

Nasal nitric oxide and nasal eosinophils decrease with levocetirizine in subjects with perennial allergic rhinitis

Angela P. Bautista, B.A., Claudia P. Eisenlohr, M.I.B., and Miguel J. Lanz, M.D.

ABSTRACT

Background: Allergic rhinitis is commonly treated with antihistamines. Monitoring improvement of airway inflammation noninvasively using nasal nitric oxide (nNO) would be clinically useful. To determine the anti-inflammatory effect of oral levocetirizine dihydrochloride (LC), we measured nNO and nasal eosinophils (nEos) in perennial allergic rhinitis (PAR) subjects.

Methods: A randomized double-blind placebo-controlled crossover design was used. Inclusion criteria consisted of subjects having a PAR history, exam and diary scores consistent with active symptoms, and positive skin testing. Subjects taking allergy medications 1 month before the study were not enrolled. After consenting, 31 subjects (24 female subjects; mean age, 29 years) were randomized to either oral LC (5 mg) or matching placebo for 2 weeks. After 2-week washout, subjects started the other 2-week treatment. At each visit, nNO was measured by aspiration at each nare using a nasal kit from NIOX (Aerocrine, Sweden) in parts per billion; nEos was collected from nasal smears and measured by microscopy using the scoring system (0–4+) and symptoms were self-reported using the Allergic Rhinitis Quality of Life Questionnaire (RQLQ). Daily allergy symptom scores (total symptom score [TSS] 4) were collected at each visit.

Results: During LC, mean baseline nNO was 807 ± 317 parts per billion (ppb; left) and 831 ± 332 ppb (right) and decreased significantly to 688 ± 266 ppb and 702 ± 286 ppb, respectively ($p < 0.05$). No significance was found during placebo treatment (778 ± 270 ppb, 808 ± 299 ppb to 802 ± 271 ppb, 813 ± 273 ppb). The mean nNO change was also significant compared with placebo (-125 ppb versus $+14$ ppb; $p < 0.05$). There was a significant decrease in nEos with LC compared with placebo (3.1 – 2.5 versus 2.9 – 2.6 ; $p < 0.05$). RQLQ scores were significantly improved with LC only. In TSS-4 scoring, a trend toward improvement during LC and significant worsening during placebo was found. Baseline nNO predicted changes in nasal eosinophils (nEos) and RQLQ.

Conclusion: We showed that oral LC therapy decreased objective markers of rhinitis inflammation, nNO and nEos, in patients with PAR. Improvement in symptom scoring was also found with LC treatment.

(Am J Rhinol Allergy 25, 383–387, 2011; doi: 10.2500/ajra.2011.25.3668)

Perennial allergic rhinitis (PAR) is a chronic atopic inflammatory disease that affects the upper respiratory airway. Generally, symptoms include nasal pruritus, ocular pruritus, rhinorrhea, sneezing, and nasal congestion. The initial step in the medical management of PAR consists of antihistamines for the acute relief of symptoms.^{1,2} For this class of medication, newer-generation antihistamines are less sedating and have less collateral systemic side effects than the first-generation antihistamines. Intranasal steroids are used in PAR as the next line of therapy to directly treat the underlying inflammation found in the nasal passages. Biopsy specimens of nasal mucosa in subjects with PAR reveal eosinophils, which decrease with anti-inflammatory therapies.^{3,4} Measurement of the inflammatory milieu in a noninvasive manner would be optimal to monitor response to medical therapy.

Nasal nitric oxide (nNO) is a noninvasive marker of inflammation that has been shown to be elevated in allergic rhinitis.^{5–7} There have been studies with nNO to show improvement of airway inflammation in PAR after anti-inflammatory medication, such as intranasal steroids.^{8,9} This reduction in NO after anti-inflammatory therapy is similar to that found in asthma. There are direct maneuvers for the collection nasally of nNO, including nasal breath holding and nasal humming, which have been used previously.^{10–12}

The newer third-generation oral antihistamine levocetirizine dihydrochloride (LC) is an active enantiomer of cetirizine with twofold higher affinity for the H₁-receptor.^{13–15} It has been shown to improve PAR symptoms including nasal congestion by subject diary scoring when compared with placebo.^{16,17} Self-reported improvement in Rhinitis Quality of Life Questionnaire (RQLQ) measures was seen with LC therapy in PAR.^{18,19} Furthermore, LC has additional anti-inflammatory properties unrelated to the antihistamine effect. Inhibition of eotaxin-induced eosinophil chemotaxis and endothelial migration *in vitro* and *in vivo* was reported with LC.^{20–24} In another *in vitro* study, LC has been suggested to modulate release of various inflammatory mediators produced by eosinophil.²⁵ Reduction of eosinophils in nasal scrapings in a study of adults with PAR after LC treatment versus placebo was also reported.¹⁹ Because of these prior findings, we proposed to evaluate *in vivo* the anti-inflammatory effect of LC on adult PAR subjects by measuring nNO directly from the nares. To show that any improvement in nNO correlated with an anti-inflammatory effect of LC, eosinophils from nasal secretions were also studied as an end point marker. Secondary end points that were to be measured were subjective in nature and consisted of diary symptom scoring, need for rescue medication, and RQLQ measures.

METHODS

This study was approved by the Institutional Review Board, RCRC-IRB, and registered at Clinicaltrials.gov (NCT00894231).

We performed a randomized, double-blind, placebo-controlled, crossover design study in adults and adolescents with PAR that were actively experiencing symptoms at the time of study entry. After written informed consent was obtained, inclusion and exclusion criteria were reviewed for subject participation. For inclusion, subjects (≥ 12 years of age) enrolled had to have a diagnosis of PAR with symptoms for over a year. Levels of measured nNO had to be elevated above 442 parts per billion (ppb) at baseline before randomization during the run-in visit to avoid subjects with chronic rhinosinusitis (CRS) and nasal polyps.²⁶ Subjects were excluded for use of

From the Department of Allergy, Asthma, and Rhinosinusitis, AAADRS Clinical Research Center, Coral Gables, Florida

Presented in part at the annual meeting of the American Academy of Allergy, Asthma, and Immunology (AAAAI), New Orleans, Louisiana, February 26 to March 2, 2010. Supported by an Investigator Initiated Study Program Grant from Sanofi-Aventis and UCB.

M. Lanz is a consultant/speaker for Sanofi-Aventis. The remaining authors have no conflicts to declare pertaining to this article.

Address correspondence and reprint requests to M.J. Lanz, M.D., AAADRS Clinical Research Center, 365 Alcazar Avenue, Coral Gables, FL 33134.

E-mail address: mjlantz@pol.net

Copyright © 2011, OceanSide Publications, Inc., U.S.A.

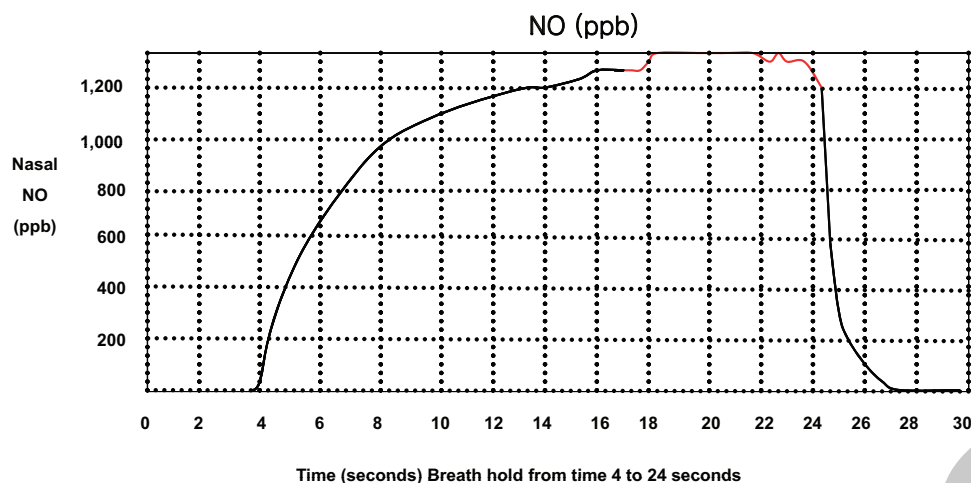


Figure 1. Example of nasal nitric oxide (nNO) tracing during breath holding (nNO = 1250 ppb and breath hold is for 20 seconds).

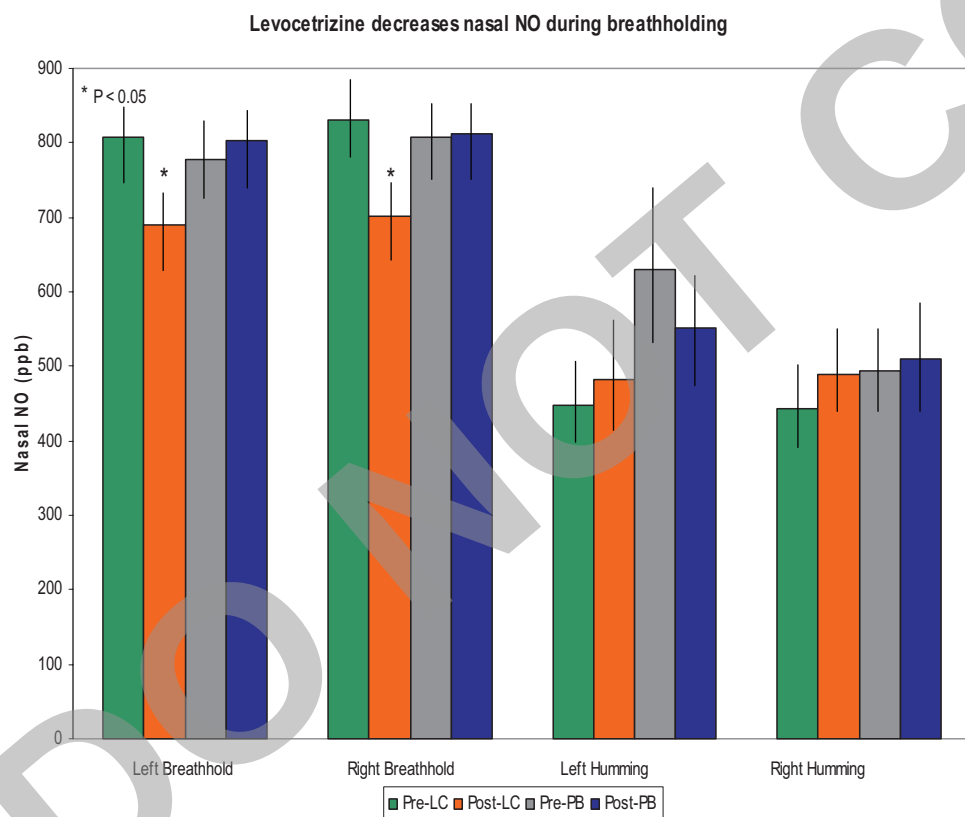


Figure 2. In perennial allergic rhinitis subjects, nasal nitric oxide during breath hold is decreased with levocetirizine, 5mg, in both nares after 2 weeks compared with placebo (* $p < 0.05$). No significant changes in nasal nitric oxide while humming were found in either treatment group.

systemic steroids within 60 days, inhaled/nasal steroids and leukotriene modifiers within 30 days, and nasal/oral antihistamines within 14 days of baseline and throughout the study other than study LC. Subjects with persistent asthma, CRS, sinus polyp disease, or acute respiratory infection were excluded. Active cigarette smokers or those on medications that would affect NO production, such as those with nitrates, were also excluded. These exclusions were set because these conditions can alter NO levels beyond what is found in PAR.

Enrolled subjects started a 1-week run-in period to assess adherence and active PAR symptoms by diary reporting (total symptom scoring [TSS]). At randomization, subjects received oral LC, 5 mg, or matching placebo tablet once daily for 2 weeks. Allowed rescue medications throughout the study were nasal saline wash irrigations and short ≤ 3 days course of nasal decongestant spray medication (phenylephrine). After the randomization visit, a 2-week treatment

period was initiated followed by a 2-week washout period and then repeated in a crossover fashion. All of the subjects returned to the clinic for study visits every 2 weeks for distribution or collection of blinded study medication, physician examination, and study procedures. Nasal NO and nEos, RQLQ, and daily TSS diary scoring were the procedures collected at each study visit.

At entry, 39 subjects complied and a resulting 31 subjects were randomized (24 female subjects; mean age, 29 years; range, 13–45 years; mean allergy history, 11 years) to either treatment arm of LC or placebo. At baseline all but four subjects were skin-prick test positive to dust mites, with the remaining four sensitized to cat or dog dander.

The nNO was measured in parts per billion using the NIOX Nasal NO software and kit (Aerocrine, New Providence, NJ). Calibration of the NIOX machine was performed as per American Thoracic Society guidelines²⁷ with calibration NO gas of 2000 ppb. A nasal olive was

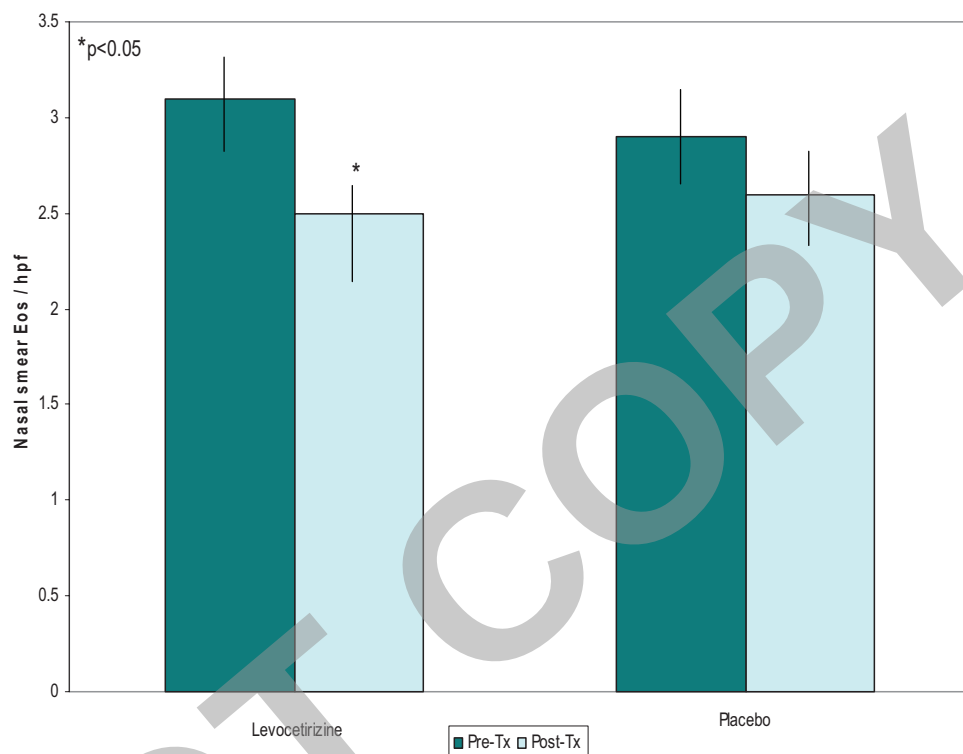


Figure 3. Nasal smear eosinophils are reduced with oral levocetirizine, 5mg, for 2 weeks compared with placebo (* $p < 0.05$).

placed at each subject's nares to directly measure the nasal NO gas. The sampling flow rate was 5 mL/s, and the nNO collection occurred over at least 20 seconds. A 5-second nNO plateau was reached for each measurement.²⁷ Immediate nNO readings were collected and recorded in triplicate for each separate nare (Fig. 1) by graders blinded to treatment arm. Nasal maneuvers for NO collection at the nare were performed in two fashions. After a deep oral inhalation, the first maneuver is a breath hold during the measurement time and the second maneuver is humming with the mouth closed during the measurement time as well. In studies regarding nasal NO collection maneuvers, it has been proposed that the first method collects NO mainly from the nasal passage and reflective of airway inflammation.^{12,28} The latter method collects NO mainly from the sinus passages and reflective of obstruction at the level of the osteomeatal complex and sinus NO milieu.

Nasal cytology smears for eosinophils were obtained with a Rhinoprobe (Arlington Scientific, Springville, UT) from the inferior turbinate. Specimens were prepared by fixing and staining with 95% ethyl alcohol and Wright Giemsa (Volu-Sol, Salt Lake City, UT), respectively. After rinsing and drying, specimens were analyzed under the light microscopy. The nEos seen under microscopy were graded for the number of eosinophils per high-power field on a scale of 0–4+ by graders blinded to treatment arm.

Subjects filled out daily diary cards to record symptoms, rescue medication use, and adverse events. Symptoms of PAR (sneezing, nasal itching, nasal secretions, ocular itching, and nasal congestion) were assessed by each subject by using a grading scale of 0–5 (0 = no symptoms, 1 = one short period, 2 = two or more short periods, 3 = most of the time but not affecting activities, 4 = most of the time and affecting activities, 5 = severe and can not perform activities). Symptom scoring was done twice daily, on awakening in the morning and at bedtime. The combined symptom score was calculated by adding the daily scores with or without the nasal congestion score (TSS-4; TSS-5). Rescue medication use was recorded in number of sprays in

the past 12 hours. Individual self-reporting of RQLQ was measured using the specific questionnaire RQLQ²⁹ at each study visit.

Skin-prick testing was performed with commercial glycerinated extracts (ALK, Port Washington, NY). The standard panel of allergens included was dust mites (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat (Fel d1), dog, hamster, horse, rabbit, feathers, house dust, mold mix, and cockroach, with a negative control (normal saline) and positive control (histamine, 1 mg/mL). Wheals were contoured with a soft marker and transferred with tape onto paper. A skin reaction of ≥ 5 mm and a histamine reaction of >3 mm greater than the negative control was considered positive.

Statistical Analysis

Statistical analyses were performed with SPSS software (SPSS, Inc., Chicago, IL). Changes between pre- and posttreatment were analyzed using paired *t*-test and a comparison between groups was made using unpaired *t*-test. Nasal cytology, diary symptom scores, and RQLQ were not normally distributed and were analyzed using nonparametric tests. To test association between baseline nNO, as a predictor, and change in outcomes measures of nEos and RQLQ, we conducted a regression analysis. Assumption of linearity was confirmed and transformation was not required. A value of $p \leq 0.05$ was considered significant.

RESULTS

Thirty-one subjects were randomized and 26 subjects completed the study. Three subjects moved to another region and two were lost to follow-up. Both respiratory maneuvers for nNO collection, breath holding and humming, were measured at each visit. In summary, significant decreases were found with the breath-holding maneuver for nNO and not with humming (Fig. 2).

With the breath hold at baseline, mean nNO was 807 ± 317 ppb in the left nare and 831 ± 332 ppb in the right nare. After LC treatment,

QOL scores improve with Levocetirizine

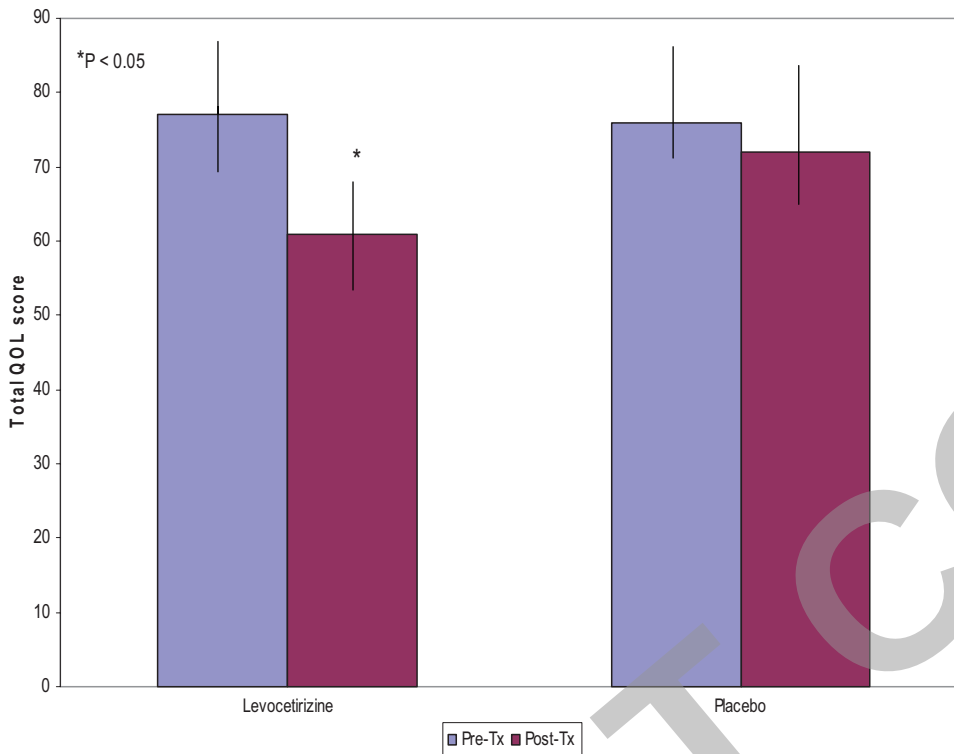


Figure 4. Rhinitis quality of life questionnaire scores are improved with oral levocetirizine, 5 mg, for 2 weeks compared with placebo (* $p < 0.05$).

the mean nNO significantly decreased to 688 ± 266 ppb in the left nare and 702 ± 286 ppb in the right nare ($p < 0.05$). During the placebo treatment, nNO at baseline was 778 ± 270 ppb and 808 ± 299 ppb (left and right) and post-nNO was 802 ± 271 ppb and 813 ± 273 ppb. Thus, no significance was found during placebo treatment. The mean change of nNO during breath hold with LC was also significant compared with placebo (-125 ppb versus $+14$ ppb; $p < 0.05$). No significance was found with any time points during either treatment arm with the humming maneuver.

In our study, there was a significant decrease in nEos with LC compared with placebo (3.1 – 2.5 with LC versus 2.9 – 2.6 with placebo; $p < 0.05$; Fig. 3). Total RQLQ scores were significantly improved with LC treatment ($p \leq 0.05$), and no improvement in RQLQ scores were found during placebo treatment (Fig. 4). Significance was found with baseline nNO as a predictor to the resulting treatment change in nEos and RQLQ. For every -100 ppb of baseline nNO, nEos decreased 0.2 and RQLQ scores decreased 4.6 , after LC treatment.

In TSS-4 symptom scoring, a trend toward improvement during LC treatment was found ($p < 0.075$). Accordingly, during the placebo treatment, a significant worsening in symptom scores was found ($p \leq 0.05$). No significant improvement was found in nasal congestion scores with LC, but significant worsening was found with placebo ($p < 0.05$). No significant differences were found in need for rescue medications or reporting of adverse events with either treatment arm. Reports of adverse events were few and mild in intensity and outcome. No serious adverse events were reported.

DISCUSSION

The need for an objective marker in PAR that is easily obtained and noninvasive is sorely needed. Our primary outcome measure, nNO, is a validated marker of airway inflammation in allergic rhinitis.^{3–6} In this double-blind, placebo-controlled crossover study, we have shown that nNO is significantly reduced after 2 weeks of daily LC. This is the first study to reveal reduction in exclusively derived nNO

with LC therapy in adults with PAR. This novel finding supports the hypothesis of the anti-inflammatory effect that this new generation antihistamine may have.

The reduction in nNO was found when subjects performed the breath-holding maneuver and not the humming maneuver. As it has been suggested, breath holding nNO collection reflects the inflammatory milieu of the nasal passages, while humming nNO reflects that of the paranasal sinuses and osteomeatal complex.¹² Our study results confirm that breath-holding collection for nNO is sensitive for treatment effect changes in PAR. Because similar nNO levels would be expected in bilateral nares with PAR, the finding that no significant differences in nNO were found between nares was expected. The reduction found in breath holding nNO was mirrored with the reduction in nEos, indicating an anti-inflammatory effect. This finding confirms one prior study¹⁹ with nasal scrapings and eosinophils with LC in PAR. These results strengthen the role of nNO in the clinical assessment of PAR inflammation.

The findings in this study are novel in that treatment improvements in nNO correspond with similar improvements in nEos and RQLQ. Additionally, baseline nNO predicted significant improvements in nEos and RQLQ. Because lower baseline nNO levels were most predictive, this may be caused by the treatment intervention being an antihistamine. Perhaps nasal steroid treatment would show comparatively greater baseline nNO to be more predictive because, theoretically, more inflammation is present. Comparative studies between antihistamine and nasal steroid with the same study design and nNO collection will be required.

The subjective outcomes in the study reflected the reductions found with nNO. Similar to previous findings,^{18,19} we showed that the subjective measures of RQLQ scores were significantly improved during LC and not placebo. A statistical trend toward improvement in total diary scores was found during LC, including four nasal/ocular scores. Significant worsening was found in all diary scores, including nasal congestion during placebo. Although no significance

was found with nasal congestion scores with LC, significant worsening of congestion with placebo is important to note. This lack of improvement with LC and worsening with placebo suggests that nasal steroids may be required for complete anti-inflammatory effect with regard to nasal congestion in PAR. Second, as is found with exhaled NO and asthma, the improvements in objective measures may precede in time any improvements that would be found with subjective measures.

The limitations of the study were that this was a small, single-site study with the majority of subjects monosensitized to dust mites. Because of the methodology issues with nNO collection, this study investigated subjects with PAR only and none with accompanying CRS.

Future focus on the clinical use of nNO will be to assess an individual's own course of disease with treatment and level of inflammation over time. Based on the concept of personalized medicine, measurements of the anti-inflammatory effects of other PAR medications will be necessary to individualize varying therapeutic responses of patients. Previous studies investigating nNO with antihistamines have been with only cetirizine or LC in seasonal allergic rhinitis subjects as an add-on therapy and were mixed in their results.^{30,31} Future studies with other oral or nasal antihistamines with nNO are needed to delineate any differences in anti-inflammatory response and efficacy.

In conclusion, objective inflammatory monitoring of PAR has been lacking for the clinician and subjective symptom reporting has been the norm. We have shown the use of an objective measure of clinical response with nNO that is similar to nEos in PAR.

REFERENCES

- Greiner AN. Allergic rhinitis: Impact of the disease and considerations for management. *Med Clin North Am* 90:17–38, 2006.
- Broide DH. Allergic rhinitis: Pathophysiology. *Allergy Asthma Proc* 31:370–374, 2010.
- Durham SR. The inflammatory nature of Allergic Disease. *Clin Exp Allergy* 28(suppl 6):20–24, 1998.
- Marple BF. Allergic rhinitis and inflammatory airway disease: Interactions within the unified airspace. *Am J Rhinol Allergy* 24:249–254, 2010.
- Arnal JF, Didier A, Rami J, et al. Nasal nitric oxide is increased in allergic rhinitis. *Clin Exp Allergy* 27:358–362, 1997.
- Struben VM, Wieringa MH, Feenstra L, et al. Nasal nitric oxide and nasal allergy. *Allergy* 61:665–670, 2006.
- Boot JD, de Kam ML, Mascelli MA, et al. Nasal nitric oxide: Longitudinal reproducibility and the effects of a nasal allergen challenge in patients with allergic rhinitis. *Allergy* 62:378–384, 2007.
- Kharitonov SA, Rajakulasingam K, O'Connor B, et al. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 99:58–64, 1997.
- Kharitonov SA, and Barnes PJ. Clinical aspects of nitric oxide. *Eur Respir J* 16:781–792, 2000.
- De Winter-de Groot K, and van der Ent C. Measurement of nasal nitric oxide: Evaluation of six different sampling methods. *Eur J Clin Invest* 39:72–77, 2009.
- Kharitonov SA, Walker L, and Barnes PJ. Repeatability of standardized nasal nitric oxide measurements in healthy and asthmatic adults and children. *Respir Med* 99:1105–1114, 2005.
- Struben V, Wieringa M, Mantingh C, et al. Nasal NO measurements by direct sampling from the nose during breath hold: Aspiration flow, nasal resistance and reproducibility. *Eur Arch Otorhinolaryngol* 263:723–728, 2006.
- Wang DY, Hanotte F, De Vos C, et al. Effect of cetirizine, levocetirizine, and dextrocetirizine on histamine-induced nasal response in healthy adult volunteers. *Allergy* 56:339–343, 2001.
- Gillard M, Van Der Perren C, Moguilevsky N, et al. Binding characteristics of cetirizine and levocetirizine to human H1 histamine receptors: Contribution of Lys (191) and Thr (194). *Mol Pharmacol* 61:391–399, 2002.
- Walsh GM. Anti-inflammatory properties of antihistamines: An update. *Clin Exp Allergy Rev* 5:21–25, 2005.
- Potter PC, and Pediatric Levocetirizine Study Group. Levocetirizine is effective for symptom relief including nasal congestion in adolescent and adult (PAR) sensitized to house dust mites. *Allergy* 58:893–899, 2003.
- Patou J, De Smedt H, van Cauwenberge P, et al. Pathophysiology of nasal obstruction and meta-analysis of early and late effects of levocetirizine. *Clin Exp Allergy* 36:972–981, 2006.
- Bachert C, Bousquet J, Canonica GW, et al. Levocetirizine improves quality of life and reduces cost in long-term management of persistent allergic rhinitis. *J Allergy Clin Immunol* 114:838–844, 2004.
- Pasquali M, Baiardini I, Rogkakou A, et al. Levocetirizine in persistent allergic rhinitis and asthma: Effects on symptoms, quality of life, and inflammatory parameters. *Clin Exp Allergy* 36:1161–1167, 2006.
- Albanesi C, Pastore S, Fanale-Belasio E, et al. Cetirizine and hydrocortisone differentially regulate ICAM-1 expression and chemokine release in cultured human keratinocytes. *Clin Exp Allergy* 28:101–109, 1998.
- Jazrawi E, Stirling R, and Chung KF. Effects of levocetirizide on eotaxin-induced eosinophil chemotaxis with or without interleukin-5 priming in vitro. *Allergy* 56(suppl 68):138, 2001.
- Thomson L, Blylock MG, Sexton DW, et al. Cetirizine and levocetirizine inhibit eotaxin-induced eosinophil transendothelial migration through human dermal or lung microvascular endothelial cells. *Clin Exp Allergy* 32:1187–1192, 2002.
- Ciprandi G, Cirillo IG, Vizzaccaro A, et al. Levocetirizine improves nasal symptoms and air flow in patients with persistent allergic rhinitis: a pilot study. *Eur Ann Allergy Clin Immunol* 37:25–29, 2005.
- Ciprandi G, Cirillo IG, Vizzaccaro A, et al. Levocetirizine improves nasal obstruction and modulates cytokine pattern in patients with seasonal allergic rhinitis: A pilot study. *Clin Exp Allergy* 34:958–964, 2004.
- Hasala H, Janka-Junttila N, Moilanen E, et al. Levocetirizine and cytokine production and apoptosis of human eosinophils. *Allergy Asthma Proc* 28:582–589, 2007.
- Bommarito L, Guida G, Heffler E, et al. Nasal nitric oxide concentration in suspected chronic rhinosinusitis. *Ann Allergy Asthma Immunol* 101:358–362, 2008.
- American Thoracic Society/European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide 2005. *Am J Respir Crit Care Med* 171:912–930, 2005.
- Lanz MJ, Prendes S, Peyrou N, et al. Nasal nitric oxide as a noninvasive marker in the antibiotic treatment of acute bacterial sinusitis. *J Allergy Clin Immunol* 121:530–531, 2008.
- Juniper EF, Guyatt GH, and Dolovich J. Assessment of quality of life in adolescents with allergic rhinoconjunctivitis: Development and testing of a questionnaire for clinical trials. *J Allergy Clin Immunol* 93:413–423, 1994.
- Barnes ML, Ward JH, Fardon TC, and Lipworth BJ. Effects of levocetirizine as add-on therapy to fluticasone in seasonal allergic rhinitis. *Clin Exp Allergy* 36:676–684, 2006.
- Ho CY, and Tan CT. Comparison of antileukotrienes and antihistamines in the treatment of allergic rhinitis. *Am J Rhinol* 21:439–443, 2007. □

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.