0.133
Violet		0.166	0.124	0.148	0.185
Yellow		0.146	0.152	0.153	0.135
TIME 300	**				
Grp 1	0.152	0.176	0.166	0.149	0.131
Green	0.189	0.177	0.147	0.143	0.133
Red	0.135	0.164	0.137	0.150	0.124
Teal	0.133	0.151	0.111	0.148	0.131
Violet	0.136	0.141	0.146	0.145	0.136
Yellow	0.137	0.160	0.140	0.150	0.132

* Time in minutes

** Actual Time = 240 min

Grp 1 = Unsensitized to allergen (n=6)

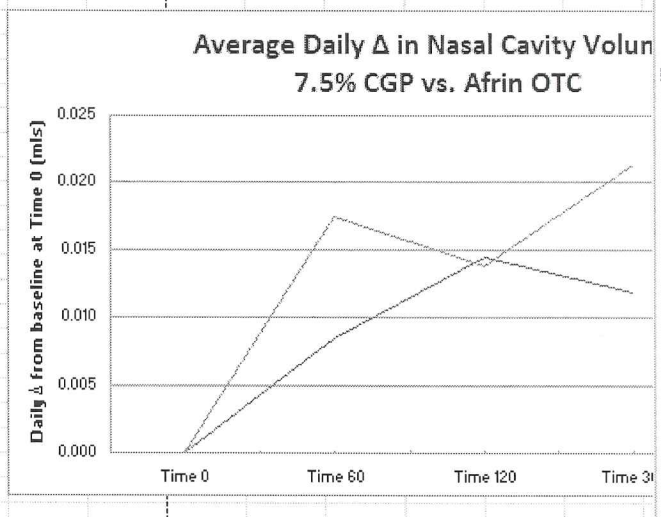
Cumulative change from placebo baseline (Red, Day 1, Time 0)

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5
Green	0.039	0.030	0.012	0.001	0.009
Red	0.025	0.054	-0.004	0.018	0.045
Teal	0.008	0.009	0.015	0.030	0.006
Violet	-0.004	0.013	0.023	-0.008	0.006
Yellow	0.049	0.002	0.000	-0.010	0.001
Grp 1	0.046	0.024	0.020	0.017	
Green	0.019	0.022	0.008	0.019	
Red	0.008	0.009	0.034	0.001	
Teal	0.034	-0.008	0.016	0.053	
Violet	0.014	0.020	0.021	0.003	
Grp 1	0.057	0.045	0.015	0.011	0.001
Green	0.003	0.032	0.005	0.018	-0.008
Red	0.001	0.019	-0.021	0.016	-0.001
Teal	0.004	0.009	0.014	0.013	0.004
Violet	0.005	0.028	0.008	0.018	0.000

Daily Δ in Nasal Cavity Volume with Treatement

7.5% Dose Formulation (Green):

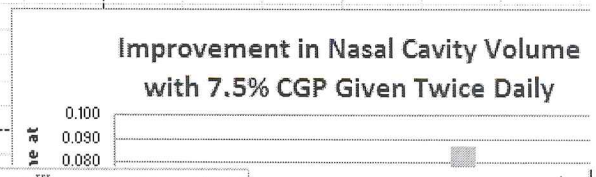
	Time 0	Time 60	Time 120	Time 300
Day 1	0.000	0.069		0.087
Day 2	0.000	0.034	0.050	0.049
Day 3	0.000	-0.026	-0.032	-0.041
Day 4	0.000	0.004	0.023	0.014
Day 5	0.000	0.006	0.014	-0.002
Ave:	0.000	0.017	0.014	0.021



Cumulative Daily Improvement in Nasal Cavity Volume with Treatment

7.5% Dose Formulation (Green):

	Time 0	Time 60	Time 120	Time 300
Day 1		0.069		0.087



Group Averages | Percent of Baseline

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Cumulative Change from Baseline (Day 1, Time 0 min)

AkP Color		Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0						
Grp 1	Unsens					
Green	7.5% CGP	0.00	0.00	0.05	0.03	0.01
Red	Sterile Water	0.00	0.04	0.01	0.04	0.01
Teal	1.875% CGP	0.00	-0.03	0.01	0.03	-0.03
Violet	3.75% CGP	0.00	0.01	-0.03	-0.05	-0.04
Yellow	Afrin OTC	0.00	-0.03	-0.02	-0.04	-0.04
TIME 60						
Grp 1	Unsens					
Green	7.5% CGP	0.07	0.06	0.04	0.02	0.04
Red	Sterile Water	0.02	0.06	0.00	0.02	0.06
Teal	1.875% CGP	-0.01	-0.01	0.00	0.01	-0.01
Violet	3.75% CGP	-0.04	-0.02	-0.02	-0.04	-0.03
Yellow	Afrin OTC	0.02	-0.03	-0.03	-0.04	-0.03
TIME 120						
Grp 1	Unsens					
Green	7.5% CGP		0.08	0.06	0.05	0.06
Red	Sterile Water		0.02	0.04	0.02	0.02
Teal	1.875% CGP		-0.01	-0.01	0.02	-0.02
Violet	3.75% CGP		0.00	-0.05	-0.02	0.02
Yellow	Afrin OTC		-0.01	-0.01	0.00	-0.02
TIME 300						
Grp 1	Unsens					
Green	7.5% CGP	0.08	0.07	0.07	0.04	0.03
Red	Sterile Water	0.01	0.04	0.01	0.02	-0.01
Teal	1.875% CGP	-0.02	0.00	-0.04	0.00	-0.02
Violet	3.75% CGP	-0.03	-0.03	-0.03	-0.03	-0.03
Yellow	Afrin OTC	-0.02	0.00	-0.01	-0.01	-0.03

Daily Change from Baseline (Time 0 min):

Day 1	Day 2	Day 3	Day 4	Day 5
+0.07	+0.06	-0.01	-0.01	+0.03
+0.02	+0.02	-0.01	-0.02	+0.05
-0.01	+0.02	-0.01	-0.02	+0.02
-0.04	-0.03	+0.01	+0.01	+0.01
+0.02	0	-0.01	0	+0.01
	+0.08	+0.01	+0.02	+0.04
	-0.02	+0.03	-0.02	+0.01
	+0.02	-0.02	-0.01	+0.01
	-0.01	-0.02	+0.03	+0.06
	+0.02	+0.01	+0.04	+0.02
Green	+0.08	+0.07	0	+0.01	+0.02
Red	+0.01	0	0	-0.02	-0.02
Teal	-0.02	+0.03	-0.05	-0.03	+0.01
violet	-0.03	-0.04	0	+0.02	+0.01
Yellow	-0.02	+0.03	+0.01	+0.03	+0.01

Cumulative Change from Baseline (Day 1, Time 0 min):

Day 1	Day 2	Day 3	Day 4	Day 5
	0	+0.05	+0.03	+0.01
	+0.04	+0.01	+0.04	+0.01
	-0.03	+0.01	+0.03	-0.03
	+0.01	-0.03	-0.05	-0.04
	-0.03	-0.02	-0.04	-0.04
+0.07	+0.06	+0.04	+0.02	+0.04
+0.02	+0.06	0	+0.02	+0.06
-0.01	-0.01	0	+0.01	-0.01
-0.04	-0.02	-0.02	-0.04	-0.03
+0.02	-0.03	-0.03	-0.04	-0.03
	+0.08	+0.06	+0.05	+0.06
	+0.02	+0.04	+0.02	+0.02
	-0.01	-0.01	+0.02	-0.02
	0	-0.05	-0.02	+0.02
	-0.01	-0.01	0	-0.02
	+0.08	+0.07	+0.07	+0.04	+0.03
	+0.01	+0.04	+0.01	+0.02	-0.01
	-0.02	0	-0.04	0	-0.02
	-0.03	-0.03	-0.03	-0.03	-0.03
	-0.02	0	-0.01	-0.01	-0.03

7.5% CGP *

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* - Day 1, Time 0 Base: 0.10 Increase: +0.03 = +30%

** - Day 1, Time 0 Base: 0.16 Decrease: -0.03 = -17%

> Difference: 47%

AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0					
Grp 1	0.10	0.13	0.16	0.16	0.15
Green	0.10	0.10	0.15	0.13	0.11
Red	0.13	0.17	0.14	0.17	0.14
Teal	0.15	0.12	0.16	0.18	0.12
Violet	0.17	0.18	0.14	0.12	0.13
Yellow	0.16	0.13	0.14	0.12	0.12
TIME 60 *					
Grp 1	0.15	0.14	0.23	0.17	0.13
Green	0.17	0.16	0.14	0.12	0.14
Red	0.15	0.19	0.13	0.15	0.19
Teal	0.14	0.14	0.15	0.16	0.14
Violet	0.13	0.15	0.15	0.13	0.14
Yellow	0.18	0.13	0.13	0.12	0.13
TIME 120 *					
Grp 1		0.17	0.11	0.18	0.13
Green		0.18	0.16	0.15	0.16
Red		0.15	0.17	0.15	0.15
Teal		0.14	0.14	0.17	0.13
Violet		0.17	0.12	0.15	0.19
Yellow		0.15	0.15	0.16	0.14
TIME 300 *	* *				
Grp 1	0.15	0.17	0.17	0.15	0.13
Green	0.18	0.17	0.15	0.14	0.13
Red	0.14	0.17	0.14	0.15	0.12
Teal	0.13	0.15	0.11	0.15	0.13
Violet	0.14	0.14	0.14	0.14	0.14
Yellow	0.14	0.16	0.15	0.15	0.13

* Time in minutes

** Actual Time = 240 min

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AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

Daily Change from Baseline (Time 0 min):

Cumulative Change from Baseline (Day 1, Time 0 min):

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0															
Grp 1	0.10	0.13	0.16	0.16	0.15										
Green	0.10	0.10	0.15	0.13	0.11							0	+0.05	+0.03	+0.01
Red	0.13	0.17	0.14	0.17	0.14							+0.04	+0.01	+0.04	+0.01
Teal	0.15	0.12	0.16	0.18	0.12							-0.03	+0.01	+0.03	-0.03
Violet	0.17	0.18	0.14	0.12	0.13							+0.01	-0.03	-0.05	-0.04
Yellow	0.16	0.13	0.14	0.12	0.12							-0.03	-0.02	-0.04	-0.04
TIME 60 *															
Grp 1	0.15	0.14	0.23	0.17	0.13										
Green	0.17	0.16	0.14	0.12	0.14	+0.07	+0.06	-0.01	-0.01	+0.03	+0.07	+0.06	+0.04	+0.02	+0.04
Red	0.15	0.19	0.13	0.15	0.19	+0.02	+0.02	-0.01	-0.02	+0.05	+0.02	+0.06	0	+0.02	+0.06
Teal	0.14	0.14	0.15	0.16	0.14	-0.01	+0.02	-0.01	-0.02	+0.02	-0.01	-0.01	0	+0.01	-0.01
Violet	0.13	0.15	0.15	0.13	0.14	-0.04	-0.03	+0.01	+0.01	+0.01	-0.04	-0.02	-0.02	-0.04	-0.03
Yellow	0.18	0.13	0.13	0.12	0.13	+0.02	0	-0.01	0	+0.01	+0.02	-0.03	-0.03	-0.04	-0.03
TIME 120 *															
Grp 1		0.17	0.11	0.18	0.13										
Green		0.18	0.16	0.15	0.16		+0.08	+0.01	+0.02	+0.04		+0.08	+0.06	+0.05	+0.06
Red		0.15	0.17	0.15	0.15		-0.02	+0.03	-0.02	+0.01		+0.02	+0.04	+0.02	+0.02
Teal		0.14	0.14	0.17	0.13		+0.02	-0.02	-0.01	+0.01		-0.01	-0.01	+0.02	-0.02
Violet		0.17	0.12	0.15	0.19		-0.01	-0.02	+0.03	+0.06		0	-0.05	-0.02	+0.02
Yellow		0.15	0.15	0.16	0.14		+0.02	+0.01	+0.04	+0.02		-0.01	-0.01	0	-0.02
TIME 300 *	**														
Grp 1	0.15	0.17	0.17	0.15	0.13										
Green	0.18	0.17	0.15	0.14	0.13	+0.08	+0.07	0	+0.01	+0.02	+0.08	+0.07	+0.07	+0.04	+0.03
Red	0.14	0.17	0.14	0.15	0.12	+0.01	0	0	-0.02	-0.02	+0.01	+0.04	+0.01	+0.02	-0.01
Teal	0.13	0.15	0.11	0.15	0.13	-0.02	+0.03	-0.05	-0.03	+0.01	-0.02	0	-0.04	0	-0.02
Violet	0.14	0.14	0.14	0.14	0.14	-0.03	-0.04	0	+0.02	+0.01	-0.03	-0.03	-0.03	-0.03	-0.03
Yellow	0.14	0.16	0.15	0.15	0.13	-0.02	+0.03	+0.01	+0.03	+0.01	-0.02	0	-0.01	-0.01	-0.03

* Time in minutes

** Actual Time = 240 min

Group Averages | Change from Average | Change from Baseline

Ready

SCRL

Re: Margaret WEis s... | Dropbox - Rhino Su... | Microsoft Excel - Rh...

9:35 AM

AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0					
Grp 1	0.10	0.13	0.16	0.16	0.15
Green	0.10	0.10	0.15	0.13	0.11
Red	0.13	0.17	0.14	0.17	0.14
Teal	0.15	0.12	0.16	0.18	0.12
Violet	0.17	0.18	0.14	0.12	0.13
Yellow	0.16	0.13	0.14	0.12	0.12
TIME 60 *					
Grp 1	0.15	0.14	0.23	0.17	0.13
Green	0.17	0.16	0.14	0.12	0.14
Red	0.15	0.19	0.13	0.15	0.19
Teal	0.14	0.14	0.15	0.16	0.14
Violet	0.13	0.15	0.15	0.13	0.14
Yellow	0.18	0.13	0.13	0.12	0.13
TIME 120 *					
Grp 1		0.17	0.11	0.18	0.13
Green		0.18	0.16	0.15	0.16
Red		0.15	0.17	0.15	0.15
Teal		0.14	0.14	0.17	0.13
Violet		0.17	0.12	0.15	0.19
Yellow		0.15	0.15	0.16	0.14
TIME 300 *	* *				
Grp 1	0.15	0.17	0.17	0.15	0.13
Green	0.18	0.17	0.15	0.14	0.13
Red	0.14	0.17	0.14	0.15	0.12
Teal	0.13	0.15	0.11	0.15	0.13
Violet	0.14	0.14	0.14	0.14	0.14
Yellow	0.14	0.16	0.15	0.15	0.13

* Time in minutes

** Actual Time = 240 min

(Dup.)

AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5	Average:
TIME 0						
Grp 1	0.10	0.13	0.16	0.16	0.15	0.14
Green	0.10	0.10	0.15	0.13	0.11	0.12
Red	0.13	0.17	0.14	0.17	0.14	0.15
Teal	0.15	0.12	0.16	0.18	0.12	0.15
Violet	0.17	0.18	0.14	0.12	0.13	0.15
Yellow	0.16	0.13	0.14	0.12	0.12	0.13
TIME 60 *						
Grp 1	0.15	0.14	0.23	0.17	0.13	0.16
Green	0.17	0.16	0.14	0.12	0.14	0.15
Red	0.15	0.19	0.13	0.15	0.19	0.16
Teal	0.14	0.14	0.15	0.16	0.14	0.15
Violet	0.13	0.15	0.15	0.13	0.14	0.14
Yellow	0.18	0.13	0.13	0.12	0.13	0.14
TIME 120 *						
Grp 1		0.17	0.11	0.18	0.13	0.15
Green		0.18	0.16	0.15	0.16	0.16
Red		0.15	0.17	0.15	0.15	0.16
Teal		0.14	0.14	0.17	0.13	0.15
Violet		0.17	0.12	0.15	0.19	0.16
Yellow		0.15	0.15	0.16	0.14	0.15
TIME 300 *	* *					
Grp 1	0.15	0.17	0.17	0.15	0.13	0.15
Green	0.18	0.17	0.15	0.14	0.13	0.15
Red	0.14	0.17	0.14	0.15	0.12	0.14
Teal	0.13	0.15	0.11	0.15	0.13	0.13
Violet	0.14	0.14	0.14	0.14	0.14	0.14
Yellow	0.14	0.16	0.15	0.15	0.13	0.15

* Time in minutes

** Actual Time = 240 min

Change from Average (Time 0 min):

+0.03
+0.01
0
-0.01
+0.01

+0.04
+0.01
0
+0.01
+0.02

+0.03
-0.01
-0.02
-0.01
+0.02

Daily Change from Baseline (Time 0 min):

Day 1	Day 2	Day 3	Day 4	Day 5
+0.07	+0.06	-0.01	-0.01	+0.03
+0.02	+0.02	-0.01	-0.02	+0.05
-0.01	+0.02	-0.01	-0.02	+0.02
-0.04	-0.03	+0.01	+0.01	+0.01
+0.02	0	-0.01	0	+0.01
	+0.08	+0.01	+0.02	+0.04
	-0.02	+0.03	-0.02	+0.01
	+0.02	-0.02	-0.01	+0.01
	-0.01	-0.02	+0.03	+0.06
	+0.02	+0.01	+0.04	+0.02
+0.08	+0.07	0	+0.01	+0.02
+0.01	0	0	-0.02	-0.02
-0.02	+0.03	-0.05	-0.03	+0.01
-0.03	-0.04	0	+0.02	+0.01
-0.02	+0.03	+0.01	+0.03	+0.01

Cumulative Change from Baseline (Day 1, Time 0 min):

Day 1	Day 2	Day 3	Day 4	Day 5
	0	+0.05	+0.03	+0.01
	+0.04	+0.01	+0.04	+0.01
	-0.03	+0.01	+0.03	-0.03
	+0.01	-0.03	-0.05	-0.04
	-0.03	-0.02	-0.04	-0.04
+0.07	+0.06	+0.04	+0.02	+0.04
+0.02	+0.06	0	+0.02	+0.06
-0.01	-0.01	0	+0.01	-0.01
-0.04	-0.02	-0.02	-0.04	-0.03
+0.02	-0.03	-0.03	-0.04	-0.03
	+0.08	+0.06	+0.05	+0.06
	+0.02	+0.04	+0.02	+0.02
	-0.01	-0.01	+0.02	-0.02
	0	-0.05	-0.02	+0.02
	-0.01	-0.01	0	-0.02
+0.08	+0.07	+0.07	+0.04	+0.03
+0.01	+0.04	+0.01	+0.02	-0.01
-0.02	0	-0.04	0	-0.02
-0.03	-0.03	-0.03	-0.03	-0.03
-0.02	0	-0.01	-0.01	-0.03

AR Rhinometry Total Nasal Cavity Volume (cm3) Group Averages

AkP Color	Day 1	Day 2	Day 3	Day 4	Day 5
TIME 0					
Grp 1	0.10	0.13	0.16	0.16	0.15
Green	0.10	0.10	0.15	0.13	0.11
Red	0.13	0.17	0.14	0.17	0.14
Teal	0.15	0.12	0.16	0.18	0.12
Violet	0.17	0.18	0.14	0.12	0.13
Yellow	0.16	0.13	0.14	0.12	0.12
TIME 60 *					
Grp 1	0.15	0.14	0.23	0.17	0.13
Green	0.17	0.16	0.14	0.12	0.14
Red	0.15	0.19	0.13	0.15	0.19
Teal	0.14	0.14	0.15	0.16	0.14
Violet	0.13	0.15	0.15	0.13	0.14
Yellow	0.18	0.13	0.13	0.12	0.13
TIME 120 *					
Grp 1		0.17	0.11	0.18	0.13
Green		0.18	0.16	0.15	0.16
Red		0.15	0.17	0.15	0.15
Teal		0.14	0.14	0.17	0.13
Violet		0.17	0.12	0.15	0.19
Yellow		0.15	0.15	0.16	0.14
TIME 300 *	* *				
Grp 1	0.15	0.17	0.17	0.15	0.13
Green	0.18	0.17	0.15	0.14	0.13
Red	0.14	0.17	0.14	0.15	0.12
Teal	0.13	0.15	0.11	0.15	0.13
Violet	0.14	0.14	0.14	0.14	0.14
Yellow	0.14	0.16	0.15	0.15	0.13

* Time in minutes

** Actual Time = 240 min