Benzalkonium chloride and nasal mucociliary clearance: A randomized, placebo-controlled, crossover, double-blind trial

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ABSTRACT

Background: *Benzalkonium chloride (BKC) has been considered an innocuous preservative for prescription drugs.*

Methods: *We performed a double-blind, placebo-controlled, randomized, crossover, single-center trial with a 3-week washout period in 43 healthy volunteers comparing the effect of 3-week use of saline nasal spray containing BKC 0.01% to preservative-free saline t.i.d. on nasal mucociliary clearance rate. Evaluations were done at the beginning and the end of each period by γ-scintigraphy with technetium^{99m}-labeled strontium.*

Results: *Nasal mucociliary clearance rate was significantly impaired by BKC with a difference of 1.23 mm·min^{-1} ($p < 0.01$) between periods.*

Conclusion: *BKC in the concentration used in nasal preparations impaired mucociliary clearance in healthy individuals after 3 weeks of use. Presently, when preservative-free alternatives are available, BKC could be a risk without benefit.*

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Among available antimicrobial preservatives, benzalkonium chloride (BKC) was first introduced in 1935 as an antiseptic agent for clinical use and later was approved by the Federal Drug Administration, in 1982, as an innocuous ingredient for prescription drugs at concentrations up to 0.1%. It is the most commonly used agent to prevent bacterial contamination and to preserve pharmacologic activity in a wide range of prescription and over-the-counter products for a large array of indications—including several topical formulations for nasal use—with millions of units sold worldwide annually.[1–3]

Although considered inert, there still is a large uncovered debate in the literature about potential harmful effects of BKC. Recently, BKC effects on nasal mucociliary clearance (NMC), mucosal histology, ciliotoxicity, and neutrophil function were reviewed with conflicting findings.[2,3] Most studies evaluating the effect of BKC on NMC—one of the main nasal defense mechanisms—were done with methodologies that depend on subjective perception as the saccharine test, usually in solutions also containing topical steroids or oxymetazoline and in patients with atopic rhinitis, all of which can introduce serious bias on evaluation. The objective of this study was to investigate the effect of BKC 0.01% saline solution on NMC rate in healthy subjects in a controlled clinical trial.

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METHODS

Study Design and Population

This was a double-blind, placebo-controlled, randomized, crossover, single-center trial devised to investigate the effects of 3-week t.i.d. use of 0.9% saline spray with or without BKC 0.01% (BKC free) on NMC rate. A 3-week washout interval was adopted between both periods. We planned our study in accordance with CONSORT statements.[4] The Institutional Review Board of the Federal University of Pernambuco approved the study and written informed consent was obtained from all participants.

Healthy volunteers, 13–50 years of age, were recruited among relatives of children attending the Pediatrics Allergy Clinic at the University Clinical Hospital. Participants received no payment except transport and meal allowances. Inclusion and exclusion criteria are listed in Table 1.

Interventions

Subjects were screened according to selection criteria (Table 1) and submitted to a basal NMC rate determination. Then, they were submitted to a sequence of two periods of blinded medication use of 3 weeks each (periods 1 and 3), with a 3 weeks washout period of no medication use between them (period 2). At the beginning of periods 1 and 3 subjects received the solution containing atomizers and were instructed to use 1 spray in each nostril t.i.d. NMC rate determinations were done at the end of periods 1, 2, and 3. Any complaints were questioned at each visit, especially those related to symptoms of upper airway infection (upper respiratory infection [URI]), such as fever, sore throat, cough, stuffy/runny nose, and malaise.

BKC saline (Sorine Infantil; batch 0302304; Aché Laboratórios Farmacêuticos SA, São Paulo, Brazil) and preservative-free (Salsep; batch 31023; Libbs Farmaceutica do Brasil, São Paulo, Brazil) solutions were purchased from the market as commercial formulations, both approved by the Brazilian reg-

Table 1 **Inclusion and exclusion**

Inclusion Criteria	Exclusion Criteria
No respiratory symptoms	Rhinosinusal complaints
Never had asthma (wheezing and shortness of breath associated with sputum) or rhinitis (recurrent sneezing and itching and watery discharge)	Important septum deviation at anterior rhinoscopy
No influenza symptoms in the past 60 days	Divers
No topical nasal medicine use	Smokers or exsmokers <5 yr
NMC > 6 mm·min^{-1}	Pregnant woman (fertile woman had β-HCG in urine)
No septum deviation occluding the nostril at anterior rhinoscopy	Refusal to sign informed consent
Signed informed consent	

β-HCG = Beta human chorionic gonadotrophin.

ulatory agency (ANVISA) and conditioned in identical sealed atomizers labeled "A" or "B" according to randomization. No difference in the appearance, taste, viscosity, or smell could be detected between the preparations.

Compliance

To check for compliance, bottles were weighed before and after use. Subjects were not informed of this procedure. Expected weight consumption was 7 g in each period based on the mean weight difference in bottles after 126 actuations of 5 atomizers.

Primary Outcome and Assessment

The primary outcome was the difference between NMC rates after 3-week use t.i.d. of nasal spray with BKC or preservative-free saline solutions. Image acquisitions and analysis for NMC rate determinations were done through a StarCam Gamma Camera (General Electric, Milwaukee, WI) 3.200 AC/T equipped with a general purpose, parallel holes, low energy collimator using a 128/128 pixels matrix. Radioactive solutions were prepared immediately before the exam by diluting 2.5 mCi of colloidal strontium labeled with tecnetium^{99m} in BKC-free 0.9% saline, resulting in 0.12–0.15 mCi of radioactivity per drop. The strontium (with a mean particle diameter of 0.03 μm) and technetium were purchased from Instituto de Pesquisas Energéticas e Nucleares, São Paulo, Brazil.

After explaining the procedure to the volunteer, a droplet of the radioactive solution was placed on the floor of the most unobstructed nostril through a pipette calibrated to deliver a 0.05-mL drop, 1.5 cm from the mucocutaneous junction. If no nostril was more patent than the other, the right was chosen. One drop of solution also was placed at the tip of the nose to create a fixed reference mark for NMC rate calculations. Immediately after, the subject was seated with the chosen side of the face in contact with the vertically positioned collimator, with the head slightly bent forward, with a chin support to minimize movements, and was instructed not to move the head, not to talk, or not to sniff.

Images were acquired in dynamic mode at 15-second intervals for 15 minutes, (total, 60 frames). At the end of acquisition, the NMC rate was calculated in millimeters per minute, measuring the length and time that elapsed between the starting point of the drop displacement and the point immediately before its fall into the pharynx. To avoid errors caused by minor head movements, the mark at the tip of the nose was used as the reference for drop displacement assessment. The calculations were performed by the same investigator and, to ascertain for accuracy, a comparison was made with a second NMC rate measurement done in 20 exams stored in the computer, randomly, and blindly chosen by a coinvestigator, before breaking the randomization codes. An example of images chosen for analysis is depicted in Fig. 1.

Sample Size

The sample size was estimated based on a pilot study undertaken in nine volunteers, which showed a mean (±SD) difference of 1.1 (±1.58) mm·min^{-1} between measurements of NMC rate taken after two periods of 2 weeks each of nasal use of 0.01% BKC saline or BKC-free control saline, with an intervening 2-week washout period (Rizzo JA, unpublished data). Accepting an α- and β-errors of 0.05 and 0.1 (two-tailed), respectively, the sample size calculated to detect a difference on NMC of 1.0 mm·min^{-1} between periods was 33 individuals for a paired clinical trial.[5] A total of 43 participants were selected to compensate for dropouts or losses because of URI, a well-known NMC rate interference cause.[6] URIs were defined based on common symptoms of cold.

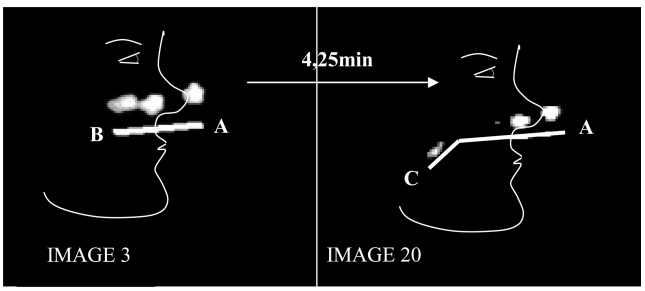

Figure 1. *Example of NMC rate calculation. Displacement distance = AC − AB; Time = final chosen image − initial chosen image; Rate = Distance/Time.*

Randomization

Randomization was accomplished using the restricted shuffle approach[5] by a third party and the sequence was kept blind to the investigators. A pharmacist conditioned the solutions according to the randomization sequence in identical sealed atomizer bottles labeled "A" and "B," which were enclosed in numbered boxes from 1 to 46. The randomization list and the atomizers were prepared at another institution and each box was intended for use by only one volunteer, bottle A first, followed by bottle B in entry sequence. Concealment was attained because it was not possible for subjects or investigators to distinguish any difference among the bottles.

Statistical Analysis

Paired *t*-test was performed on log-transformed data for comparison between NMC rates after solutions use. Unpaired *t*-test was used for comparison between groups. Log transformation was needed to normalize NMC rate distribution. Proportions were compared by chi-squared or Fisher's exact tests. Subjects who had URI symptoms during the study period were excluded from final analysis. Period and carryover effects were evaluated according to Pocock.[7]

RESULTS

Volunteers Information

Forty-three volunteers were randomized into the study. Demographics and distribution characteristics are depicted in Table 2. Twenty-one subjects were allocated to group A (to use preservative-free saline first) and 22 to group B (receiving BKC saline first).

During the trial nine subjects had URI: four subjects had URI in the first period (one on preservative-free and three on BKC saline solutions), three subjects had URI during the washout period (all after BKC-free saline), and two subjects had URI in the second period (both on preservative-free saline). Of these, all but one subject—who refused final NMC evaluation—completed the planned observations. At the end, 17 individuals in each group (our accrual goal) completed the study period without URIs. There was no association between solutions and respiratory infection ($p > 0.05$, Fisher's exact test).

Baseline and Postwashout NMC Rate Measurements

The distributions of baseline and postwashout NMC rate measurements of the 34 subjects included in the analysis are depicted in the steam-and-leaf plot in Fig. 2. There was no difference ($p > 0.05$) between these periods, because there were no carryover effects. When subjects who had URI were included in this analysis, a statistical significant difference was observed between these two periods (8.7 mm·min^{-1} versus 7.6 mm·min^{-1}; $p < 0.05$), which demanded their exclusion from the final analysis.

Table 2 **Baseline characteristics of participants**

No. of patients	43
Age (yr)	30
Median and limits	13–54
Gender (M/F)	18/25
BKC-free solution First (group A)	21
Saline with BKC first (group B)	22
Basal NMC	8.8 mm·min^{-1}
Mean (CI 95%)	7.9, 9.5

Effect of BKC on NMC Rate

There was no difference between basal NMC rate compared with that after preservative-free solution period (mean ± SD, respectively: 8.5 ± 1.3 mm·min^{-1} and 8.5 ± 1.4 mm·min^{-1}; $p > 0.05$). After BKC-containing saline, NMC mean rate was 6.9 ± 1.3 mm·min^{-1}, $p < 0.01$ compared to basal and BKC-free saline period (Fig. 3).

Compliance Evaluation

In the first period of nasal spray use, 2/34 volunteers used <50% of the expected dose (both on BKC saline) and 5/34 used >150% of prescribed dose (two on preservative-free solutions and three on BKC saline). In the subsequent crossover period 2/34 subjects used <50% (both on preservative-free saline) and 7/34 used >150% of expected dose (four on preservative-free solution and three on BKC saline; Fig. 4). It is interesting to note that all individuals that used >150% of medication in the first did so in the second period. In the last period two more subjects exceeded the recommended dose, one in each solution. There was no significant difference between groups in medication use ($p > 0.05$, Fisher's exact test). Difference in NMC rate remained significant even excluding these overusers from analysis ($p < 0.05$).

NMC Rate Measurement Consistency

The test–retest consistency of NMC rate determination was very high in the 20 repeated measurements by the investiga-

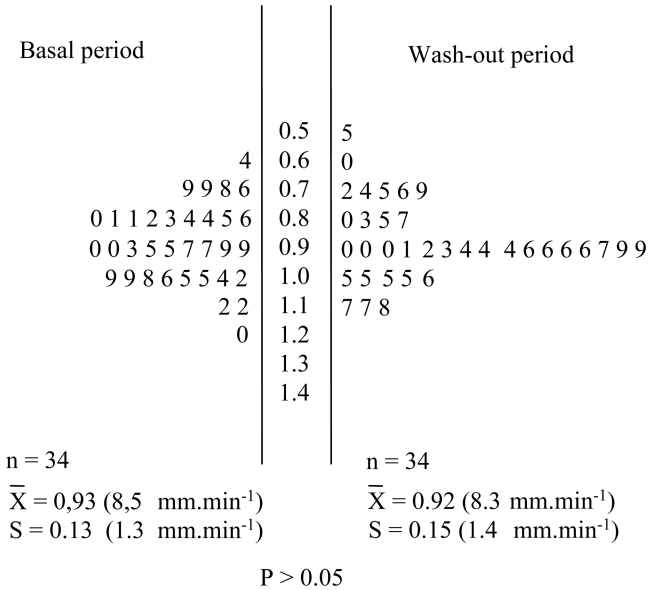

Figure 2. *Steam-and-leaf plot of log of NMC rates at basal (left) and after washout period (right). Numbers in parenthesis are antilogged values.*

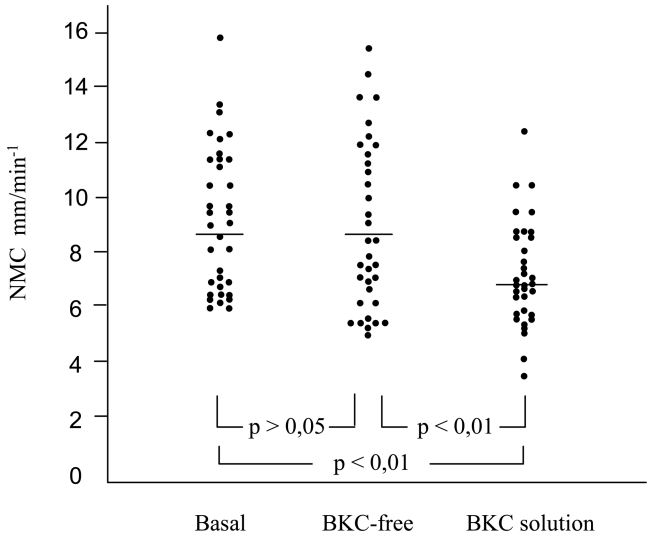

Figure 3. *NMC rate (mean ± SD) at basal evaluation (8.5 ± 1.3 mm·min^{-1}) and after BKC-free solution (8.5 ± 1.4 mm·min^{-1}) or BKC containing solution periods (6.9 ± 1.3 mm·min^{-1}).*

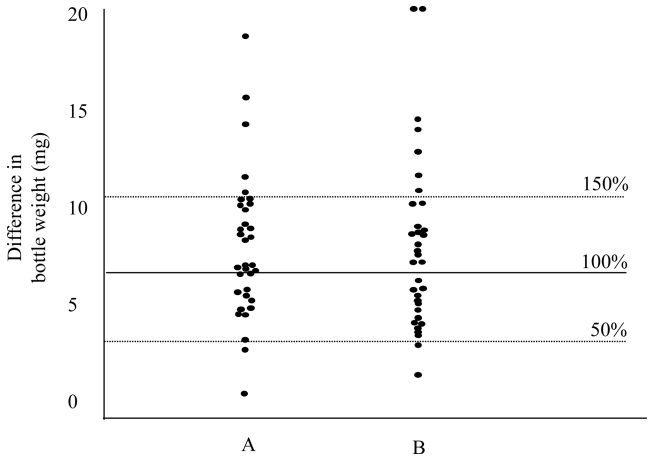

Figure 4. *Use of Solutions by volunteers measured as difference in weight of bottles before and after use period. (A) First period and (B) second period. Expected differences, 7 mg (full line). Fifty percent use (3.5 mg) and 150% use (10.5 mg) are represented as dotted lines. There was a value of $p > 0.05$ between solution weights at periods A and B.*

tor, with a mean difference of 0.04 mm·min^{-1} (95% CI, −0.44, 0.52).

DISCUSSION

This randomized, placebo-controlled, crossover, double-blind trial in healthy volunteers showed that 1 puff of 0.01% BKC saline in each nostril t.i.d. for 3 weeks impaired the NMC rate. The huge difference in NMC rates observed before and after URIs in the nine subjects who had the infection during the trial (6.1 mm·min^{-1}) interfered in NMC after washout, leading to a period effect and demanded their exclusion from final analysis, although their inclusion in an intention-to-treat basis did not change the results.

A variety of methods, end points, and different techniques were used in the few studies on adverse effects of BKC on nasal function, generating conflicting data and opinions.[2,3] The radioactive method we adopted is considered to provide the most physiological and reliable information about NMC in humans.[8] In fact, Naclerio *et al.*, using a similar method to compare NMC in allergic rhinitis patients on topical steroid solutions for 2 weeks, with or without BKC, also found a significant reduction on nasal clearance in the group using preservative-containing solutions.[9]

A recent authoritative review of the literature[10] discussing the adverse effects of BKC as a preservative in topical nasal preparations concluded that this conservant causes no significant damage to the nasal mucosal, even with prolonged use, although based in an *in vivo* small amount of data and recognizing that the *in vitro* data suggest deleterious effects. Unfortunately, the great majority of those *in vivo* studies deserve a critical look.

Since 1995, Graf *et al.* have shown, in a randomized double-blind parallel study in 20 healthy volunteers, that BKC added to oxymetazoline in nasal spray for 30 days accentuated the severity of rhinitis medicamentosa with a mean increase in rebound swelling and worse evening symptoms score.[11] He also showed that 28-day use of BKC nasal spray *without* oxymetazoline also was capable of inducing mucosal swelling.[12]

Lebe[13] performed an experimental *in vivo* study to investigate symptom manifestations (sneezing and nasal rubbing) and histological changes induced by administration of BKC 0.01% to the nasal mucosa of rats for 1 and 4 weeks. Symptoms were more intense after the 6th day and both light and electron microscopy showed mucosal lesions that were more pronounced with prolonged administration.

Recently, Riechelmann *et al.*[14] assessed the ciliotoxicity of BKC in isolated human nasal epithelium from 15 donors. They also measured the effects of nasal 0.05% BKC saline (4 × 200 μL/day for 8 days) on saccharin transport time, inflammatory cells populations, cytokine levels in nasal secretions, and nasal symptom scores in 16 healthy volunteers, in a randomized, double-blind crossover trial. BKC exposure showed ciliotoxicity ($p < 0.0001$) *in vitro* but, *in vivo*, BKC containing solution did not alter saccharin transport time ($p > 0.8$) and no proinflammatory effects were observed. The short-term BKC exposure could not be sufficient to reflect the observed histological ciliotoxic changes. In contrast, a well-designed human nasal mucosa *in vitro* study has established that steroid nasal sprays containing fluticasone or mometasone, both with BKC, caused slowing or paralysis of ciliary movements, depending on the concentration.[15]

It is very important to emphasize that the statistically significant differences found in our research may not be clinically relevant. However, some studies indicate that BKC in nasal decongestant sprays affects the nasal mucosa even after short-term use (10 days) and sustained use of BKC alone can induce nasal mucosal swelling.[16,17]

Nasal saline spray with BKC also was toxic to human neutrophils at concentrations far lower than those found in commercially available formulations.[18] Bernstein,[2] in a less recent review article, concluded that both animal and human *in vitro* data suggest that BKC promotes ciliostasis and reduction in NMC that may be partially masked by absorption and

dilution effects because of respiratory mucus. His recommendation is that the use of BKC-free glucocorticosteroid formulations should be considered, particularly in patients who complain of nasal burning, dryness, or irritation.

Possible confounding factors that may account for the discordances are inconsistent methods of study, poor compliance, insufficient length of exposure, and variation in solution concentrations. We tried to minimize confounding factors by adopting a reliable NMC rate determination method, checking consistency of measurements and compliance. In addition, concentration, doses, and length of use of the study solutions were planned to replicate real-world prescriptions.

Nasal medications containing BKC are used worldwide and topical nasal steroids containing this preservative are prescribed for months, sometimes for years. BKC-containing saline solutions are commonly prescribed as adjuvants in the treatment of rhinitis and sinusitis for nasal irrigation, often many times a day and sometimes for long periods. In Brazil, an epidemiological study showed that by the 3rd month of life 20% of all infants had used some medicine for 1 month or longer, among which the most frequently prescribed was 0.9% nasal saline containing BKC.[19]

In conclusion, our work shows that 3 weeks use of saline nasal spray containing BKC as preservative slows down NMC rate. The clinical significance of these findings remains to be established but the potential risk of short- and long-term use of BKC-containing solutions needs considerations, especially when we have enough device technologies that make it possible to deliver nasal medicines without preservatives and represent a more reasonable alternative.[3] We agree with Verse *et al.*'s[20] opinion that, nowadays, when preservative-free alternatives are available, preserved nasal sprays are obsolete.

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