A Proof-of-concept Study Using Quantitative Sensory Threshold Analysis to Compare Two Intraoral Topical Anesthetics

Elliot V. Hersh, DMD, MS, PhD[1]; Stacey Secreto, CCRC[1]; Steven Wang, DMD, MD, MPH[1]; Helen Giannakopoulos, DDS, MD[1]; Mohammad Mousavian, BS[1]; Bret Lesavoy, BS[1]; Matthew C. Hutcheson, MS[2]; John T. Farrar, MD, PhD[3]; Ping Wang, PhD[4]; Geraldine Doyle, PhD[5]; and Stephen A. Cooper, DMD, PhD[6]

[1]Department of Oral Surgery and Pharmacology, University of Pennsylvania School of Dental Medicine, Philadelphia, PA, USA; [2]Tegra Analytics, Doylestown, PA, USA; [3]Departments of Epidemiology/Biostatistics and Neurology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [4]Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, USA; [5]Clinical Research/Medical Affairs Consultant, Chatham, NJ, USA; and [6]Stephen A. Cooper DMD, PhD, LLC, Palm Beach Gardens, FL, USA

ABSTRACT

Purpose: CTY-5339A is an investigational topical anesthetic spray containing 14% benzocaine/2% tetracaine in a metered canister. Each spray delivers ~0.2 mL of solution. This double-blind, randomized, crossover study compared the local anesthetic effect of CTY-5339A versus 14% benzocaine alone by using 2 quantitative sensory threshold experimental pain paradigms on the maxillary gingiva: pin prick test pain intensity (PPT PI) and heat pain threshold (HPT).

Methods: : American Society of Anesthesiology Class 1 and 2 subjects (N = 50) were enrolled in this study. To qualify for the study, subjects were tested on the anterior maxillary gingiva with both PPT and HPT. Subjects had to report a PPT PI of ≥3 on a 0 to 10 numeric pain intensity scale on 1 of 2 consecutive pin pricks separated by 10 s, with at least one score ≥4. After PPT, mean HPT following 2 ramps in the same location had to be ≤ 46.5 °C, with each ramp beginning at 35 °C and an automatic cutoff of 50.6 °C. For treatment visits, subjects were randomly administered either 1 spray of CTY-5339A or 14% benzocaine to the anterior maxillary gingiva within 3 weeks of screening and then the alternative treatment 5 days to 2 weeks later. PPT PI and HPT were recorded immediately before drug application. After drug administration, PPT PI was recorded every minute through 5 min. Commencing at 5 min, PPT PI and HPT were recorded every 5 min through 60 min. For assessment of methemoglobin concentrations, venous blood (5 mL) was drawn from the antecubital fossa both before and 60 min after drug application. Oxygen saturation was recorded via pulse oximetry at baseline and every 10 min.

Findings: The AUCs for pain intensity difference from 0−30 and 0−60 min after PPT and HPT differences were significantly greater ($P < 0.0001$) for CTY-5339A compared with 14% benzocaine. Multiple time points on the time−action curves for PPT PI difference and HPT difference statistically ($P < 0.05$) favored CTY-5399A. Methemoglobin and oxygen saturation levels did not change compared with baseline after dosing with either treatment.

Implications: Recommended doses of CTY-5339A provided significantly more profound and sustained local anesthesia than 14% benzocaine when applied to the maxillary gingiva. Significant changes in methemoglobin or oxygen saturation concentrations

Accepted for publication December 10, 2018
https://doi.org/10.1016/j.clinthera.2018.12.009
0149-2918/$ - see front matter

did not occur for either drug. ClinicalTrials.gov identifier: NCT03233737. (*Clin Ther*. 2019;41:291–302)

Key Words: benzocaine, methemoglobinemia, quantitative sensory threshold analysis, tetracaine, topical anesthetics.

INTRODUCTION

The combination of 14% benzocaine/2% tetracaine/2% butamben with a chloroflurocarbon (CFC) propellant in an unmetered canister is currently marketed as Cetacaine® spray (Cetylite Inc, Pennsauken, New Jersey) and has been on the US market place since 1960. It came on the market 2 years before the Kefauver Harris Drug Control Act, which mandated that all drugs be proven safe and effective before approval by the US Food and Drug Administration (FDA). It currently possesses Drug Efficacy Study Implementation status, meaning that confirmation of its safety and efficacy are still pending. It is indicated to produce anesthesia of all accessible mucous membranes except the eyes, and it is used to control pain and gagging, including surgical, endoscopic, and other procedures in the ear, nose, mouth, pharynx, larynx, trachea, bronchi, and esophagus.[1] Another frequent use of this and other topical anesthetic formulations is to provide anesthesia for minor soft tissue dental procedures such as scaling and root planing (dental cleanings) and minor gingival surgery.[2]

Package insert dosing instructions state that the cannula should be depressed for ≤1 s, delivering ~200 mg of product equal to 28 mg of benzocaine plus 4 mg of tetracaine.[1] In no instance should the product be administered for >2 s (400 mg of total product or 56 mg of benzocaine plus 8 mg of tetracaine). In the investigational CTY-5339A formulation, the butamben has been removed, as has the CFC propellant because of the FDA ban on CFCs due to their ozone-depleting effects.[3] By 2020, all products containing CFCs such as asthmatic inhalers must have this propellant removed.[4] In addition, CTY-5339A is contained in a metered canister, with each application expressing ~0.2 mL of drug (28-mg benzocaine plus 4-mg tetracaine); thus, clinicians can better control how much drug is expressed.

As with other investigational drugs, the FDA is now requesting that CTY-5339A undergo rigorous studies of safety and efficacy.[5] Quantitative sensory threshold (QST) analysis has been widely used to evaluate somatosensory function by quantifying a patient's subjective response to systematically applied and quantifiable stimuli.[6,7] In clinical practice and research, QSTs including mechanical pressure, pin prick test (PPT) pain, noxious heat and cold, and electrical stimuli can be applied to the skin or the oral mucosa to establish threshold responses or to establish stimulus-response function.[8] Of most relevance to this report, QST has previously been used to evaluate topical intraoral local anesthetic agents. These have included randomized, double-blind studies evaluating topical eutectic mixtures of 2.5% lidocaine/2.5% prilocaine (EMLA) cream versus placebo[9] and/or other agents.[10,11] EMLA significantly increased pressure pain thresholds on the maxillary gingiva compared with placebo[9,10] and compared with 10% benzocaine, 1% dyclonine, 10% lidocaine, or 10% cocaine.[10] Compared with 5% lidocaine, EMLA produced a significantly longer duration of topical anesthesia as measured by PPT pain intensity (PPT PI) and a significantly greater AUC for pressure pain threshold as measured on both the maxillary and mandibular gingiva.[11]

Because CTY-5339A is a 2-component product (14% benzocaine/2% tetracaine), the FDA requested that a clinical trial be conducted to show that this combination is more efficacious than 14% benzocaine alone. As in a previously published study,[12] the FDA requested continuing safety monitoring of both products, in particular to detect any significant elevations in methemoglobin levels, which have been reported with excessive doses of benzocaine and tetracaine-containing products.[13–23] The present study compared the anesthetic effects of CTY-5339A versus those of 14% benzocaine alone by using QST and their effects on methemoglobin and oxygen saturation concentrations in American Society of Anesthesiology (ASA) Class 1 and 2 adult volunteers.

SUBJECTS AND METHODS

ASA Class 1 and 2 volunteers (N = 50) of both sexes without contraindications to topical anesthetics and laboratory values within normal limits at screening could enroll in the study. The protocol and informed consent document were approved by the University of Pennsylvania Institutional Review Board, and

the trial was listed on www.clinicaltrials.gov (NCT03233737). Subjects had to read and sign the informed consent document as witnessed by the research coordinator or the principal investigator before any research-related procedures (including screening procedures) were commenced. A negative result on a urine pregnancy test was required of all childbearing female subjects at screening and at both dosing visits before the administration of study drug.

At screening, subjects also had to meet minimum PPT PIs and heat pain thresholds (HPTs) as specified in the protocol to be eligible for study drug dosing. PPT PI was assessed by using a 90-mm, 26-gauge pencil-point spinal needle. To standardize the methods, all tests were performed by holding the needle at its hub and orienting it perpendicular to the gingival mucosa surface. Pressure was applied until the needle shaft bowed slightly. Two PPTs were administered by the same research coordinator (S.S.) ~10 s apart on the maxillary gingival tissue above the central and lateral incisors. PPT PI was reported by the subject as 0 (no pain) to 10 (severe pain) on a numerical rating scale (NRS). For continued study participation, subjects must have rated each PPT PI at least a 3, with one of those scores being 4 or higher.

For HPT, a Thermal Sensory Analyzer 2 (TSA-II; Medoc Ltd, Ramat Yishai, Israel) was held against the same maxillary gingival surface and delivered heat stimuli in 2 repetitions, with interstimulus intervals of 30 s. The basal thermode temperature was set at a comfortable 35 °C. The rate at which the thermode heated up was programmed at 0.5 °C per second. Based on the temperature elevation ramp speed, the maximum temperature cutoff was set automatically by the device at 50.6 °C. Subjects were instructed to depress a computer mouse when they first experienced any "burning" pain, which initiated an automatic thermode cool down of 8 °C per second. Reaching the cutoff temperature also initiated this cooldown. HPT data were automatically transferred to a laptop connected to the TSA-II and displayed as histograms (Figure 1). The 2 screening HPT ramp periods were averaged, and the mean HPT for continued study participation had to be $\leq$ 46.5 °C (to allow ample room for improvement after subsequent study drug administration).

Five milliliters of blood were drawn from the antecubital fossa for hematology and blood chemistry assessments. Values needed to be within the normal

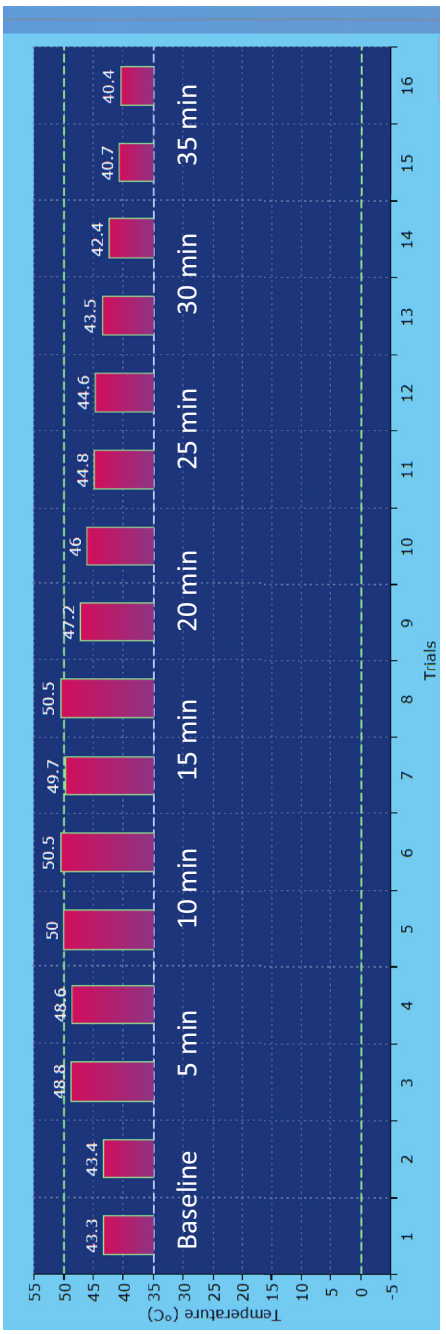

Figure 1. Computer display of heat pain thresholds of research participant at baseline and at 5, 10, 15, 20, 25, 30, and 35 min' postdrug application.

range or, if out of range, considered clinically nonsignificant by the principal investigator (E.V.H.) or S.W. for continued study participation. A urine drug screen for substances of abuse and compounds that could affect efficacy measures (eg, opioids) was also obtained at screening and immediately before dosing. The results had to be negative with the exception for a drug that a subject had been on a stable dose for at least 1 month for a legitimate medical condition (eg, a positive test for amphetamines in a subject with a history of attention-deficit/hyperactivity disorder). Subjects who "passed" screening (50 of 87 consented and screened) were then scheduled for their first blinded dosing visit within 30 days of their screening visit (Figure 2).

The dosing visits involved a double-blind, randomized, active-control (14% benzocaine), crossover design. A randomized listing was generated by using SAS software (SAS Institute, Inc, Cary, North Carolina) and sent to the party responsible for packaging and labeling the study medications. In addition, a master code was kept

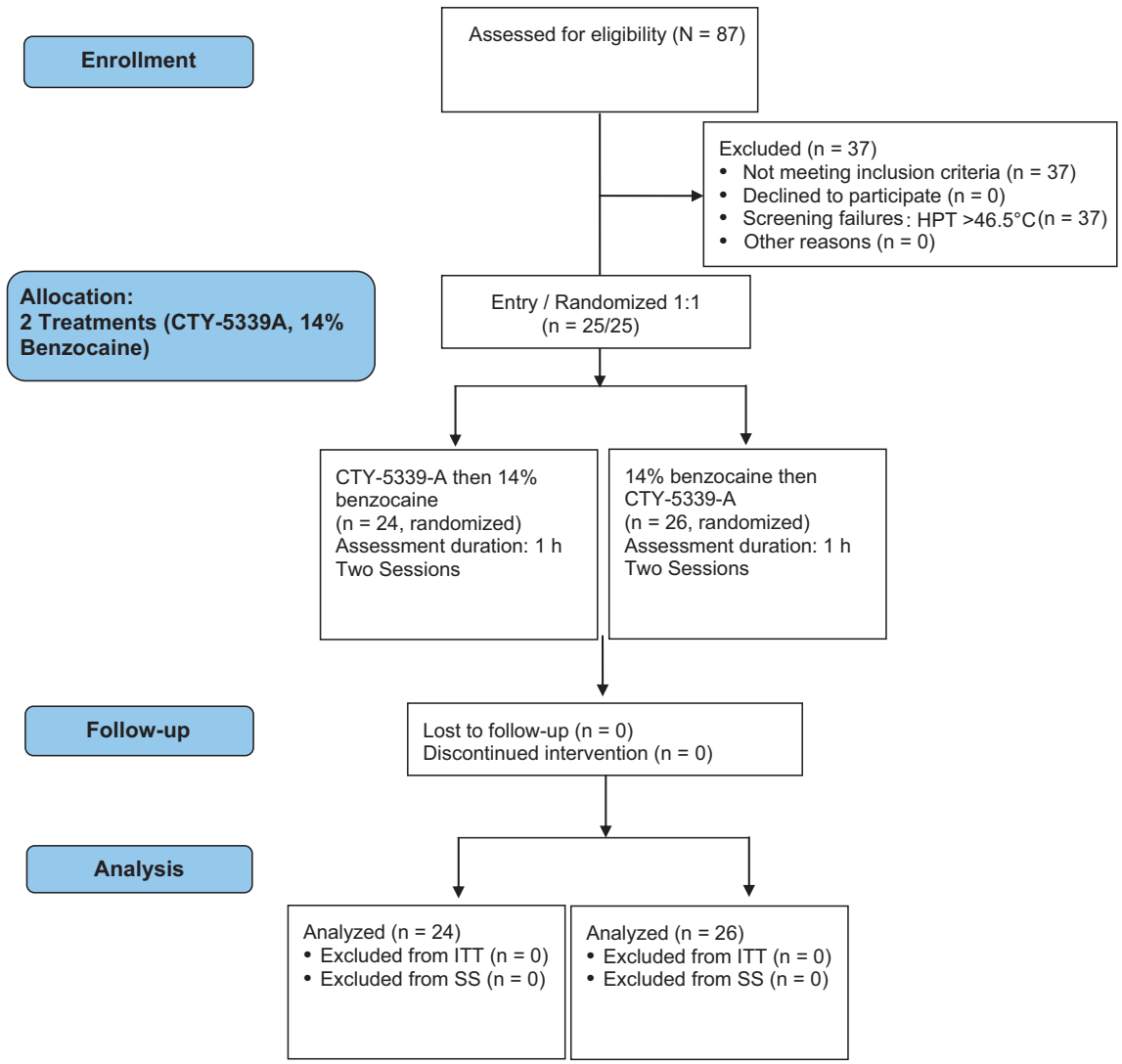

Figure 2. CONSORT diagram of study flow. HPT = heat pain threshold; ITT = intention-to-treat; SS = safety population.

by the sponsor and at the study site (in the unlikely event of the need to break the code for a serious adverse event [AE]). The study site master code had individually sealed envelopes identifying the drug in each cannister for each subject enrolled in the study.

Dosing visits were separated by at least 5−14 days. A circular area ~1 cm in diameter was drawn on the maxillary anterior gingiva as a target for experimental pain testing and study drug dosing. Study medication for subjects randomized to receive CTY-5339-A in one treatment session and 14% benzocaine in the other treatment session was double-blinded and packaged individually for each subject. Study medication for each subject consisted of 1 box, which contained 1 cannister of CTY-5339-A and 1 cannister of 14% benzocaine. Each cannister was labeled with the appropriate treatment session (A or B) and which side of the gingiva the study medication should be applied to (left or right), according to the randomization schedule.

As with screening, mean baseline PPT PI scores and HPTs were obtained with the same requirements as screening to remain in the study. Before dosing, each cannister was primed 3 times immediately before use. Study anesthetic spray was administered by the research coordinator into the predefined target by simply depressing the nozzle of the study drug canister once. Commencing at 1 min postdrug application, and every minute until 5 min, PPT PI was assessed by asking the subjects to rate their PI on the 0 to 10 NRS. At 5 min, HPT was then determined via 2 ramps separated by 30 s. Subsequently, PPT PI followed immediately by HPT was repeated every 5 min for up to 60 min. PPT was discontinued if the mean PI score of the paired PPTs returned to baseline or worse at 2 consecutive time points.

For safety assessments, oxygen saturation was recorded via an automated pulse oximeter/blood pressure unit (Series 596NT3; Criticare Systems Inc, Waukesha, Wisconsin) at baseline and every 10 min through 60 min. Immediately before and 60 min after drug administration, 5 mL of venous blood was drawn for methemoglobin concentrations into sodium heparin vacutainer tubes and placed into a refrigerator (37 °F) before being transported within 72 h to the Hospital of the University of Pennsylvania Core Laboratory. Methemoglobin concentrations were measured by using a multi-wavelength oximeter (ABL837; Radiometer America, Brea, California). The fraction of methemoglobin was derived by using the following calculation: Met-Hb % = [Met-Hb/(O_2-Hb + deoxy-Hb + CO-Hb + Met-Hb)]. Per protocol and with agreement from the FDA, if the methemoglobin level in any individual increased to >5%, it was to be considered a serious AE and the study would be terminated.

This analysis was a proof-of-concept study to determine if CTY-5339A had at least 1 relevant efficacy advantage over 14% benzocaine. A reasonable efficacy signal was defined per protocol as a mean increase in duration of anesthesia of $\geq$5 min for CTY-5339A compared with 14% benzocaine. Thus, the co-primary efficacy variables were the comparison of CTY-5339A versus 14% benzocaine for duration of effect for PPT and HPT. Duration of effect was defined as the time (in minutes) from onset to treatment failure or a maximum of 60 min. For PPT, onset time was first of 2 consecutive time points in which mean PPT PI was reduced by at least 1 unit from baseline PI on the 0 to 10 numeric pain rating scale. Treatment failure occurred at the first of 2 consecutive time points at which mean PPT PI was the same or greater than the mean of baseline PPT PI. For HPT, onset time was defined as the first of 2 consecutive time points when the average HPT was increased by at least 3 °C; treatment failure occurred at the first of two consecutive time points when the average HPT was less than 3 °C above the average HPT obtained at baseline. A sample size of 50 subjects using a crossover design was calculated to provide greater than 80% power assuming a 5-min duration advantage existed for CTY-5339A compared with 14% benzocaine. These end points were analyzed via an ANOVA model with treatment effects assessed at each time point.

Exploratory analyses of the 30- and 60-min AUCs for PPT pain intensity difference (PID) and HPT difference from baseline (HPTD) were decided to be undertaken before breaking the blind. Individual PPT PID scores were calculated by subtracting each mean postdosing PPT PI from mean baseline PPT PI. These were then summed and time-weighted to calculate AUC (SPID). The individual HPTD were calculated by subtracting each mean postdosing HPT from mean baseline HPT and then summing these values. Likewise, these were summed to calculate the area under the HPTD time−action curve (SHPTD). Time-action curves of

PPT PIDs and HPTDs were constructed, and individual time points were compared between treatments by using 2-way ANOVAs.

AE analyses included all AEs that initially occurred, or worsened, after treatment (ie, treatment-emergent signs and symptoms). AEs were summarized according to the Medical Dictionary for Regulatory Activities preferred term and by system organ class. Methemoglobin concentrations were expressed in percentages and were compared predose and at 60 min' postdose by using a paired *t* test for each treatment group. Oxygen saturation values were also expressed in percentages and averaged. The Wilcoxon signed rank test was applied to oxygen saturation values because all recorded measurements were 96%, 97%, 98%, or 99% during the study.

All statistical tests were 2-sided, and differences were declared statistically significant if the probability of a random occurrence of a difference was ≤5% ($P \leq 0.05$).

RESULTS

Eighty-seven subjects signed informed consent forms for study participation, and 50 of these subjects were enrolled. All screening failures were due to participants displaying a mean HPT >46.5 °C (Figure 2). The overall demographic characteristics of the study population revealed a relatively young group of volunteers (mean age, 23.9 years) with a preponderance (72%) of female subjects enrolling. Importantly, because this study had a crossover design, the demographic characteristics based on the order of medication administration were similar, with the largest differences appearing in sex (Table I). Table II displays the results for the 2 co-primary efficacy end points: duration of response for PPT PI

Table I. Summary of demographic characteristics.

Category	CTY-5339A, 14% Benzocaine (n = 24)	14% Benzocaine, CTY-5339A (n = 26)	All Treatment Sequences (N = 50)
Age, y			
Mean (SD)	24.3 (4.9)	23.5 (1.8)	23.9 (3.6)
Median	23.0	23.5	23.0
Minimum, maximum	18, 43	21, 27	18, 43
Sex, no. (%)			
Male	9 (37.5)	5 (19.2)	14 (28.0)
Female	15 (62.5)	21 (80.8)	36 (72.0)
Ethnicity			
Hispanic or Latino	0	3 (11.5)	3 (6.0)
Not Hispanic or Latino	24 (100.0)	23 (88.5)	47 (94.0)
Race, no. (%)			
White	13 (54.2)	12 (46.2)	25 (50.0)
Black or African American	1 (4.2)	0	1 (2.0)
Asian	8 (33.3)	12 (46.2)	20 (40.0)
Other	2 (8.3)	2 (7.7)	4 (8.0)
Weight, lb			
Mean (SD)	154.1 (33.0)	141.2 (27.6)	147.4 (30.7)
Median	148.5	134.0	135.0
Minimum, maximum	105, 215	103, 240	103, 240
Height, inches			
Mean (SD)	67.3 (4.1)	65.8 (2.6)	66.5 (3.5)
Median	66.5	66.0	66.0
Minimum, maximum	61, 76	62, 72	61, 76

Table II. Duration of response (minutes).

Variable	CTY-5339-A (N = 50)	14% Benzocaine (N = 50)
Pin prick test		
Mean (SD)	45.5 (13.63)	40.8 (16.30)
Median	47	39
Minimum, maximum	1, 59	0, 59
Treatment differences		
Least squares mean	4.8	
SE	2.19	
95% CI	0.35 to 9.16	
P	0.035	
Heat pain threshold		
Mean (SD)	30.9 (22.82)	23.2 (21.91)
Median	29	19
Minimum, maximum	0, 59	0, 59
Treatment differences		
Least squares mean	7.6	
SE	3.89	
95% CI	−0.22 to 15.43	
P	0.056	

For the pin prick test, ≥1 unit decrease in pain intensity represented a response. For heat pain threshold, ≥3 °C increase represented a response.

and HPT. For PPT PI, CTY-5339A displayed a significantly ($P = 0.035$) longer duration of effect than 14% benzocaine. For HPT, CTY-5339A provided a numerically superior duration; however, this difference did not reach statistical significance ($P = 0.056$).

Because classical postoperative analgesic trials often use differences from baseline PI (PID) and their respective AUCs (SPID) as secondary and primary end points, respectively,[24–27] exploratory analyses were performed to evaluate these surrogate end points in the present study. Figures 3 and 4 illustrate the treatment time–action curves for PPT PID and HPTD, respectively. Baseline PPT PI (mean [SEM], 5.5 [0.2] for both CTY-5339A and 14% benzocaine) and HPT (41.8 °C [0.3 °C] and 42.0 °C [0.3 °C] for both treatments, respectively) did not differ statistically. For most time points beginning at 2 min' postdrug dosing for PPT PID and 5 min for HPTD through 45 and 40 min, CTY-5339A was statistically superior ($P < 0.05$) to 14% benzocaine. Likewise, 30- and 60-min AUC measures (Figures 5 and 6) were also significantly more efficacious for CTY-5339A ($P < 0.0001$).

Only a few treatment-emergent AEs were reported in this study. None was considered a serious AE, and all completely resolved. One subject after exposure to CTY-5339A experienced sore throat, oropharyngeal pain, nasal congestion, and a rash, while a second subject reported a headache. One subject developed streptococcal pharyngitis after exposure to 14% benzocaine. All events were judged to be either unrelated or unlikely to be related to study medication before breaking the blind. No subject had a pulse oximetry reading <96% at any point during the study, and no subject had a methemoglobinemia level >0.9% after administration of either treatment. There were no clinically significant or statistically significant changes in either pulse oximetry or methemoglobin concentrations.

DISCUSSION

The results of this study show the superiority in topical anesthetic efficacy of CTY-5339A compared with 14% benzocaine for a variety of primary and exploratory end points. Twenty percent benzocaine applied to the tip of the tongue in humans employing an electrical stimulation pain model reportedly has a very rapid onset time of 12 s but an anesthetic duration of only 4.3 min.[28] Conversely, in the same model, the onset of 2% tetracaine was slower, with an onset time of 11 min, but exhibited a much longer anesthetic duration of 48.6 min. Likewise, in a dog conjunctival model of topical anesthesia, tetracaine had the slowest onset of all topical anesthetics tested (mean [SD], 4 [1.4] minutes) but the longest duration of action (145 [25] minutes) second only to dibucaine (190 [15] minutes).[29] Tetracaine's relatively high pKA (8.3) but vigorous neuronal protein binding probably most contributes to its anesthetic lag but long duration of action.[30] Thus, CTY-5339A combines the

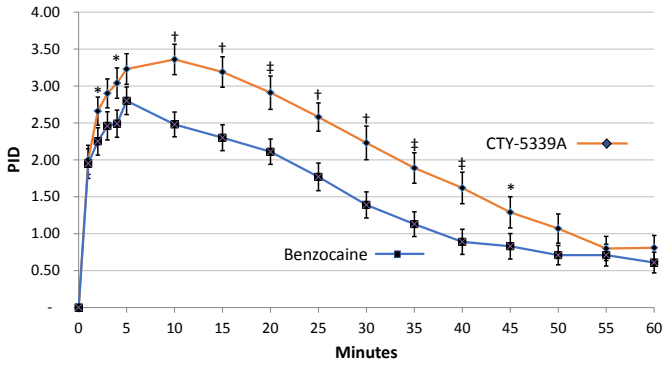

Figure 3. Time-action curves of CTY-5339A versus 14% benzocaine for pain intensity difference (PID) via the pin prick test (PPT). $^{*}P < 0.05$, $^{\dagger}P < 0.001$, $^{\ddagger}P < 0.01$.

rapid topical anesthetic onset of benzocaine with the extended duration of tetracaine.

The exploratory end points of PPT PID and HTPD, and their respective area under the time action curves, seem to display greater assay sensitivity than simply measuring duration of effect when comparing these topical anesthetics. The probable reason for this theory is that PPT PID and HPTD take into account not only the duration of effect but also the profundity of anesthesia at each time point. For example, for PPT PID, a 3-unit decrease in baseline pain carries more weight than a 1-unit decrease at a given postdrug administration time point, even though both reductions would be considered responders in our primary analyses of treatment duration. Based on a study by Farrar et al,[31] an increase of 2 points in the PID on a 0 to 10 NRS is considered clinically important for a subject. This outcome was achieved on average for both treatments by 5 min but was maintained for the CTY-5339A for almost 35 min compared with only slightly more than 20 min for 14% benzocaine (Figure 3). Likewise, for HPTD, an average 5 °C increase from baseline is weighted more heavily than a 3 °C increase, although both would be considered responders based on our predefined responder definition. There are several published reports demonstrating significantly greater topical anesthetic profundity with topical tetracaine compared with topical lidocaine in patients undergoing transnasal fiber-optic laryngoscopy and in normal volunteers experiencing experimental nasal mucosal pain via Semmes-Weinstein monofilaments. In both situations, the topical tetracaine group displayed statistically ($P < 0.05$) lower pain scores than those who received topical lidocaine.[32–34] This study also confirms previous data that various aspects of QST can be used to demonstrate efficacy differences of topical anesthetic drugs applied to the oral mucous membranes.[9–11]

As in our previously published report,[12] methemoglobin and oxygen saturation concentrations did not change with the doses of benzocaine and tetracaine we administered in this study. However, it must be stressed that there are numerous reports of benzocaine and combinations of benzocaine with tetracaine inducing methemoglobinemia when excessive doses of these agents are delivered to the oropharyngeal membranes.[13–22] In fact, the FDA has recently published a warning to avoid the use of over-the-counter topical benzocaine products in teething infants.[35] Like many drug-induced toxicities, the dose necessary to produce methemoglobinemia is weight-based, a factor that can make over-the-counter benzocaine products especially hazardous in teething infants.[36] The fact that CTY-5339A is contained in a metered canister and will only be marketed as an in-office product should reduce the possibility of this overdose phenomenon.

Weaknesses of the present study include the absence of a placebo control treatment, which would have further confirmed the assay sensitivity of the methods.[24,25] In terms of safety assessments, all subjects were ASA Class 1 or 2, and thus safety data on this population cannot be generalized to medically compromised individuals. Although the dosage

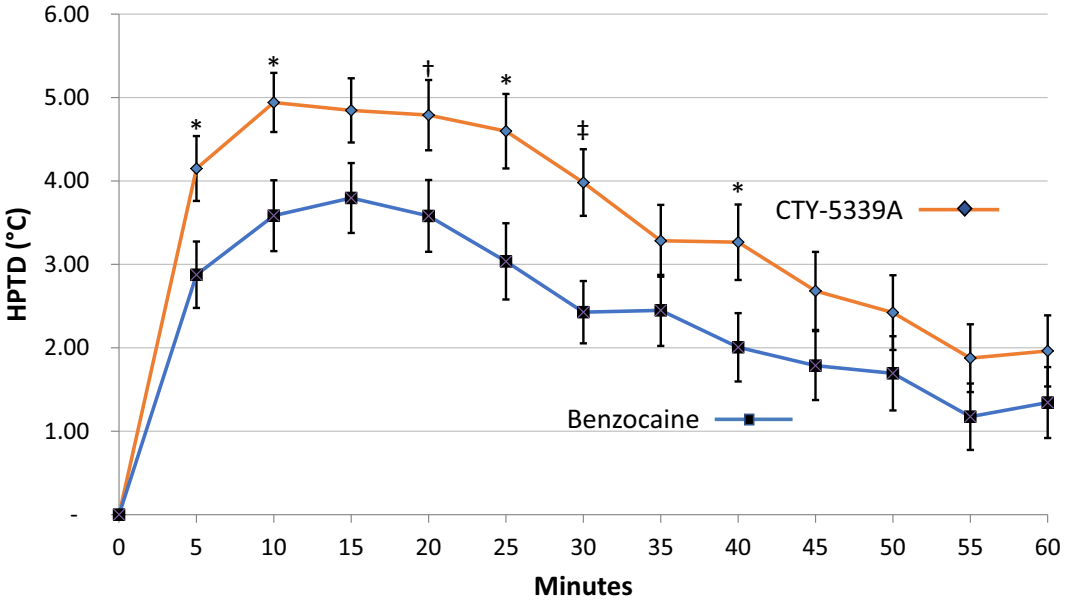

Figure 4. Time-action curves of CTY-5339A versus 14% benzocaine for heat pain threshold difference (HPTD) via the Thermal Sensory Analyzer II. *$P < 0.01$, †$P < 0.05$, ‡$P < 0.001$.

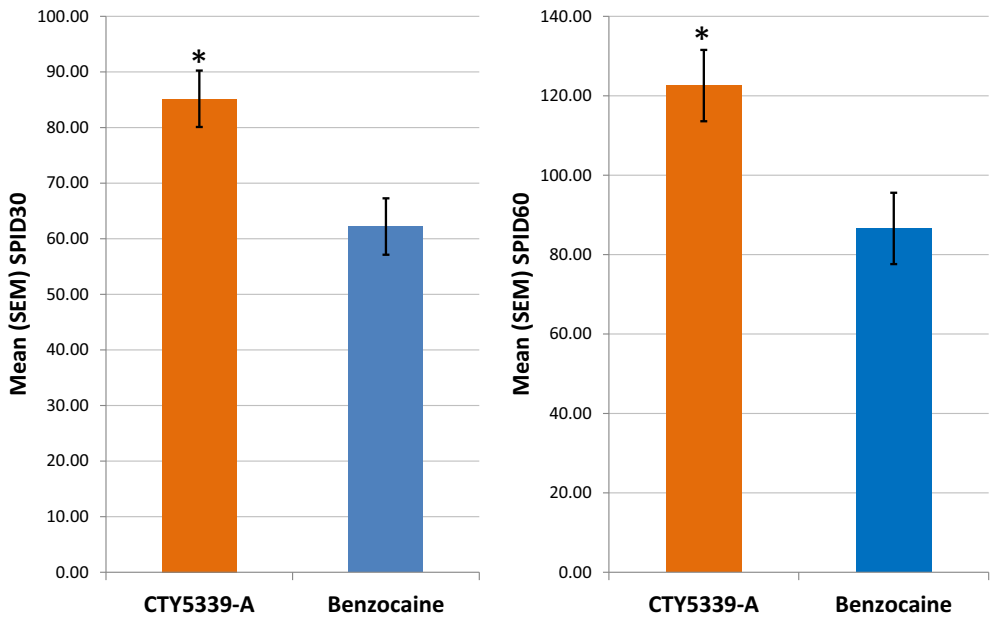

Figure 5. Area under the curves (SPID) of CTY-5339A versus 14% benzocaine for pain intensity difference from 0–30 and 0–60 minutes via the pin prick test. *$P < 0.0001$.

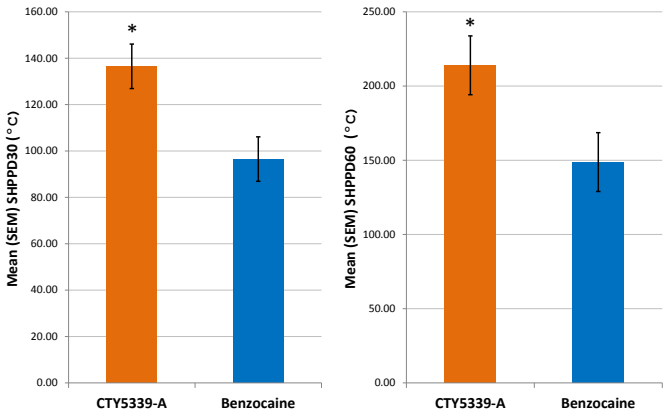

Figure 6. Area under the curves (SHPTD) of CTY-5339A versus 14% benzocaine for heat pain threshold difference from 0−30 and 0−60 minutes via the Thermal Sensory Analyzer II. *$P < 0.0001$.

exposure to both benzocaine and tetracaine used in this study followed recommended package insert guidelines,[1] it would be of interest to evaluate higher doses of benzocaine plus tetracaine and their effects on methemoglobin and oxygen saturation concentrations.

CONCLUSIONS

Over 60 minutes, CTY-5339A containing 2% tetracaine plus 14% benzocaine provided more profound gingival anesthesia than 14% benzocaine in two experimental pain paradigms in healthy volunteers. Both drugs were well tolerated at the doses employed in this study.

ACKNOWLEDGMENTS

Dr. Hersh wrote the initial draft of the manuscript, reviewed all comments and edits by coauthors, performed all the institutional review board−related regulatory duties (including development of the informed consent (ICF) document), reviewed and revised the initial protocol, reviewed laboratory and other study inclusion criteria, helped construct the figures, and reviewed all biostatistical analyses. He, along with his research coordinator, programmed the TSA-II. He made the decision along with Dr. Cooper before the code break to analyze the data set by classic postoperative pain PID- and SPID-like parameters. Ms. Secreto was research coordinator for the study. She reviewed and approved the final version of the paper, and she reviewed and commented on the original draft protocol. She along with Dr. Hersh programmed the TSA-II. She performed the informed consent procedure, drew the blood samples, collected the urine, recorded the pulse oximetry readings, and administered the test drugs and the experimental pain tests. Dr. S. Wang reviewed, approved, and performed some minor editing of the manuscript before accepting the final version of the paper. He reviewed and commented on the protocol, and he also reviewed and approved screening laboratory assessments on study participants necessary for participant study continuation. Dr. Giannakopoulos reviewed, approved, and performed some minor editing of the manuscript before accepting the final version of the paper. She reviewed and commented on the original draft protocol, and she also reviewed and approved screening laboratory assessments necessary for study participant continuation.

Mr. Mousavian (at the time a third-year dental student) reviewed and approved the manuscript; he also reviewed and commented on the final protocol. He also aided Dr. Hersh's research coordinator in preparing urine and blood samples for laboratory analyses and in collecting data from the PPTs and HPT tests (which were going at rapid-fire for 60 min). Mr. Lesavoy (at the time a third-year dental student) reviewed and approved the manuscript. He also reviewed and commented on the final protocol. He also aided Dr. Hersh's research coordinator in preparing urine and blood samples for laboratory analyses and in collecting data from the PPTs and HPT tests (which were going at rapid-fire for 60 min). Mr. Hutcheson reviewed, edited, and commented on

the initial and subsequent drafts of the manuscript before finally approving it. He played a pivotal role in writing the biostatistics section of the protocol, and he constructed all the electronic data capture forms. He performed all the biostatistics in this study and worked closely with the principal investigator in the development of the CONSORT diagram and the rest of the figures. The original draft of the figures was his, under Dr. Hersh's guidance (who made them prettier and added some necessary elements).

Dr. Farrar reviewed, edited, and commented on the initial and subsequent drafts of the manuscript before approving it. He reviewed and made edits to the initial protocol. His input into the experimental pain paradigms was invaluable because he had some experience in QST analyses. He worked closely with the principal investigator (Dr. Hersh) and his research coordinator (Ms. Secreto) on many of the study's logistics. Dr. P. Wang reviewed, made some minor edits to earlier drafts, and approved the final version of the manuscript. She was responsible for all the screening laboratory evaluations and, even more importantly, the methemoglobin concentration analyses. She wrote that section of the paper and edited that section of the protocol (describing how it is done). Dr. Doyle reviewed and commented on an earlier draft before approving the final version of the manuscript. She prepared up-to-date data listings of all the blinded data during various time points in the study, and she also reviewed and edited the original draft protocol. She reviewed blinded data from the Thermal Sensory Analyzer Lap Top with Dr. Hersh's research coordinator and on paper case report forms to ensure that the data were transferred accurately to electronic data capture forms. She also made sure that we (University of Pennsylvania) were meeting our regulatory obligations. Dr. Cooper reviewed, edited, and commented on the initial and subsequent drafts of the manuscript before approving it. He constructed the initial draft of the research protocol and closely reviewed the biostatistical analysis and report of Mr. Hutcheson. He made the decision with Dr. Hersh before the code break to also analyze the data set with classic PID- and SPID-like parameters.

CONFLICTS OF INTEREST

Dr. Hersh, representing the Trustees of the University of Pennsylvania, received grant funding from Cetylite Industries Inc for his work in preparing the protocol and conducting the research study. Drs. Cooper and Doyle were paid consultants of Cetylite Industries Inc for their work in preparing the protocol and monitoring the study. Mr. Hutcheson was a paid biostatistical consultant for Cetylite Industries Inc.

The authors received no other help in writing or revising this manuscript. The study sponsors had no role in the study design, writing of the manuscript or in the decision as to when and where to submit the manuscript.

There was no additional funding from the University or Federal Agencies for the conduct of this study.

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

REFERENCES

1. Cetacaine® topical anesthetic. At http://www.cetacaine.com/dental/about/prescribing-information. Accessed July 12, 2017.
2. Meechan JG. Intraoral topical anesthesia. *Periodontol 2000*. 2008;46:56–79.
3. Use of ozone-depleting substance. Federal Register. At https://www.federalregister.gov/documents/2016/10/26/2016-25852/use-of-ozone-depleting-substances. Accessed July 12, 2017.
4. US Food and Drug Administration. Phase-out of CFC metered-dose inhalers containing flunisolide, triamcinolone, metaproterenol, pirbuterol, albuterol and ipratropium in combination, cromolyn, and nedocromil—questions and answers. At https://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm208138.htm. Accessed July 13, 2017.
5. US Food and Drug Administration. CPG Sec. 440.100 Marketed new drugs without approved NDAs and ANDAs. At https://www.fda.gov/iceci/compliancemanuals/compliancepolicyguidancemanual/ucm074382.htm. Accessed July 13, 2017.
6. Hansson P, Backonja M, Bouhassira D. Usefulness and limitations of quantitative sensory testing: clinical and research application in neuropathic pain states. *Pain*. 2007;129:256–259.
7. Cruz-Almeida YF, Filligim RB. Can quantitative sensory threshold testing move us closer to mechanism-based pain management? *Pain Med*. 2014;15:61–72.
8. Zhou P, Chen Y, Zhang J, Wang K, Svensson P. Quantitative sensory testing for assessment of somatosensory function in human oral mucosa. *Acta Odontol Scand*. 2018;76:13–20.
9. Barcohana N, Duperon DF, Yashar M. The relationship of application time to EMLA efficacy. *J Dent Child (Chic)*. 2003;70:51–54.

10. Roghani S, Duperon DF, Barcohana N. Evaluating the efficacy of commonly used topical anesthetics. *Pediatr Dent*. 1999;21:197—200.
11. McMillan AS, Walshaw D, Meechan JG. The efficacy of EMLA and 5% lignocaine gel for anaesthesia of human gingival mucosa. *Br J Oral Maxillofac Surg*. 2000;38:58—61.
12. Wang S, Giannakopoulos H, Lowstetter J, et al. Lack of methemoglobin elevations after topical applications of benzocaine alone or benzocaine plus tetracaine to the oral mucosa. *Clin Ther*. 2017;39:2103—2108.
13. King A, Menke N, Katz K. Toxic hemoglobinopathies in the emergency department. *EM Crit Care*. 2013;3:1—17.
14. Aepfelbacher FC, Breen P, Manning WJ. Methemoglobinemia and topical pharyngeal anesthesia. *N Engl J Med*. 2003;348:85—86.
15. Nguyen ST, Cabes RE, Bashour CA, et al. Benzocaine-induced methemoglobinemia. *Anesth Analg*. 2000;90:369—371.
16. Ellis FD, Seiler JG, Pahnore MM. Methemoglobinemia: a complication after fiberoptic orotracheal intubation with benzocaine spray. A case report. *J Bone Jt Surg Am*. 1995;77:937—939.
17. Udeh C, Bittikofer J, Sum-Ping ST. Severe methemoglobinemia on re-exposure to benzocaine. *J Clin Anesth*. 2001;13:128—130.
18. Gupta PM, Lala DS, Arsura EL. Benzocaine-induced methemoglobinemia. *South Med J*. 2000;93:83—86.
19. Guay J. Methemoglobinemia related to local anesthetics: a summary of 242 episodes. *Anesth Analg*. 2009;108:837—845.
20. Gregory PJ, Matsuda K. Cetacaine spray-induced methemoglobinemia after transesophageal echocardiography. *Ann Pharmacother*. 2000;34:1077.
21. Ferraro L, Zeichner S, Greenblott G, Groeger JS. Cetacaine-induced acute methemoglobinemia. *Anesthesiology*. 1988;69:614—615.
22. Whited C, Cohen SM. Nasal tetracaine spray-induced methemoglobinemia. *Otolaryngol Head Neck Surg*. 2012;146:678—679.
23. Lavergne S, Darmon M, Levy V, Azoulay E. Methemoglobinemia and acute hemolysis after tetracaine lozenge use. *J Crit Care*. 2006;21:112—114.
24. Cooper SA, Beaver WT. A model to evaluate mild analgesics in oral surgery outpatients. *Clin Pharmacol Ther*. 1976;20:242—250.
25. Cooper SA, Desjardins PJ, Turk DC, et al. Research design considerations for single-dose analgesic clinical trials in acute pain: IMMPACT recommendations. *Pain*. 2016;157:288—301.
26. Hersh EV, Levin LM, Adamson D, et al. Dose-ranging analgesic study of Prosorb diclofenac potassium in postsurgical dental pain. *Clin Ther*. 2004;26:1215—1227.
27. Hersh EV, Levin LM, Cooper SA, et al. Ibuprofen liquigel for oral surgery pain. *Clin Ther*. 2000;22:1306—1318.
28. Adriani J, Zepernick R, Arens J, Authement E. The comparative potency and effectiveness of topical anesthetics in man. *Clin Pharmacol Ther*. 1964;5:49—62.
29. Defalque RJ, Stoelting VK. Latency and duration of action of common local anesthetics: an experimental evaluation. *Anesth Analg*. 1967;46:311—314.
30. Covino BG. Pharmacology of local anaesthetic agents. *Br J Anaesth*. 1986;58:701—716.
31. Farrar JT, Portenoy RK, Berlin JA, Kinman JL, Strom BL. Defining the clinically important difference in pain outcome measures. *Pain*. 2000;88:287—294.
32. Bourolias C, Gkotsis A, Kontaxakis A, Tsoukarelis P. Lidocaine spray vs tetracaine solution for transnasal fiber-optic laryngoscopy. *Am J Otolaryngol*. 2010;31:114—116.
33. Noorily AD, Noorily SH, Otto RA. Cocaine, lidocaine, tetracaine: which is best for topical nasal anesthesia? *Anesth Analg*. 1995;81:724—727.
34. Noorily AD, Otto RA, Noorily SH. Intranasal anesthetic effects of lidocaine and tetracaine compared. *Otolaryngol Head Neck Surg*. 1995;113:370—374.
35. FDA Drug Safety Communication: reports of a rare, but serious and potentially fatal adverse effect with the use of over-the-counter (OTC) benzocaine gels and liquids applied to the gums or mouth. At https://www.fda.gov/Drugs/DrugSafety/ucm250024.htm. Accessed June 19, 2018.
36. Townes PL, Geertsma MA, White MR. Benzocaine-induced methemoglobinemia. *Am J Dis Child*. 1977;131:697—698.

Address correspondence to: Elliot V. Hersh, DMD, MS, PhD, University of Pennsylvania School of Dental Medicine, 240 South 40th Street, Philadelphia, PA 19104-6030, USA. E-mail: evhersh@upenn.edu