



Clinical standardization of two controlled allergen challenge facilities

The Environmental Exposure Unit and the Biogenics Research Chamber

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ARTICLE INFO

Article history:

Received for publication November 5, 2018.

Received in revised form February 9, 2019.

Accepted for publication March 6, 2019.

ABSTRACT

Background: Controlled allergen challenge facilities (CACF), in disparate geographic regions with dissimilar engineering and base populations, have historically functioned as single, independent sites in clinical allergy trials. We aimed to demonstrate “between-unit reproducibility” to allow controlled challenge trials of participants using 2 CACFs.

Objective: To compare and standardize 2 CACFs located in Kingston, Ontario, Canada, and San Antonio, Texas, by examining participant-reported symptom severity during qualifying and treatment visits and evaluating response to treatment, while using the same allergen.

Methods: At 2 different CACFs, participants were enrolled in a double-blind, placebo-controlled, crossover intervention trial with cetirizine 10 mg. Different distribution devices delivered common short ragweed pollen via laminar air flow and maintained an airborne concentration of 3500 ± 700 grains/m³ in both facilities. A 1-hour “sham” run with no pollen release preceded a priming exposure of 3 hours and was followed 3 days later by a qualifying/treatment 5-hour exposure. At least 14 days later, another priming exposure was followed by the crossover exposure and treatment.

Results: Forty-eight and 43 subjects completed the study at Kingston and San Antonio, respectively. Demographics were similar. Fewer than 10% exhibited symptoms with sham exposure. No significant differences were found between the 2 facilities in maximal total rhinoconjunctivitis symptom score, total nasal symptom score, and total ocular symptom score, nor in areas under the curve. In both facilities, no significant effects of cetirizine 10 mg over placebo were detected.

Conclusion: The results were equivalent, demonstrating that the 2 CACFs can be used together in dual-center clinical trials and show the possibility of multicenter trials involving multiple CACFs.

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Disclosures: Dr. Anne K. Ellis has participated in advisory boards for ALK Abello, Circassia Ltd, GlaxoSmithKline, Merck, and Novartis, and has been a speaker for Aralez, AstraZeneca, Merck, Mylan, Novartis, Padiapharm, Pfizer, and Takeda. Her institution has received research grants from Circassia Ltd, Green Cross Pharmaceuticals, GlaxoSmithKline, Sun Pharma Advanced Research Corporation, Merck, Novartis, Sanofi, and Pfizer. Dr. Ellis is also a consultant to Bayer Consumer Health

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Division and Johnson and Johnson. Mark Tenn, Lisa Steacy, Daniel Adams, Terry Walker, Cynthia Visness, and Robert James have no conflicts of interest to disclose. Drs. Charles P. Andrews, Robert L. Jacobs, and Daniel A. Ramirez are co-owners of Biogenics Research Chamber. Cynthia G. Rather is the Executive Director of Biogenics Research Chamber. There are no other conflicts of interest.

Funding Sources: This study was funded by the Kingston Allergy Research Trust, the Biogenics Research Chamber, and through a NIAID Grant (UM2AI117870) only. The funding sources are correctly listed as is. The list referenced above (GlaxoSmithKline, Merck, Novartis, Pfizer, and Sanofi) are the relevant disclosures of the first author (Dr. Anne K. Ellis).

Introduction

To overcome the confounding factors present in natural exposure allergy studies, controlled allergen challenge facilities (CACFs) were developed to provide exposures under tightly regulated conditions.¹ Controlled allergen challenge facilities' engineering allows the creation of stable, controlled environments for studies in disparate geographical regions to mitigate the confounding factors of traditional natural exposure studies of varying aeroallergens, wind, rain, humidity, and temperature, and differences in the magnitude of pollen exposure between study participants caused by individual exposures.^{1–3} These characteristics of CACFs allow for larger numbers of subjects to be simultaneously studied in shorter time frames at multiple sites.

Since the development of the first CACF in 1987 (The Vienna Challenge Chamber, Austria), multiple facilities have been created worldwide, such as the Environmental Exposure Unit in Kingston, Canada, and the Biogenics Research Chamber in San Antonio, Texas.⁴ Although these facilities have been developed using dissimilar engineering and different pollen dispersal technologies, they are capable of achieving temporally stable and homogenous pollen distribution under standardized conditions.^{5,6} However, CACFs have traditionally operated as single study sites.

A workshop at the US National Institute of Allergy and Infectious Diseases convened in 2010⁷ to initiate a discussion on design of a validation protocol to assure reliability of outcomes between facilities to use multiple locations as a new era of personalized medicine evolves and novel drugs and treatments are developed that are best studied in controlled environments. Several questions were posed during this meeting that required elucidation before attempts could be made to standardize across facilities. Would subjects in 1 geographic area, exposed during the natural setting to a different species of a given plant, respond adequately to a single nondominant species used in the allergen chamber studies? What are the relationships of signs and symptoms in controlled studies within an exposure facility vs those seen during the natural setting exposures? What are the "between facilities" variabilities in the clinical outcomes of allergen exposures under standardized conditions that would be applied to all participant exposure facilities? Other questions and observations have surfaced since that meeting, including meetings in 2015 in which representatives of European and North American chambers met and published concepts of harmonizing these facilities.⁴

To address these issues, suggestions have been made to validate and compare CACFs to allow studies to be performed at more than 1 site.^{4,8} Harmonizing study protocols and establishing comparable clinical outcomes between CACFs may allow for clinical efficacy studies to be conducted across multiple facilities. Such trials have a great potential to be superior to the single-center CACF trials conducted to date. Studies have already demonstrated the reproducibility of symptoms across different CACF visits (intra-CACF variability) and their correlation to symptoms elicited from natural outdoor exposure.^{9–14} However, the variability between facilities (inter-CACF variability) has not been assessed. This translates into the question of whether allergen exposure–induced symptoms and the effects of various treatments obtained in different CACFs are comparable, when identical protocols are used. The aim of the current study was to standardize and compare 2 CACFs, one located in Kingston, Ontario, Canada, and the second in San Antonio, Texas, by examining participant-reported symptom and treatment responses at both sites under controlled conditions using the same allergen.

Methods

Study Participants

The trial enrolled healthy males and females 18 to 65 years of age with a history of at least 2 years of ragweed season correlative

seasonal allergic rhinoconjunctivitis (SAR) and a positive skin prick test (SPT) (defined as a wheal diameter ≥ 5 mm larger than the negative control wheal) to common short ragweed (*Ambrosia artemisiifolia*) pollen. Key exclusion criteria included a history of receiving immunotherapy containing short ragweed within the last 3 years and any signs or symptoms of active seasonal or perennial rhinitis.

Allergen Challenge Facilities

Participants were exposed to common short ragweed pollen (*A. artemisiifolia*, Greer Laboratories, Lenoir, NC) in Kingston and San Antonio during a series of challenge visits. In both facilities, ventilation systems provided 100% fresh filtered outdoor air, which was directly exhausted outside. Humidity and temperature were maintained at comparable levels across both study sites (40% and 50%–52%; 22–24°C and 21–22°C in the Kingston and San Antonio sites, respectively). Airborne pollen levels were maintained at 3500 ± 700 grains/m³ in both facilities. To ensure even distribution and steady concentration of airborne pollen, airborne pollen levels were measured at 30-minute intervals at stations positioned in the respective facilities; 7 Rotorod samplers and a Kanomax Handheld laser particle counter in Kingston, and 5 pollen counting stations using manual Allergenco cassette systems in San Antonio (Figs 1 and 2).

Study Design and Treatment

This was a randomized, dual-center, double-blind, placebo-controlled, 2-way crossover study ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study?term=NCT02543346) identifier NCT02543346) that took place at 2 separate CACFs; in Kingston, Ontario, Canada, and in San Antonio, Texas. The protocol, amendments, and informed consent documents were granted ethical clearance by the Queen's University Health Sciences and Affiliated Teaching Hospital Research Ethics Board and the IntegReview Institutional Review Board. All participants provided written informed consent, and the study was conducted according to Good Clinical Practice standards. It was conducted in accordance with the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013) and the International Council for Harmonisation Guideline on Good Clinical Practice.

The study comprised 3 phases: run-in and priming, qualifying/treatment, and priming/crossover treatment (Fig 3).

A screening visit was conducted to assess participant eligibility. Eligible participants then entered the run-in phase and recorded instantaneous nasal and ocular symptoms twice daily for 4 days before and including the morning of the priming visit. Participants who achieved a mean total nasal symptom score (TNSS) above 5 during the run-in phase were excluded from the study. The remaining participants entered the CACF for the sham/priming visit. For the first 60 minutes, pollen dispersal equipment was activated without delivering pollen (sham run). At 60 minutes, dispersal of ragweed pollen was initiated and continued for the remaining 180 minutes of the priming exposure. Participants recorded nasal and ocular symptoms at baseline and then at 30-minute intervals during the challenge. Participants self-assessed TNSS—runny nose, itchy nose, sneezing, nasal congestion—and total ocular symptom score (TOSS)—itchy eyes, watery eyes, and red/burning eyes—on a scale of 0–3, using a 4-point Likert scale (0, none; 1, mild; 2, moderate; 3, severe) (eTable 1 in the Online Repository). The TNSS and TOSS scores were combined to calculate the total rhinoconjunctivitis symptom score (TRSS) (eTable 2 in the Online Repository). Participants who achieved a TNSS of 6 or greater during the sham run were excluded from the study. The remaining participants were required to obtain a TNSS ≥ 6 at any point during the priming exposure period (60–240 minutes) to proceed to the qualifying/treatment phase.

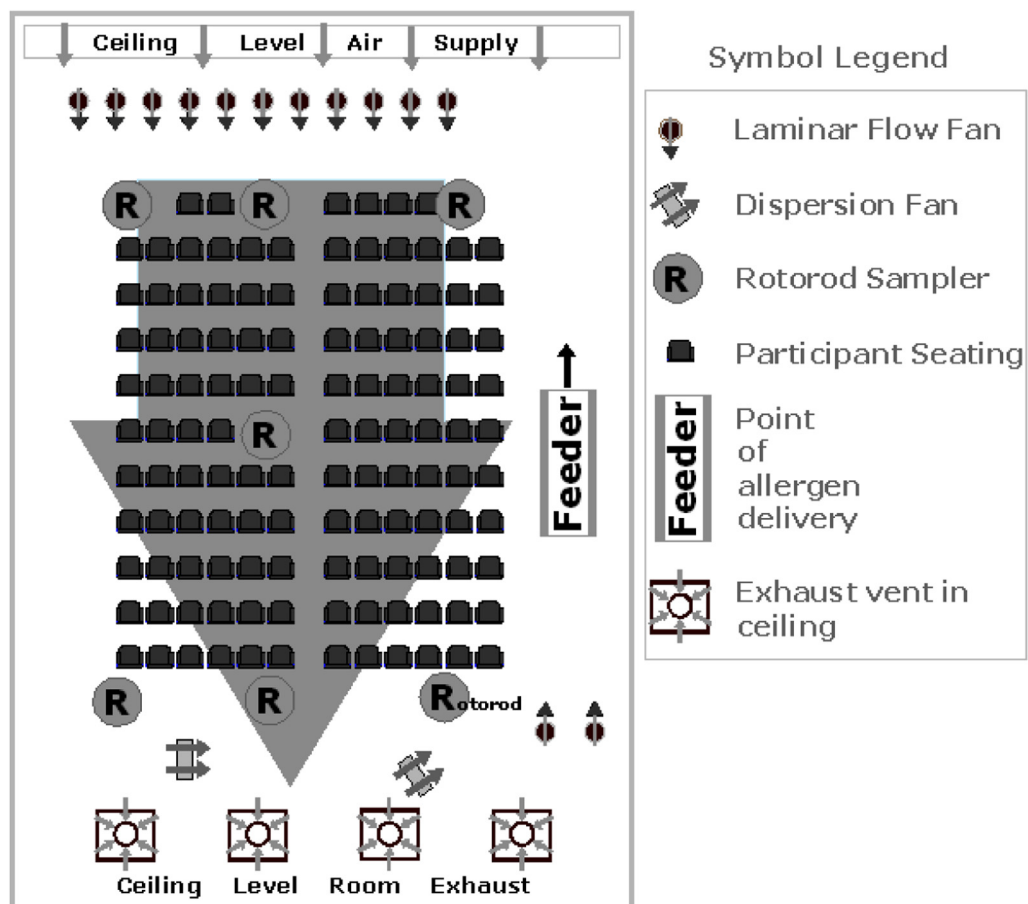


Figure 1. Floor schematic of the Environmental Exposure Unit.

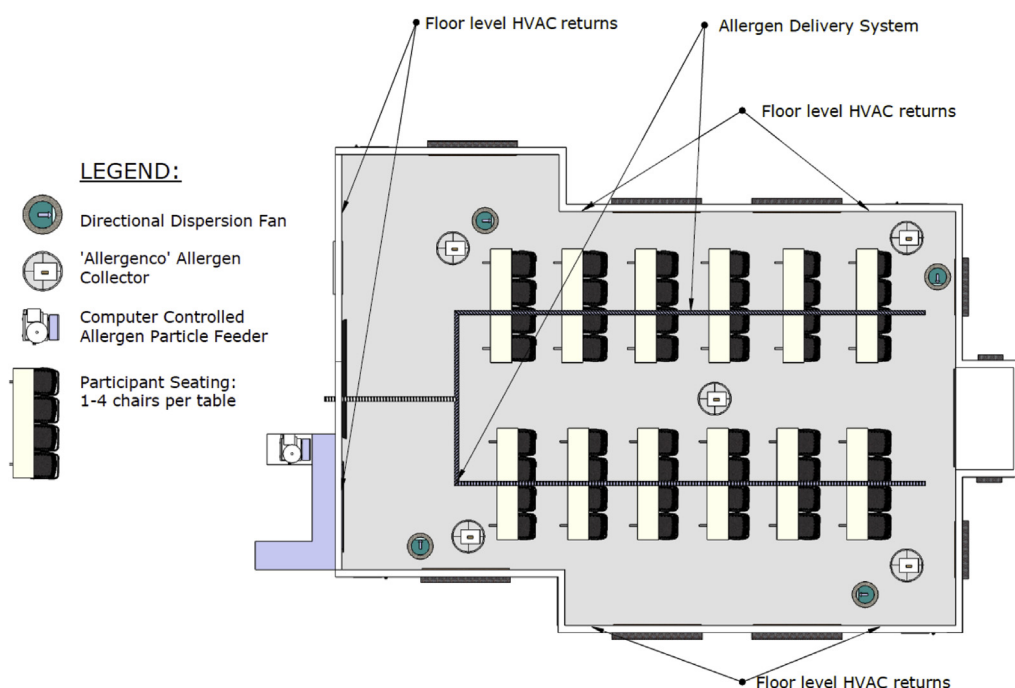


Figure 2. Floor schematic of the Biogenics Research Chamber.

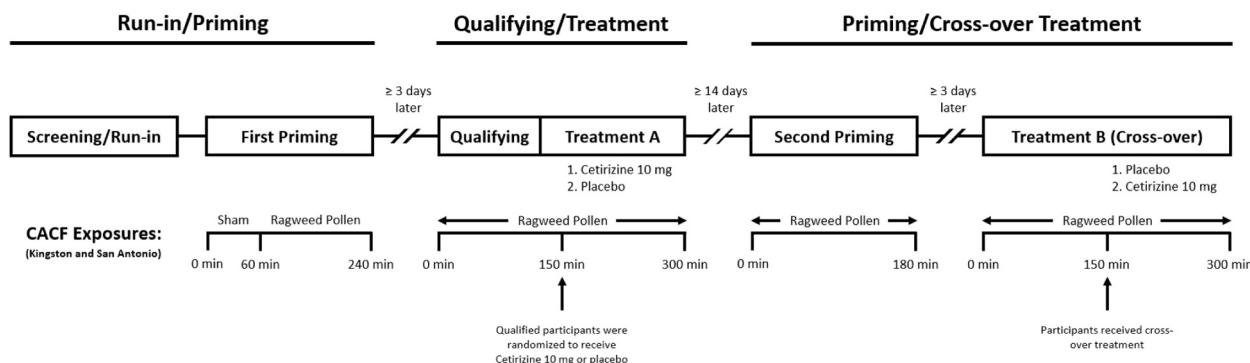


Figure 3. Study diagram.

Three days later, participants returned to the study site for the qualifying/treatment phase composed of a 5-hour (300 minutes) ragweed pollen challenge in the CACF. Nasal and ocular symptoms were recorded at baseline and at 30-minute intervals during the challenge. Participants were required to achieve a TNSS ≥ 6 at any point within the first 120 minutes of pollen exposure to remain in the study. Those who did not achieve a TNSS ≥ 6 within this period were disqualified from the study and considered screen failures. Qualifying participants were randomized in a 1:1 ratio to either a single dose of oral cetirizine 10 mg or placebo that they received at 150 minutes. Visual analog scale (VAS) scores and global rating of change scores (GRCS) were collected at the end of the pollen challenge to assess symptom severity global scores (eTable 3 in the Online Repository).

At least 14 days later, participants returned to the CACF for the crossover phase. Participants were evaluated to determine continued eligibility and then entered the second priming exposure consisting of a 3-hour ragweed pollen challenge. They returned to the study site 3 days after priming, were evaluated for continued eligibility, and underwent a 5-hour ragweed pollen challenge, during which they received crossover study treatment, as described previously.

Study Outcomes

The primary outcome of the study was the comparison of the estimated treatment area under the concentration-time curve (AUC) for TRSS between facilities in the cetirizine and placebo treatment arms. The AUC was calculated using the linear trapezoidal rule from 2.5 to 5 hours after the start of pollen exposure.

Secondary outcomes included the comparisons of estimated treatment AUCs for TNSS, TOSS, VAS, and GRCS in the cetirizine and placebo treatment arms.

Safety was assessed through physical examination findings and vital sign measurements (screening visit only), urine pregnancy tests, and incidence and severity of adverse events (AEs).

Statistical Analyses

Using data from previous studies, a mean difference (standard deviation [SD]) in the TRSS AUC of 1 (1.6) unit between active and placebo was determined to be a clinically meaningful effect. Therefore, a sample size of 42 participants per CACF (total of 84 participants between study sites) would provide 80% power at a 2-sided α level of .05 to detect a 1-unit difference in the AUC between active and placebo treatment arms. Assuming a 20% loss to follow-up, the estimated sample size was 51 participants for each CACF.

The safety population consisted of all participants deemed eligible during the screening visit. The intention-to-treat population was included in the efficacy analyses and consisted of all randomized participants who received 1 or more doses of study

medication (cetirizine or placebo). The CACF facilities were tested for equivalence with respect to the primary and secondary AUC outcomes using linear mixed effects repeated measures models controlled for their symptom scores at the start of treatment (2.5 hours post pollen exposure). The AUC equivalence between the facilities was inferred when the 90% confidence interval of their AUC measure's difference between the facilities was completely contained within $\pm 20\%$ of the mean AUC of the 2 facilities. Additional detail about the model is provided in the [Methods](#) section in this article's Online Repository. A 2-sided P value of $\leq .05$ was considered significant. Statistical tests were performed using SAS statistical software (SAS Institute, Cary, NC) and GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA).

Results

Participant Demographics

A total of 98 participants were randomized into the study (51 at Kingston, 47 at San Antonio) (eFig 1 in the Online Repository). The mean age was 42.4 and 46.1 years at the Kingston and San Antonio sites, respectively. More than half of the participants at both sites were female (Kingston, 62.7%; San Antonio, 53.2%), and a greater proportion were of Hispanic or Latino background at the San Antonio site (55.3%) vs the Kingston site (2.0%) (Table 1). The mean baseline ragweed-specific IgE levels (8.4 KU_A/L vs 5.2 KU_A/L) and ragweed-induced SPT wheal size (11.7 mm vs 8.9 mm) were greater in participants from the Kingston site vs the San Antonio site. The mean years of ragweed-induced SAR were comparable between the Kingston (27.6 years) and San Antonio (27.4 years) sites (Table 1). Mean pollen counts for both sites were within the pre-specified range of 3500 ± 700 grains/m³ during the exposure periods (Fig 4).

Sham Exposure

During the 60-minute sham run of the initial priming visit, Kingston disqualified 5 of 64 (7.8%) and San Antonio disqualified 6 of 69 (8.7%) participants for excessive symptoms. Per protocol, these participants were excluded from the study.

Primary Outcome Analyses

Site equivalence was demonstrated for TRSS between the 2 chambers in both placebo and cetirizine arms (Table 2). The TRSS AUCs were also statistically equivalent between the 2 chambers in both treatment arms (placebo, 11.9 ± 0.49 vs 11.7 ± 0.51 , $P = .81$; cetirizine, 11.5 ± 0.49 vs 10.8 ± 0.52 , $P = .33$). In neither facility was a statistically significant treatment difference observed for cetirizine on the AUC for TRSS (Kingston, $P = .59$; San Antonio, $P = .18$) (Table 2 and Fig 5), emphasizing the equivalence of the sites.

Table 1
Participant Characteristics

Characteristics	Kingston (n = 51)	San Antonio (n = 47)	Overall (n = 98)
Mean age in years (SD)	42.4 (11.2)	46.1 (12.2)	44.2 (11.8)
Female (%)	32 (62.7)	25 (53.2)	57 (58.2)
Race (%)			
Hispanic or Latino	1 (2.0)	26 (55.3)	27 (27.6)
Non-Hispanic or Latino	50 (98.0)	21 (44.7)	71 (72.4)
Mean ragweed-specific IgE (SD) ^a	8.4 (10.5)	5.2 (10.9)	6.9 (10.8)
Mean ragweed-induced wheal size (in mm, SD)	11.7 (4.8)	8.9 (4.0)	10.4 (4.6)
Mean years of ragweed-induced SAR (SD) ^b	27.6 (12.1)	27.4 (13.3)	27.5 (12.6)

IgE, immunoglobulin E; SAR, seasonal allergic rhinoconjunctivitis; SD, standard deviation.

^aKingston site, n = 50; San Antonio, n = 47; IgE levels listed in KU/L.

^bKingston, n = 50; San Antonio, n = 45.

Secondary Outcome Analyses

Between the 2 chambers, site equivalence was only demonstrated for TNSS in the placebo and cetirizine arms (Table 2). Site equivalence results for TOSS were inconclusive. After allergen exposure, both TNSS AUC and TOSS AUC were statistically equivalent between both chambers in the placebo (TNSS, 7.3 ± 0.30 vs 7.5 ± 0.32 , $P = .62$; TOSS, 4.6 ± 0.23 vs 4.2 ± 0.24 , $P = .32$) and cetirizine (TNSS, 7.1 ± 0.30 vs 6.9 ± 0.32 , $P = .66$; TOSS, 4.4 ± 0.23 vs 3.9 ± 0.24 , $P = .19$) arms. Statistically significant treatment differences were not observed for TNSS (Kingston, $P = .73$; San Antonio, $P = .16$) or TOSS (Kingston, $P = .48$; San Antonio, $P = .29$) in either facility (Table 2; Fig 5).

For VAS and GRCS, site equivalence could not be demonstrated (Table 2). The VAS AUC and GRCS AUC were statistically equivalent between the 2 chambers in both the placebo (VAS, 52.7 ± 3.98 vs 47.7 ± 4.18 , $P = .40$; GRCS, 1.2 ± 0.55 vs -0.1 ± 0.57 , $P = .10$) and cetirizine arms (VAS, 45.3 ± 4.00 vs 44.1 ± 4.19 , $P = .84$; GRCS, 1.8 ± 0.55 vs 0.9 ± 0.58 , $P = .26$). Likewise, statistically significant treatment differences were not observed in either of the 2 facilities (VAS: Kingston, $P = .11$; San Antonio, $P = .46$ and GRCS: Kingston, $P = .39$; San Antonio, $P = .17$) (Table 2).

Safety

A total of 144 study participants (75 at the Kingston site, 69 at the San Antonio site) were included in the safety population. Neither serious nor severe AEs were reported by either facility on exposure to allergen or in relation to treatment.

Discussion

This is the first study using an identical protocol and the same batch of allergen to directly compare 2 CACFs according to participant-reported symptom severity and treatment outcomes to an anti-allergic intervention. Site equivalence was established under Food and Drug Administration rules, opening a path for dual-site trials involving these 2 facilities. Such trials may require fewer participants per site than conventional field studies and effectively eliminate many confounding factors. This has the potential of accelerating the development of new SAR, and possibly asthma, interventions. Additionally, this study sets the stage for other CACFs that may wish to establish site equivalence.

Geography, Climate, Demographics

This study was conducted at 2 sites with dramatically contrasting differences in climate, natural exposures, and subject population. San Antonio is a warm dry temperate climate with 3 ragweed species (giant, false, and western), whereas Kingston is a cool temperate climate with short ragweed as the dominant species. The natural seasons of ragweed pollination differ between the 2 geographic areas. The peak of ragweed season in Kingston is in early September, whereas in San Antonio the peak occurs in early October.³ The subjects of the Kingston area are of European descent, and more than 50% of the subjects of San Antonio were Hispanics of Mexican descent. The study was done at both sites outside of the local ragweed season (mid-winter in Kingston and late-fall in San Antonio). This study confirms, in a comparative manner, a previous report that subjects in disparate geographic locations, of different genetic backgrounds, with natural exposure to different species of a plant will react similarly to a common genus allergen.¹³

Chamber Characteristics

The CACFs maintained a clean, stable atmosphere and delivered uniform amounts of allergen throughout the course of an exposure despite differences in technology and engineering.^{5,6} Both facilities had certain similar characteristics, using clean room techniques and materials. Flooring and walls were constructed with smooth, seamless materials that were washable. Ceilings and light fixtures were constructed with clean room material that is sealed and fixed in place. Entry and exit doors were tight fitting to prevent leakage and to conform to local building codes. Observation windows were 2-way, airtight, and allowed for full view of the exposure area. Pass-through windows were used to allow for transfer of small items between the exposure area and control room. Adequate communication systems, both audio and video, allowed for instant messaging between the control room and the exposure area. The air handling systems had the capacity to cool, or heat, and maintain a fixed temperature, humidity, and carbon dioxide level at maximal operational capacity under extreme outside weather conditions.

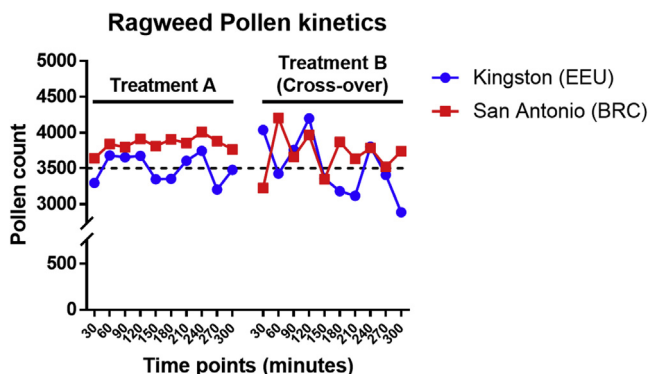


Figure 4. Ragweed pollen counts during the treatment and crossover exposure periods in the Environmental Exposure Unit and Biogenics Research Chamber. Ragweed pollen counts were recorded at specified time points during the 5-hour ragweed pollen challenges in both the Kingston (EEU) and San Antonio (BRC) facilities. Pollen counts at each time point were calculated as an average over 7 Rotorod samplers in the EEU and 5 pollen counting stations in the BRC. Horizontal dotted line represents the target airborne pollen concentration of 3500 grains/m³. BRC, Biogenics Research Chamber; EEU, Environmental Exposure Unit.

Table 2

Treatment Effect and Site Equivalence between the Kingston and San Antonio Controlled Allergen Challenge Facilities for All Efficacy Endpoints

	Placebo	Cetirizine	Overall (ITT)	Treatment difference
Total rhinoconjunctivitis symptom score				
Kingston ^a	11.9 (0.49)	11.5 (0.49)	11.7 (0.39)	-0.3 (0.59)
San Antonio ^a	11.7 (0.51)	10.8 (0.52)	11.3 (0.41)	-0.9 (0.63)
Site difference ^b	0.2 (0.72; -1.02, 1.37)	0.7 (0.73; -0.49, 1.91)	0.4 (0.58; -0.52, 1.41)	<i>P</i> = .59
Site equivalence Boundary ^c	± 2.36	± 2.23	± 2.30	<i>P</i> = .18
Site equivalence	Yes	Yes	Yes	
Total Ocular Symptom Score				
Kingston ^a	4.6 (0.23)	4.4 (0.23)	4.5 (0.19)	-0.2 (0.26)
San Antonio ^a	4.2 (0.24)	3.9 (0.24)	4.1 (0.20)	-0.3 (0.28)
Site Difference ^b	0.3 (0.34; -0.22, 0.90)	0.4 (0.34; -0.12, 1.01)	0.4 (0.28; -0.07, 0.86)	<i>P</i> = .29
Site equivalence Boundary ^c	± 0.88	± 0.83	± 0.86	
Site equivalence	Inconclusive	Inconclusive	Inconclusive	
Total nasal symptom score				
Kingston ^a	7.3 (0.30)	7.1 (0.30)	7.2 (0.24)	-0.1 (0.38)
San Antonio ^a	7.5 (0.32)	6.9 (0.32)	7.2 (0.25)	-0.6 (0.40)
Site Difference ^b	0.2 (0.46; -0.98, 0.53)	0.2 (0.45; -0.55, 0.95)	0.0 (0.36; -0.62, 0.59)	<i>P</i> = .73
Site equivalence boundary ^c	± 1.48	± 1.40	± 1.44	<i>P</i> = .16
Site equivalence	Yes	Yes	Yes	
Visual Analog Scale Score Population				
Kingston ^a	52.7 (3.98)	45.3 (4.00)	49.0 (3.30)	-7.3 (4.51)
San Antonio ^a	47.7 (4.18)	44.1 (4.19)	45.9 (3.45)	-3.5 (4.74)
Site Difference ^b	5.0 (5.89; -4.75, 14.74)	1.2 (5.90; -8.57, 10.97)	3.1 (4.90; -5.05, 11.25)	<i>P</i> = .11
Site equivalence boundary ^c	± 10.04	± 8.94	± 9.49	<i>P</i> = .46
Site equivalence	Inconclusive	Inconclusive	Inconclusive	
Global Rating of Change Score				
Kingston ^a	1.2 (0.55)	1.8 (0.55)	1.5 (0.42)	0.6 (0.70)
San Antonio ^a	-0.1 (0.57)	0.9 (0.58)	0.4 (0.44)	<i>P</i> = .39
Site difference ^b	1.3 (0.81; -0.01, 2.66)	0.9 (0.81; -0.42, 2.26)	1.1 (0.63; 0.08, 2.17)	1.0 (0.74)
Site equivalence boundary ^c	± 0.11	± 0.27	± 0.19	<i>P</i> = .17
Site equivalence	Inconclusive	No	Inconclusive	

Results are from a linear mixed effects repeated measures model controlled for initial symptom scores at dosing (2.5 hours post-pollen exposure).

ITT, intention-to-treat; SE, standard error; CI, confidence interval.

^aEstimate (SE).^bEstimate (SE, 90% CI).^cBecause neither site was a "gold standard," boundaries were defined as $\pm 20\%$ of the mean estimate between the 2 sites.

Pollen feeders delivered accurate and adjustable amounts of pollen into the dispersal system, with pollen measured by weight in San Antonio and by laser methods in Kingston. In San Antonio, pollen was distributed throughout the chamber via a ceiling-height tube system with a vertical laminar airflow. In Kingston, fans were used to disburse pollen with a horizontal laminar flow. Both facilities used dispersal fans to move pollen around the periphery of the chamber. Pollen collectors were placed at the nasal level of sitting subjects at strategic locations within the chambers to monitor pollen counts, using the Allergenco cassette system in San Antonio and Rotorod system in Kingston. Other chambers use somewhat different design characteristics and pollen delivery and dispersal technologies, yet seem to achieve similar results.^{9,15,16}

Allergen Counts

Despite differences in engineering and technology between the 2 CAFCs, both maintained pollen levels throughout the exposures at the specified goals. The variations that occurred between sampling

periods may be attributed to factors associated with a dynamic environment within the chambers, including symptoms of coughing and sneezing and movement of subjects or staff that may disturb settled pollen grains. Precisely accurate counting of large numbers of pollen grains on greased slides and Rotorod strips was difficult and also may have contributed to minor differences in the counts.

Sham Response

During the sham section of the initial priming exposure, an equal percentage of participants at each site recorded a TNSS ≥ 6 when not exposed to the allergen, and were therefore disqualified for this response. This nocebo response may have been psychological, related to the mental state of the subject, or a physiological reaction related to underlying nasal nonspecific hyper-responsiveness.^{17,18} Although the percentage of nocebo responders is low, clearing these participants from the study population is important to allow for a more precise characterization of the true effect of the allergen exposure and the response to an intervention.

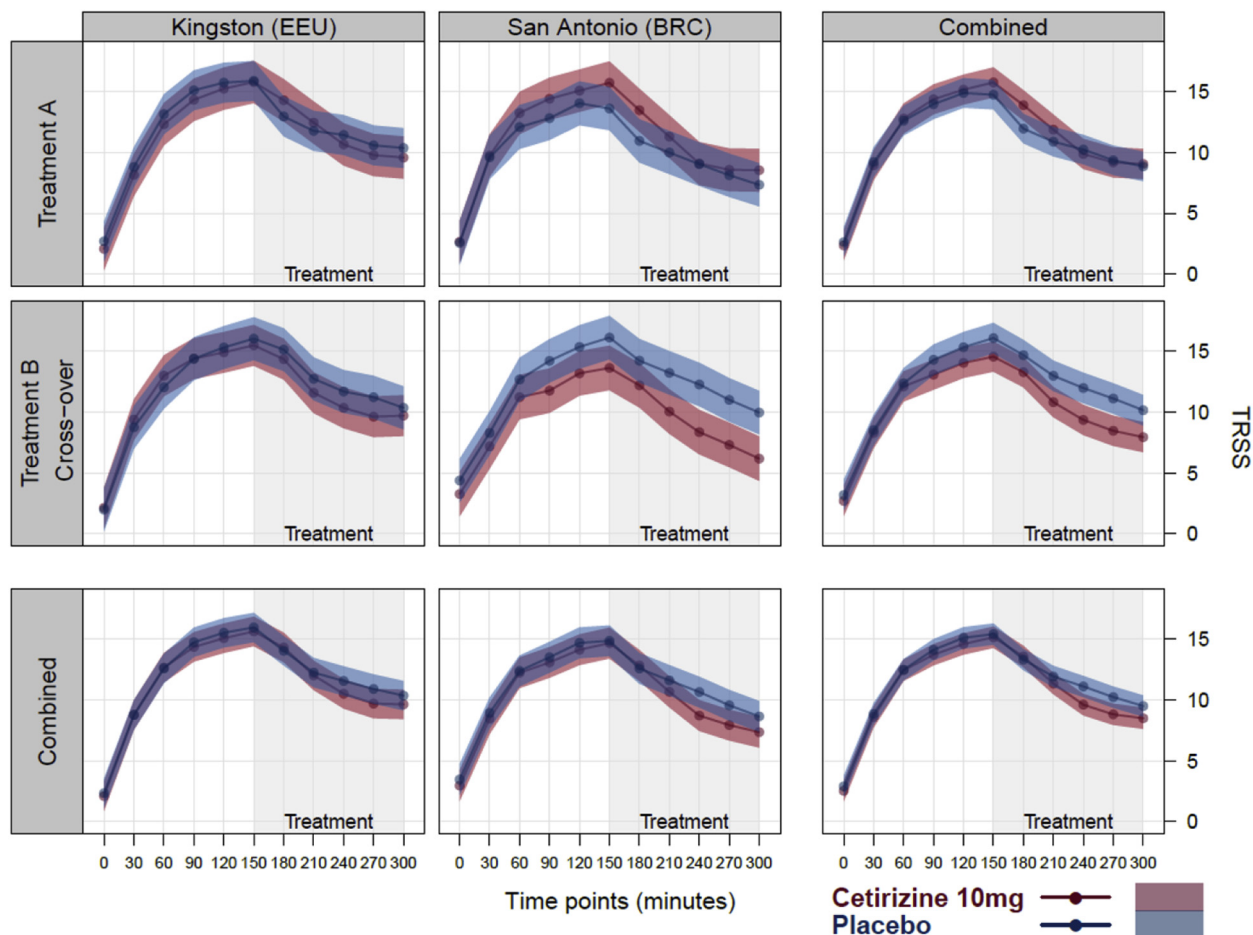


Figure 5. Change in nasal and ocular symptom scores during challenge after treatment with cetirizine or placebo. Both cetirizine and placebo groups demonstrated a decrease in TRSS after dosing during allergen challenge in both Kingston (EEU) and San Antonio (BRC) facilities. Data are represented as means. Shaded regions indicate the 90% CIs. BRC, Biogenics Research Chamber; CI, confidence interval; EEU, Environmental Exposure Unit; TRSS, total rhinoconjunctivitis symptom score.

Response to Challenge

Site equivalence between the 2 chambers was demonstrated for TRSS (primary endpoint) and TNSS (a secondary endpoint) in both treatment arms and overall. In Table 2, for TOSS, VAS, and GRCS, the site equivalence outcomes were reported as “inconclusive” because of the 90% confidence intervals of the site differences not fitting either completely within or completely outside of the site equivalence boundaries. For these inconclusive measures, a larger sample size would tighten the confidence intervals, potentially leading to conclusive site equivalence (yes or no).

Response to Treatment

Although symptom outcomes in the active and placebo groups were comparable across both chambers, a numerically stronger response to cetirizine was observed in the San Antonio population compared with the Kingston population. This may be attributable to the lower sIgE levels and ragweed-induced SPT wheals observed in participants from San Antonio compared with the Kingston cohort.

A notable placebo response was also observed across both chambers. The lack of a symptomatic benefit of cetirizine over placebo contrasts previous studies performed in the Environmental Exposure Unit^{19–21} and may be attributable to the study's sample size and efficacy parameters used to assess SAR symptoms. Although the study was adequately powered to detect a treatment effect based on a priori assumptions, a larger sample size would be

required to overcome the placebo response often seen in CACF trials. This is well described in past CACF studies, which had dramatically larger participant cohorts ($n > 200$).^{20–24} Efficacy parameters for SAR research are often self-reported by participants. The parameters used in this study were all reliant on the participant's perception of his or her own symptoms. Although composite scores such as TRSS and TNSS are well-established outcome measures used to evaluate the efficacy of SAR treatments, they are highly subjective, and they may have magnified the observed placebo response.

Phase III Trials

Systematic clinical validation of CACF units is necessary to enable the use of CACF technology in pivotal phase III efficacy trials. Studies have already made strides in demonstrating high reproducibility in clinical symptom generated across multiple CACF visits using several different allergens (grass pollen, ragweed pollen, house dust mite, and others) in single chambers.^{8,10–12,25,26} One of the primary concerns with CACF validation is the comparability of results from such studies to those of traditional natural exposure trials. According to a recent position paper by the European Academy of Allergy and Clinical Immunology, a direct comparison between the 2 is difficult because of the inability to accurately quantify natural pollen exposure levels on a per individual basis.⁴ However, a recent study demonstrated a good correlation between symptom scores (nasal and ocular) elicited during ragweed

season and scores obtained during an out-of-season ragweed challenge in a CACF setting.¹³ Moreover, another study demonstrated comparable treatment effects across in- and out-of-season CACF challenges after combination treatment (cetirizine and pseudoephedrine) in the same cohort of grass-allergic participants.²⁷

In summary, this is the first study to use an identical clinical protocol to directly compare 2 CACFs according to participant-reported symptom severity and treatment outcomes with an anti-allergic medication. Symptom outcomes were comparable across both facilities, and site equivalence was demonstrated for TRSS and TNSS. These data suggest that multicenter trials involving multiple CACFs are possible, as long as similar studies are conducted to establish equivalence as we have done here. Systematic clinical validation and comparison of CACFs is a necessary step toward the use of these facilities in phase III multi-center trials for SAR treatments. We have demonstrated that 2 facilities could reliably be used simultaneously for a dual-center CACF trial. This study can furthermore serve as a precedent for future studies to evaluate equivalence between CACFs.

Acknowledgments

The authors thank Omid Aghamirian and Jenny Thiele for their contribution to the successful conduct of this trial at the Kingston EEU site.

Supplementary Data

Supplementary data related to this article can be found online at <https://doi.org/10.1016/j.anai.2019.03.007>.

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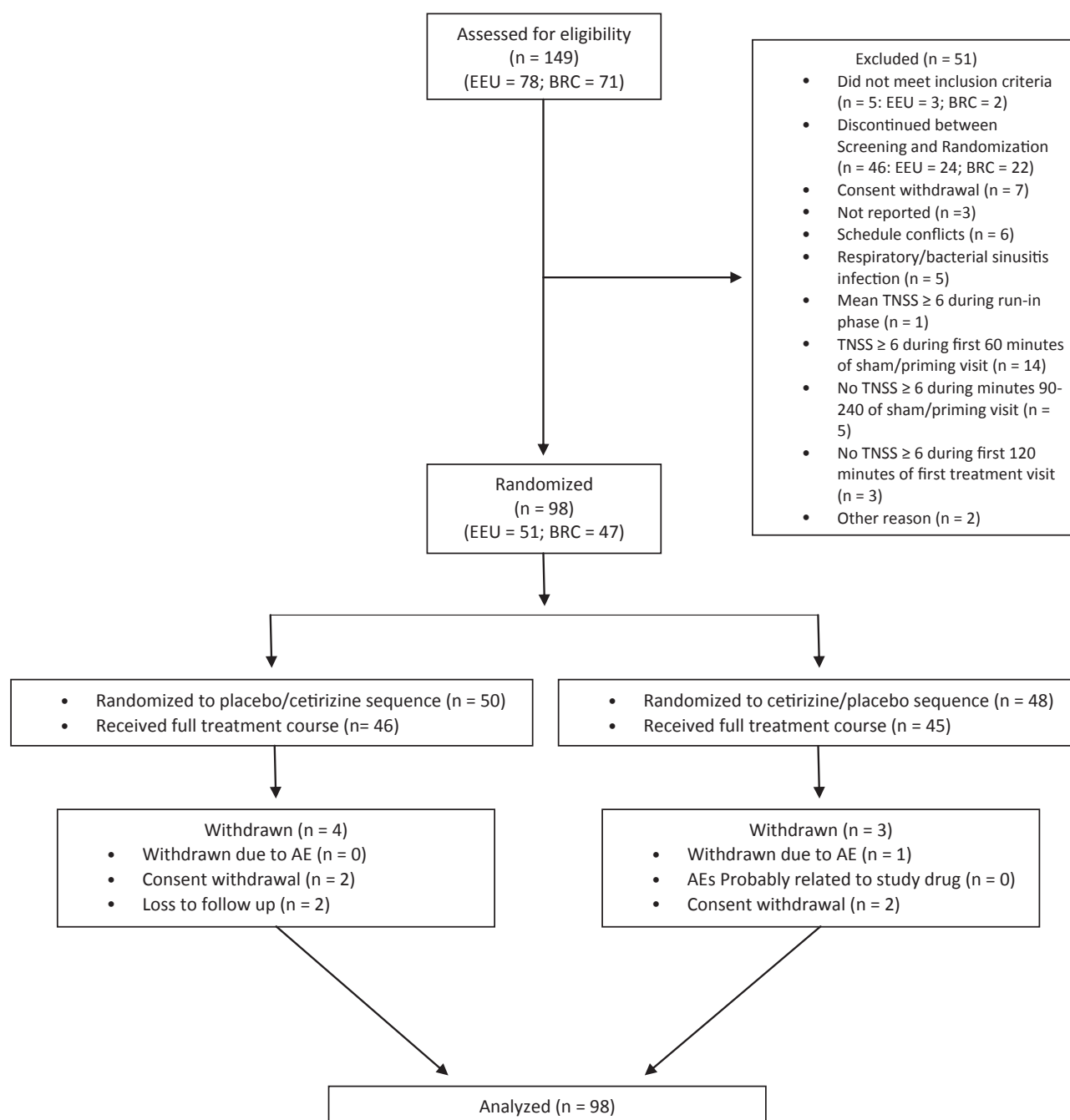
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eSupplement

Methods

Statistical Analyses

As adapted from Chow and Liu,^{E1} the primary criterion for demonstrating site equivalence between Kingston and San Antonio was less than 20% difference in the estimated treatment TRSS AUC, tested separately in the active and placebo arms. The equivalence boundaries were centered on the average symptom score AUC across both facilities, because neither could have served as the “gold standard” or “reference” measurement. Site equivalence was demonstrated if the 90% CI of the site difference was completely contained within the equivalence boundaries, was inconclusive if only 1 end (upper or lower limit) of the 90% CI was contained, and was not demonstrated if both limits exceeded the boundaries. Identical criteria were used to demonstrate site equivalence using estimated treatment AUCs for TNSS, TOSS, VAS, and GRCS. Because VAS and GRCS were measured only once at the end of both treatment challenges, the raw single values were evaluated for equivalence using the same linear mixed-effects repeated-measures model.



eFigure 1. Participant disposition.

eTable 1
Likert 4-Point Severity Scale for Seasonal Allergic Rhinoconjunctivitis Symptoms

Score	Grade	Guideline
0	None	No sign/symptom is evident
1	Mild	Sign/symptom clearly present, but minimal awareness; easily tolerated
2	Moderate	Definite awareness of sign/symptom that is bothersome, but tolerable
3	Severe	Sign/symptom that is hard to tolerate; causes interference with activities during the challenge session

eTable 3
Global Rating of Change Scale

Rating	Description
-7	A very great deal worse
-6	A great deal worse
-5	A good deal worse
-4	Moderately worse
-3	Somewhat worse
-2	A little worse
-1	Almost the same, hardly any worse at all
0	No change
+1	Almost the same, hardly any better at all
+2	A little better
+3	Somewhat better
+4	Moderately better
+5	A good deal better
+6	A great deal better
+7	A very great deal better

eTable 2
Nasal and Ocular Symptoms of Seasonal Allergic Rhinoconjunctivitis^a

Symptom	TNSS	TOSS	TRSS
Runny nose (rhinorrhea)	X		X
Sneezing	X		X
Nasal itching	X		X
Nasal congestion	X		X
Itchy eyes		X	X
Watery eyes		X	X
Red/burning eyes		X	X

TNSS, total nasal symptom score; TOSS, total ocular symptom score; TRSS, total rhinoconjunctivitis symptom score.

^aTotal composite scores were the sum of each individual symptom score (rated between 0 and 3); TNSS (0-12), TOSS (0-9), TRSS (0-21).

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E1. Chow S-C, Liu J. *Design and Analysis of Bioavailability and Bioequivalence Studies*. Second Edition. Boca Raton, FL: CRC Press; 1999.