

REVIEW ARTICLE

Safety review of benzalkonium chloride used as a preservative in intranasal solutions: An overview of conflicting data and opinions

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BACKGROUND: For most multiuse aqueous nasal, ophthalmic, and otic products, benzalkonium chloride (BKC) is the preservative of choice. The American College of Toxicology has concluded that BKC can be safely used as an antimicrobial agent at concentrations up to 0.1%. BKC has been in clinical use since 1935 and is contained in a wide variety of prescription and over-the-counter products. However, over the past several years there have been conflicting reports of damage to human nasal epithelia and/or exacerbation of rhinitis medicamentosa associated with intranasal products containing BKC.

OBJECTIVE: We sought to review the published literature and determine whether there is sufficient, clinically significant data that would confirm that intranasal products containing BKC are likely to damage human nasal epithelia or exacerbate rhinitis medicamentosa.

METHODS: A literature search was conducted for in vivo and in vitro studies that evaluated the effects of BKC on human nasal epithelia.

RESULTS: A total of 18 studies (14 in vivo, 4 in vitro) were identified that evaluated short- and long-term exposure of concentrations of BKC in concentrations ranging from 0.00045% to 0.1%. Eight studies, including a 6-month and 1-year long-term treatment study, demonstrated no toxic effects associated with BKC, indicating that BKC was neither harmful to nasal tissue nor prone to exacerbate

rhinitis medicamentosa. Furthermore, of the 10 studies that concluded that BKC resulted in degenerative changes in human nasal epithelia (eg, ciliary beat frequency, ciliary morphology, mucociliary clearance, epithelial thinning and/or destruction) or that BKC exacerbates rhinitis medicamentosa, only 2 (it was 2 according to the Results section) of these studies were supported by statistically significant differences between BKC and placebo or active control groups were compared. It is important to note that in both of these studies, the protocol incorporated the use of oxymetazoline in some or all of the subjects. Oxymetazoline is associated with rhinitis medicamentosa.

CONCLUSION: Intranasal products containing the preservative BKC appear to be safe and well tolerated for both long- and short-term clinical use. (*Otolaryngol Head Neck Surg* 2004;130:131-41.)

Benzalkonium chloride (BKC) is a quaternary ammonium compound that has been in clinical use since 1935¹ as an antimicrobial additive. It has been used to maintain the sterility of a variety of prescription and over-the-counter products, such as cosmetics, infant care products, and pharmaceutical nasal sprays, ophthalmic solutions, and otic drops.² As reported in the *Journal of American College of Toxicology*, the Cosmetic Ingredient Review panel concluded that BKC can be safely used as an antimicrobial agent at concentrations up to 0.1%.² However, over the past several years, reports of damage to human nasal epithelia and/or exacerbation of rhinitis medicamentosa associated with intranasal products containing BKC have emerged.³⁻⁷

The objective of this article was to review the published literature specific to these safety issues to determine whether sufficient, clinically significant data exist to confirm that intranasal products containing BKC cause actual damage to human

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nasal epithelia or exacerbate rhinitis medicamentosa.

MATERIALS AND METHODS

A MEDLINE literature search was conducted from 1980 to February 2003 for in vivo and in vitro studies that evaluated the effects of BKC on nasal epithelia. The search identified a total of 18 preclinical and clinical studies. An overview of the study methods are presented as follows:

In Vivo Studies

There were 14 in vivo studies, with 11 using human subjects and 3 using animal subjects. A variety of different techniques and methods were used in each of the studies. Nasal biopsy samples, when taken, were harvested from different nasal locations. Changes in ultrastructural ciliary form and function were determined by various types of microscopy, including light microscopy (LM),⁸⁻¹³ transmission electron microscopy (TEM),^{9,12} scanning electron microscopy (SEM),^{9,12} and inverted phase microscopy (IPM).¹⁴ Direct mucociliary clearance was evaluated via indigo carmine saccharine transport time (ICST)^{10,15} or saccharine clearance time (SCT).¹⁴ Exacerbation of rhinitis medicamentosa was determined by changes in nasal epithelia thickness^{15,16} (Tables 1 and 2).

In Vitro Studies

There were 4 in vitro studies. As with the in vivo studies, a variety of methodologies were used. Indirect mucociliary clearance via ciliary beat frequency was determined using cultured ciliated chick embryo tracheas or human ciliated adenoid or nasal epithelial tissue. Changes in ultrastructural ciliary form and function were determined by various types of microscopy, including LM,^{12,17} TEM,¹² SEM,¹² and IPM (Tables 1 and 2).

RESULTS: SUMMARY OF PUBLISHED STUDIES

Of the 18 studies identified, 8 concluded there were no toxic effects associated with BKC and 10 concluded that BKC was detrimental to nasal epithelium or exacerbated rhinitis medicamentosa at concentrations of BKC ranging from 0.1 mg/mL to 0.02%.

The studies that concluded there were no toxic effects associated with BKC are summarized in Table 1. All were in vivo (7 human, 1 animal) and were well powered to detect statistically significant differences in nasal epithelium histology or function due to exposure to BKC 0.1% to 0.02%. Within this group of studies, no statistically significant differences were noted between treatment groups that would indicate BKC was either harmful to nasal tissue or exacerbated rhinitis medicamentosa. The study that was longest in duration compared mucosal biopsy samples from patients receiving BKC-containing intranasal steroid sprays versus oral antihistamines for a period of 6 months. No significant differences were noted between any of the treatment arms, and no adverse effects on nasal mucosa were observed after 6 months of treatment with triamcinolone acetonide (n = 21) or beclomethasone dipropionate (n = 26) aqueous nasal sprays containing BKC 0.02%.⁸

The 10 studies that concluded there were toxic effects associated with BKC are summarized in Table 2 (human: 4 in vivo, 3 in vitro; animal: 2 in vivo, 1 in vitro). Of particular interest, Graf et al^{18,19} conducted 2 controlled studies that examined the long-term effect of BKC on the nasal mucosa. In the first trial, patients were treated with either oxymetazoline containing BKC 0.01% or BKC-free oxymetazoline for a period of 30 days. Nasal mucosal swelling was indirectly measured using rhinostereometry and nasal reactivity was measured via histamine provocation. Patients treated with the oxymetazoline/BKC combination demonstrated significantly greater nasal mucosal swelling ($P < .05$). Unfortunately, interpretation of these data is unclear given that both groups that were evaluated had confirmed nasal mucosal swelling after 30 days and no placebo group was included for comparison.²⁰ The second study by Graf et al¹⁸ challenged the authors' interpretation of their earlier results by showing that patients treated with BKC-free oxymetazoline had significantly more nasal stuffiness than those treated with BKC alone and those treated with placebo. With regard to nasal mucosal swelling, an ANOVA analysis yielded no significant difference among the groups.¹⁸ Further corroboration of these results was provided in a short-term study by Graf et al¹⁹ in 1999 that concluded that oxymeta-

zoline and xylometazoline nasal spray with or without BKC may be safely used for up to 10 days in patients with chronic untreated vasomotor rhinitis. In this study, there was no difference in the degree of nasal mucosa swelling and nasal stuffiness between patients treated with oxymetazoline containing BKC and patients treated with BKC-free oxymetazoline.¹⁹

In another example, a preclinical placebo-controlled study with Sprague-Dawley rats exposed to both high (0.1%) and low (0.01%) BKC with and without triamcinolone acetonide showed BKC-associated nasal epithelial changes after 1 week of exposure. However, the editor stated that this study was underpowered and that the standard deviations were too large and therefore statistically valid conclusions could not be drawn.¹⁶

Overall, only 2 of the 10 studies that concluded BKC to be detrimental to nasal mucosa and/or exacerbated rhinitis medicamentosa via swelling of nasal tissues were supported by significantly different results from placebo or active controls. Of interest, both of these studies also included the use of oxymetazoline, which is well known for its association with rhinitis medicamentosa.^{20,21}

Discussion

Maintaining a sterile environment within multidose medication delivery systems is a challenge fundamental to patient safety. Failure to provide such an environment risks patient inoculation with fungal, bacterial, and viral pathogens, which can lead to life- and health-threatening consequences. This issue has prompted health regulation organizations, such as the US and European pharmacopoeias, to issue strict criteria regarding maintenance of product sterility. Unfortunately, very few new antimicrobial preservatives have been introduced to the market over the course of the past 4 decades. During the same time period many older preservatives have been withdrawn from the market due to concerns of tissue toxicity.²² BKC, which has been in clinical use since 1935 and approved for use as a preservative by the Food and Drug Administration since 1982, has been used effectively in its role as a preservative.¹ Its use in a variety of prescription and over-the-counter products (eg, cosmetic, including infant care; pharmaceutical and over-the-counter nasal sprays,

ophthalmic solutions, and otic products) has offered a long history demonstrating both safety and effectiveness.

Review of the current published literature, however, reveals an emerging concern that exposure of nasal epithelia to BKC may lead to induction of pathologic or histologic changes within nasal epithelial tissue or possibly exacerbate rhinitis medicamentosa by causing increased swelling of nasal epithelium. Further, if this concern is valid, it follows that these effects might be time or concentration dependent. The impact of these issues to patient safety is of obvious concern to practitioners.

A number of studies have been designed and performed over the course of the past 3 decades in an attempt to address these concerns. Unfortunately, a number of different confounding issues have led to continued confusion surrounding safety concerns of BKC. Differences in study design, analysis of data, and choice of outcome parameters are among only some of the factors that have contributed to a lack of consensus regarding the safety of BKC. One striking and relatively consistent difference that emerges when these studies are cumulatively reviewed is the discrepancy between *in vitro* and *in vivo* data. Although examination of data provided by *in vitro* studies raises some concern regarding the safety of BKC,^{3,12,17,23} examination of available *in vivo* data favors the safety of BKC.^{8-10,14,15,18,20,24,25}

Several factors that may lead to differences have been observed between basic science and clinical studies. One major contributing factor to this problem is the lack of a universally accepted *in vitro* model for standard evaluation of the effects of preservatives on human nasal epithelium. The *in vitro* studies reviewed in the preparation of this report made use of a variety of different models and methods for evaluating BKC effects, giving rise to the possibility of significant differences in outcomes. As an example, ciliated cells cultured from human adenoids used in some studies were noted to be less susceptible to the ciliotoxic effects of preservatives than were chicken embryo tracheas used in other studies.²⁶ In the end, researchers have been unable to duplicate results obtained from other studies, resulting in differing conclusions (Tables 1 and 2).

Table 1. Summary of studies finding no toxic effects associated with benzalkonium chloride (BKC)

Author	Design	Treatment materials/regimen
Ainge et al ²⁴	In vivo, nasal mucosa of rats (n = 24) and monkeys (n = 8) treated for 28 d	Monkeys: FPANS 2x right nostril QID (n = 4), control 5% glucose (n = 4) Rats: BDPANS 1 h via snout-only inhalation chamber (n = 12), control air only (n = 12).
Batts et al ¹⁵	In vivo, single-dose, single-center, active controlled, clinical study	0.9% NaCl nasal solution with 0.01% thiomersol or 0.01% BKC, or 0.1% EDTA PBO, 0.9% NaCl
Braat et al ¹⁷	In vivo, 6-week, single-center, randomized, double-blind, nasal biopsy study	PBO run-in: 2 sprays, BID each nostril × 2 wk; FPANS 200 µg/spray (n = 8) or PBO w/BKC (n = 8) or PBO (n = 6) BID each nostril × 6 wk
Klossek et al ²⁵	In vivo, 24-week, randomized, prospective, parallel-group, active controlled, open study	TAAANS 2 × 55 µg sprays each nostril QD (n = 29); CTZ 10 mg orally QD (n = 30); BDPANS 50 µg spray each nostril QID (n = 31)
Laliberte et al ⁸	In vivo, 6-month, multicenter, randomized, parallel-group, open study	TAAANS (n = 21); BDPANS (n = 26); CTZ (n = 23) × 6 mo
McMahon et al ²⁵	In vivo, 2-part, randomized, double-blind, placebo-controlled, study: part 1, 2-arm, 2-way crossover, part 2, 3-arm, 2 wk	Part 1: NaCl 0.9% or NaCl 0.9% + 0.02% PKC 2 × 100 µL per nostril; 1 week between treatments, then crossover (n = 27) Part 2: NaCl 0.9% (n = 20), FPANS (n = 23), or FPANS vehicle (n = 15), 2 squirts each nostril BID × 2 wk
Storraas et al ²⁷	In vivo, 2-part, single-treatment, study: part 1, acute BKC exposure; part 2, sustained BKC exposure	Part 1: 10 min nasal pool exposure NS and NS + 0.1 mg/mL (n = 10) Part 2: 100 µL each nostril TID × 10 d (n = 12)
Holm et al ³¹	In vivo, double-blind, parallel-group study comparing FPANS BID vs placebo. Duration of 1 y	FPANS 100 µcg BID vs placebo. (n = 42)

α_2 -MG, α_2 -Macroglobulin; BDPANS, beclomethasone dipropionate aqueous nasal spray (BDP 0.2%, sodium citrate 0.038%, citric acid monohydrate 0.0195%, chlorocresol 0.01%, sodium chloride 0.9%, BKC 0.01%, polysorbate-80 0.0008%, distilled water q.s. 100 g); BKC, benzalkonium chloride; CBF, ciliary beat frequency; DTPA, diethylenetriaminepentaacetic acid; FPANS, fluticasone propionate aqueous nasal spray; ICST, indigocarmine saccharine transport time; LM, light microscopy; NS, normal saline (0.9%); PBO, placebo; SCT, saccharine clearance time; SEM, scanning electron microscopy; TAAANS, triamcinolone acetonide aqueous nasal spray; TEM, transmission electron microscopy.

Table 1. Continued

Evaluation method(s)	BKC (%)	Results
LM: epithelium, number of ciliated cells; ciliated cells SEM and TEM: ultrastructure	0.02% Monkeys, 0.01% rats	LM: monkeys, no effect; rats, lower incidence of lymphoid tissue in upper airway of treated rats SEM and TEM: no abnormalities or differences between treated and control monkeys and rats
Evaluate nasal clearance with radiolabeled (⁹⁹ Tc-DTPC) saccharine nasal spray, 1 h after preservative or PBO nasal drops	0.01%	No significant differences in either CI rate or proportion of radiolabeled nasal spray at 10, 20, 30, 60, and 90 min after administration with any preservative compared with PBO (<i>P</i> > 0.05, for both)
ICST q 2 wk, before, during, and after treatment	0.02%	No significant differences between groups; no statistical relationship between number of ciliated cells and treatment; SEM and TEM showed no BKC effects
ICST, endoscopic evaluation, nasal mucosal thickness (NMT)	0.02%	For all ITT treatment groups; no statistically significant difference in NMT; no quantitative or qualitative treatment-related differences in nasal epithelium; biopsies showed no destruction of epithelium; no major or minor endoscopic findings
Endoscopic evaluation of nasal cavities, biopsies of posterior inferior nasal turbinate	0.02%	Endoscopy: for all treatments, all nasal tissue were still normal after 6 mo of therapy LM: no significant difference in ET between all 3 treatment groups (<i>P</i> < 0.06), all showed decreased ET from PT compared with EOT; qualitative an LM: no significant difference in ET between all 3 treatment groups (<i>P</i> = 0.06), all showed decreased ET from PT compared with EOT; qualitative analysis showed no significant change in individual biopsies before and after treatment; biopsies never showed epithelium destruction. Long-term Treatment with BKC showed no adverse effects on nasal mucosa
Part 1: Immediate effect on SCT	0.02%	Part 1: neither treatment showed a significant difference in SCT (<i>P</i> > 0.05)
Part 2: effect before and after 2 wk exposure, on SCT, CBF, acoustic rhinometry (Amin), & symptom scores (SS)		Part 2: no significant differences in measurements (ie, CBF, SCT, Amin, SS) between treatments after 2 weeks (<i>P</i> > 0.053); all treatments showed a significant decrease in CBF (<i>P</i> ≤ 0.013); SCT tended to increase but not significantly (<i>P</i> ≥ 0.20); no evidence of any ciliotoxicity during 2 wk regular therapy
Part 1: Nasal pain (0 = none to 3 = several); α ₂ -MG; fucose	0.1 mg/mL	Part 1: pain scores: 0.3 ± 0.2 NS, 1.2 ± 0.2 BKC (<i>P</i> < 0.01); BKC significantly increased fucose secretion (<i>P</i> < 0.05); α ₂ -MG unaffected
Part 2: nasal symptoms (sneezes, blockage, rhinorrhea, and pain; 0 = none to 3 = severe); α ₂ -MG; fucose; histamine challenge before and after BKC exposure		Part 2: no nasal pain on frequent BKC administration; nasal secretion/blockage infrequent; nasal baseline scores 0.4 ± 0.2 before and after BKC (<i>P</i> = 0.79); histamine increased nasal symptoms before and after BKC (<i>P</i> < 0.01); histamine increased α ₂ -MG before and after 10 d BKC (<i>P</i> < 0.01) but plasma exudation of α ₂ -MG was unaffected. BKC in concentrations for OTC products is not associated with exudative hyperresponsiveness or airway inflammation
Nasal biopsies were obtained at entry and end of treatment period	0.02%	Improvement in tissue edema. No detrimental effects to epithelium, cellular inflammation, or sinusoidal nasal dilation

Beyond the obvious problems posed by comparison of differing in vitro methodologies, other problems arise when attempting to predict in vivo

outcomes based on in vitro results.²⁶ Discrepancies between conclusions derived from in vitro and in vivo studies may occur as a result of nu-

Table 2. Summary of studies finding toxic effects associated with benzalkonium chloride (BKC)

Author	Design	Treatment Materials/Regimen
Berg et al ²⁸	Two 3 wk animal in vivo experiments (E1 and E2)	E1: 10 μ L right nostril FLANS (n = 10) or BDPANS (n = 10) or BUDANS-PF (n = 10); left nostril 10 μ L PBO (n = 30) E2: 10 μ L right nostril BDPANS (n = 10) or BUDANS-PF (n = 10); left nostril 10 μ L PBO (n = 20) PBO, NaCl 0.9%
Berg et al ²⁹	Three in vitro 2-wk experiments with cultured human ciliated epithelium exposed to varied concentrations of BKC for 1 to 30 min/d	Undiluted oxymetazoline NS (ONS) and 3%, 10%, and 30% diluted ONS in NaCl
Cho et al ¹²	In vivo, placebo-controlled, 4-wk preclinical study	80 Sprague-Dawley rats exposed to low and high concentrations of preservatives: 0.01% BKC or 0.1% PS (n = 9); 0.1% BKC or 5.0% PS (n = 9); TAA 0.15% w/0.01% BKC or 0.1% PS (n = 9); TAA 0.15% w/0.1% BKC or 5.0% PS (n = 9); PBO (NS) (n = 4); 7 μ L in each nostril BID \leq 4 wk
Graf et al ⁹	In vivo, randomized, double-blind, parallel-group clinical study	OMZ 0.5 mg/mL (n = 10) or OMZ 0.5 mg/mL + 0.1 mg/mL BKC (n = 10); 0.1 mL each nostril TID \times 30 d
Graf and Hallen ¹⁰	In vivo, randomized, placebo-controlled, double-blind, parallel-grouped, clinical study	OMZ 0.5 mg/mL (n = 10), 0.1 mg/mL BKC (n = 10), or PBO (NS + Na phosphate + EDTA) (n = 10); 0.1 mL each nostril TID \times 30 d
Graf et al ¹¹	In vivo, randomized, double-blind, parallel-grouped, clinical study	OMZ 0.5 mg/mL (n = 17) or OMZ 0.5 mg/mL + 0.1 mg/mL BKC (n = 18); 0.1 mL each nostril TID \times 10 d

BDPANS, Beclomethasone dipropionate aqueous nasal spray; *BKC*, benzalkonium chloride; *BTN spray-PF*, betamethasone, tramazoline, neomycin spray-preservative-free; *BUDANS-PF*, budesonide aqueous nasal spray-preservative-free; *EDTA*, ethylenediamine tetraacetic acid; *FLANS*, flunisolide aqueous nasal spray; *FPANS*, fluticasone propionate aqueous nasal spray; *IPM*, inverted phase microscopy; *LM*, light microscopy; *Na phosphate*, sodium phosphate; *OMZ*, oxymetazoline; *PBO*, placebo; *PS*, potassium sorbate; *SEM*, scanning electron microscope; *TEM*, transmission electron microscope.

Table 2. Continued

Evaluation Method(s)	BKC %	Results
Forty-eight of 50 heads, fixed, decalcified, and sectioned; steroid treated right side compared with left side PBO control for histologic changes in nasal mucosa	FLANS 310 µg/ml (0.031%); BUDANS 220 µg/mL (0.022%)	BUDANS-PF: no histologic difference compared with PBO, although both treatments showed frequent openings in cilia carpet by goblet cells FLANS, and BDPANS: morphologic alterations, epithelium, height reduced with pleomorphism of some cells, few cells with cilia; number of goblet cells reduced, and no mucus covering epithelium; no changes in subepithelium; greatest changes were anterior/septum, lesser changes away from vestibulum. No statistical analyses were performed.
Cultured adenoid tissue from adenoidectomies w/beating, ciliated epithelia (288 samples: 8 × 12/ experiment); exposed QD × 14 d to undiluted ONS for 1, 3, 10, and 30 min each, and diluted ONS for 10 min Control, 10 min in NaCl (8 groups total) Tissue changes evaluated by IPM, LM, SEM, and TEM	Undiluted: 0.15 mg/mL (0.015%); 30%: 0.045 mg/mL (0.0045%); 10%: 0.015 mg/mL (0.0015%); 3%: 0.0045 mg/mL (0.00045%)	BKC 0.015% to 0.0015% showed a gradual loss of continuous epithelial lining was concentration and time dependent. BKC 0.00045% showed no morphologic changes compared with NaCl control solution
Weeks 1, 2, and 4 (n = 3, each Tx): nasal cavities sectioned and epithelial tissue examined	0.01% 0.1%	BKC: week 1, increased proliferation of intraepithelial glands inflammatory cell infiltration regardless of TAA; week 4, less histologic changes w/TAA compared with BKC alone and decreased epithelial thickness. PS: similar trend in results. Editor's note states study underpowered and SDs too large to "draw statistically valid conclusions."
Nasal swelling via rhinostereometry, and nasal reactivity via histamine challenge with 1.0, 2.0, and 4.0 mg/mL at baseline and end of study Nasal symptoms (ie, dry nose, runny nose, irritation, nasal bleeding, and/or a cold) and nasal stuffiness via VAS (0 clear to 100 blocked) daily	0.01%	After 30 d, O MZ + BKC showed greater mucosal swelling, and stuffiness was greater in PM at week 4 ($P < 0.05$, for both); unpaired <i>t</i> tests showed no difference in mucosal swelling between groups at any histamine provocation level before or after treatment. No differences in nasal symptoms scores were found between the 2 groups.
Nasal swelling and nasal reactivity at baseline and end of study, and daily nasal symptoms (see Graf et al, 1995)	0.01%	Only BKC group had significantly greater mucosal swelling ($P < 0.05$, paired <i>t</i> test) at 30 d. However, ANOVA showed no significant differences in mucosal swelling between groups. All groups had increased reactivity to histamine but BKC and PBO were significantly higher at 2 mg/mL concentration ($P < 0.05$, for both)
Nasal swelling (acoustic and stereometric) and nasal reactivity at baseline and end of study and daily nasal symptoms (see Graf et al, 1995)	0.01%	No difference in mucosal swelling between groups (unpaired <i>t</i> test). Symptom scores were also very similar before and after treatment: 50/49 with BKC and 48/51 without BKC

Table 2. Continued

Author	Design	Treatment Materials/Regimen
Griffen and Cote ³⁰	Experimental in vitro (human)	Betamethasone drops Betamethasone + neomycin drops BTN spray-PF
Merkus and Vande donk ³	Experimental in vitro (animal)	Various prescription and nonprescription intranasal products
Hallen and Graf ¹³	In vivo, randomized, double-blind, parallel-grouped, clinical study	OMZ 0.5 mg/mL (n = 10) or OMZ 0.5 mg/mL + 0.1 mg/mL BKC (n = 9); 0.1 ml each nostril TID ×10 d (3 mo earlier, same patients previously used same products for 4 wk)
Steinsvag et al ¹⁶	Experimental in vitro (human adenoid tissue)	BDPANS 500 µg/mL FLANS 250 µg/mL BUDANS 2 mg/mL

merous protective mechanisms intrinsic to the local nasal environment. Nasal secretions and active mucociliary clearance cause varying dilutions of preservatives, while simultaneously serving as a mechanical barrier to protect ciliated nasal epithelium from the detrimental effects of substances that are introduced into the nose.^{15,17,24,27} Nasal mucus is a complex aqueous mixture of glycoproteins, lipids, salts, and other cellular constituents that normally protects nasal epithelia from a wide variety of environmental insults. Aside from its function as a simple mechanical barrier, nasal mucus likely plays an active role through inactivation of many substances that gain access to the nose. This characteristic of nasal mucus has been found to adversely affect the absorption and action of some intranasal formulations.²⁸ Ainge et al⁹ suggested that various proteins contained within nasal mucus possess the ability to rapidly inactivate quaternary ammonium compounds such as BKC. In the specific case of quaternary ammonium compounds, BKC can be inactivated by non-ionic surfactants,²⁹ raising the possibility that BKC may be neutralized by the surfactant properties of nasal mucus.^{6,28}

Another local factor that might contribute to amelioration of the reported in vivo results of BKC is the acidic environment of the nasal cavity.

pH plays an important role in the antimicrobial activity of weak acids by influencing the nondissociated fraction of the molecules that are the most effective in preservative activity.²⁹ In an in vitro study by van de Donk et al,²⁶ the negative effect of BKC on ciliary beat frequency was significantly attenuated with decreases in pH. Moreover, this effect was noted with rather mild reductions of the pH from 7.4 to 6.0. Given the fact that the normal pH of the nose is slightly acidic, ranging from 5.5 to 6.0,³⁰ it is reasonable that this factor might contribute to the disparity found when comparing in vivo and in vitro results.^{26,31}

It has been proposed that different regions within the nasal cavity might be more susceptible to the effects of BKC toxicity, due to either concentration of BKC or innate susceptibility to insult. Such areas within the nasal vestibule would include the nasal septum, and anterior aspects of the inferior and middle turbinates, which are directly challenged by the application of nasal sprays. To assess this, Ainge et al⁹ specifically studied histologic specimens obtained from the anterior head of the inferior turbinates in primates and rats exposed to 28 days of fluticasone dipropionate or beclomethasone dipropionate, both containing BKC. No histologic differences were noted compared with control animals, with the exception

Table 2. Continued

Evaluation Method(s)	BKC %	Results
CBF of human nasal cilia (obtained by brush technique) after exposure to increased dilutions of products	0.006%	BKC 0.006% + EDTA 0.1%, and thiomersal 0.005% caused ciliostasis; a $\times 25$ dilution was not ciliotoxic; human nasal cilia may be more sensitive to effects of preservatives than animal cilia or human adenoidal cilia
CBF of chicken trachea after 60-min exposure: rated based on percent recovery of CBF Cilio-friendly, $\geq 75\%$, cilio-inhibiting, 25% 75%, cilostatic, $\leq 25\%$	0.01%-0.02%	Products with BKC had a cilio-inhibiting effect. Products with 0.02% BKC were often ciliostatic effect (no statistical analysis of results)
Nasal symptom scores, mucosa swelling, mucosa reactivity (see Graf studies)	0.01%	Symptom scores and nasal reactivity were not statistically different between groups, although patients treated with OMZ + BKC had higher symptom scores; nasal mucosa swelling was significantly higher w/BKC ($P > 0.05$) and difference was significant between groups ($P > 0.05$)
CBF, changes in cell morphology	0.031%, 0.022%, 0.020%	Changes in CBF and cell morphology increased w/increased concentrations of BKC; no statistical analyses were performed

of a decrease in submucosal lymphoid tissue in the treatment groups.

Based on these considerations, the significant differences that are observed when comparing in vitro and in vivo data are less confusing. Given the protective effects of the barrier action of nasal mucus, varying dilution of BKC concentration, potential enzymatic degradation of BKC, and pH effect on BKC activity, it is reasonable to conceive that the protective mechanisms that are intrinsic to the nasal environment during in vivo studies can compensate for toxic effects observed with BKC in some in vitro studies. Quite simply, the end result is that the nose provides an environment within which nasal epithelial exposure to BKC is minimized.

In preparation of this review, a total of 18 studies (14 in vivo, 4 in vitro) were identified that evaluated short- and long-term clinical exposure of concentrations of BKC ranging from 0.00045% to 0.1%. Eight of these studies, including a 6-month long-term treatment study, demonstrated no significant differences, indicating that BKC was either harmful to nasal tissue or prone to exacerbate rhinitis medicamentosa. Of the 10 studies that concluded that BKC resulted in degenerative changes in human nasal epithelia or exacerbated rhinitis medicamentosa, only 2 demonstrated statis-

tical significance when treatment differences between BKC and placebo or active control groups were compared. Both of these studies included the use of oxymetazoline, which is associated with rhinitis medicamentosa. In its present state, the current body of literature addressing the issue of BKC safety is confusing and logically has led to fueling debate regarding its safety. Ultimately, interpretation of the information regarding the safety of BKC requires aggregate consideration of the entire body of information related to this compound.

What appears to have been overlooked in the controversy surrounding the issues of BKC safety is the need for preservatives in order to maintain the safety of multidose topical preparations. Failure to incorporate an antimicrobial preservative in cosmetic or pharmaceutical products leads to an increased risk of inoculation and colonization by fungal, bacterial, and viral pathogens, which could result in health and/or life threatening consequences. Alternative aqueous preservatives do exist, but a greater potential for either toxicity or product spoilage from microbial growth may occur with their use.³² To address the issue of preservative efficacy, Hodges et al tested the response of 3 common topical nasal sprays (with differing preservatives) to inoculation with bacteria and fungus. The combination of BKC and phenylethyl

alcohol was found to be far superior to the combinations of BKC and disodium edetate or potassium sorbate and disodium edetate.²² Therefore, at the present time, unless a better substitute can be found, BKC remains the best choice to ensure that multidose aqueous nasal sprays remain pathogen free.

Novel to discussions concerning BKC is the concept that this compound may possess attributes beneficial to patients with chronic mucosal pathology. BKC, like other quaternary ammonium compounds, is chemically a cationic detergent and has surfactant properties. The extent to which these surfactant properties contribute to the potential benefits BKC remains largely unexplored. Theoretically, BKC might enhance penetration of the delivery of an accompanying active ingredient. For example, the surface tension of ocular medications, when combined with BKC, is significantly lower than that of physiologic lacrimal fluid,³³ thus aiding in delivery of the medication. Surfactants also reduce aggregate formation within an aerosol and allow for easy resuspension on shaking, which also contributes to better medication dispersal.³⁴ To carry this a step further, Scadding³⁵ suggested that the intrinsic antibiotic effect of BKC might lead to a reduction in exacerbations of recurrent infections. Unfortunately, this hypothesis is still unproved, as no patients within their study (experimental or control) experienced recurrence of infection. Aside from its innate antibacterial effect, BKC adversely affects bacterial cellular respiration as a likely result of uncoupling of oxidative phosphorylation.³⁶ It is currently held that this property, in the future, may help to disrupt and eliminate biofilms, an emerging concern in patients with chronic bacterial disease processes. So, although currently premature, it appears that the future may hold additional new horizons for preparations that possess the safety history and potential therapeutic characteristics of BKC.

Given the limited choices that are currently available to serve as preservatives, as well as the years of clinical safety that BKC has demonstrated, careful risk-to-benefit analysis should be considered before dismissal of BKC as preservative option. Further, careful review of the currently available literature has failed to establish a

clear cause and effect relationship between BKC at concentrations less than 0.1% and clinically significant detrimental effects on nasal epithelium.

CONCLUSION

Review of the current literature reveals a limited amount of data that demonstrate statistically significant safety concerns. Further analysis of these data reveals that the studies that raise any concern are largely limited to in vitro design. On the other hand, in vivo data suggest that even prolonged use of topical nasal preparations containing BKC causes no significant damage to the nasal mucosa.

As a result, although continued attention should be directed toward safety issues surrounding all medication, at the present time insufficient evidence exists to justify categorical dismissal of BKC for use in aqueous nasal sprays. Instead, it is hoped that a standardized test using a more representative model for evaluating preservatives can be devised and accepted. It appears that intranasal products containing the preservative BKC are safe and well tolerated for both short- and long-term use.

REFERENCES

1. Block SS. Disinfection, sterilization and preservation, 4th edition. Philadelphia: Lea & Febiger; 1991.
2. Liebert MA. Final report on the safety assessment of benzalkonium chloride. *J Am Coll Toxicol* 1989;8:589-625.
3. Merkus FWHM, van de donk HJM. Does the intranasal application of drugs damage ciliary movement? [abstract]. *Clin Pharmacol Ther* 1982;31:250.
4. Graf P. Rhinitis medicamentosa: aspects of pathophysiology and treatment. *Allergy* 1997;52:28-34.
5. Graf P. Adverse effects of benzalkonium chloride on the nasal mucosa: allergic rhinitis and rhinitis medicamentosa. *Clin Ther* 1999;21:1749-55.
6. Richards DH. Preservation of nasal sprays [letter]. *J Allergy Clin Immunol* 2000;106:596.
7. Graf P. Benzalkonium chloride as a preservative in nasal solutions: re-examining the data. *Respir Med* 2001;95:728-33.
8. Laliberte F, Laliberte MF, Lecart S, et al. Clinical and pathologic methods to assess the long-term safety of nasal corticosteroids. *Allergy* 2000;55:718-22.
9. Ainge G, Bowles JA, McCormick SG, et al. Lack of deleterious effects of corticosteroid sprays containing benzalkonium chloride on nasal ciliated epithelium. *Drug Invest* 1994;8:127-33.
10. Klossek JM, Laliberte F, Laliberte MF, et al. Local safety of intranasal triamcinolone acetonide: clinical and histological aspects of nasal mucosa in the long term treatment of perennial allergic rhinitis. *Rhinology* 2001;39:17-22.

11. Berg OH, Lie K, Steinsvag SK. The effects of topical nasal steroids on rat respiratory mucosa in vivo, with special reference to benzalkonium chloride. *Allergy* 1997;52:627-32.
12. Berg OH, Henriksen RN, Steinsvag SK. The effect of a benzalkonium chloride-containing nasal spray on human respiratory mucosa in vitro as a function of concentration and time of action. *Pharmacol Toxicol* 1995;76:245-9.
13. Holm AF, Fokkens WJ, Godthelp T, et al. A 1-year placebo-controlled study of intranasal fluticasone propionate aqueous nasal spray in patients with perennial allergic rhinitis: a safety and biopsy study. *Clin Otolaryngol* 1998;23:69-73.
14. McMahan C, Darby Y, Ryan R, et al. Immediate and short-term effects of benzalkonium chloride on the human nasal mucosa in vivo. *Clin Otolaryngol* 1997;22:318-22.
15. Braat JP, Ainge G, Bowles JA, et al. The lack of effect of benzalkonium chloride on the cilia of the nasal mucosa in patients with perennial allergic rhinitis: a combined functional, light, scanning and transmission electron microscopy study. *Clin Exp Allergy* 1995;25:957-65.
16. Cho JH, Kwun YS, Jang HS, et al. Long-term use of preservatives on rat nasal respiratory mucosa: effects of benzalkonium chloride and potassium sorbate. *Laryngoscope* 2000;110:312-7.
17. Steinsvag S, Bjerknes R, Berg OH. Effects of topical nasal steroids on human respiratory mucosa and human granulocytes in vitro. *Acta Otolaryngol* 1996;116:868-75.
18. Graf P, Hallen H. Effect on the nasal mucosa of long-term treatment with oxymetazoline, benzalkonium chloride, and placebo nasal sprays. *Laryngoscope* 1996;106:605-9.
19. Graf P, Enerdal J, Hallen H. Ten days' use of oxymetazoline nasal spray with or without benzalkonium chloride in patients with vasomotor rhinitis. *Arch Otolaryngol Head Neck Surg* 1999;125:1128-32.
20. Graf P, Hallen H, Juto JE. Benzalkonium chloride in a decongestant nasal spray aggravates rhinitis medicamentosa in healthy volunteers. *Clin Exp Allergy* 1995;25:395-400.
21. Hallen H, Graf P. Benzalkonium chloride in nasal decongestive sprays has a long-lasting adverse effect on the nasal mucosa of healthy volunteers. *Clin Exp Allergy* 1995;25:401-5.
22. Hodges NA, Denver SP, Hanlon GW, et al. Preservative efficacy tests in formulated nasal products: reproducibility and factors affecting preservative activity. *J Pharm Pharmacol* 1996;48:1237-42.
23. Griffen WM, Cole PJ. Effect of topical nasal medications on human nasal cilia [abstract].
24. Batts AH, Marriott C, Martin GP, et al. The use of a radiolabelled saccharin solution to monitor the effect of the preservatives thiomersal, benzalkonium chloride and EDTA on human nasal clearance. *J Pharm Pharmacol* 1991;43:180-5.
25. Storaas T, Andersson M, Persson CG, et al. Effects of benzalkonium chloride on innate immunity physiology of the human nasal mucosa in vivo. *Laryngoscope* 2000;110:1543-7.
26. van de donk HJM. Nasal medication and ciliary movement. *Pharma Weekblad Sci Ed* 1983;5:32-3.
27. Stanley PJ, Griffin WM, Wilson R, et al. Effect of beta-methasone and betamethasone with neomycin nasal drops on human nasal mucociliary clearance and ciliary beat frequency. *Thorax* 1985;40:607-12.
28. Khanvilkar K, Donovan MD, Flanagan DR. Drug transfer through mucus. *Adv Drug Deliv Rev* 2001;48:173-93.
29. Hodges NA, Denyer SP, Hanlon GW, et al. Preservative efficacy tests in formulated nasal products: reproducibility and factors affecting preservative activity. *J Pharm Pharmacol* 1996;48:1237-42.
30. England RJ, Homer JJ, Knight LC, et al. Nasal pH measurement: a reliable and repeatable parameter. *Clin Otolaryngol* 1999;24:67-8.
31. Bernstein IL. Is the use of benzalkonium chloride as a preservative for nasal formulations a safety concern?. A cautionary note based on compromised mucociliary transport. *J Allergy Clin Immunol* 2000;105:39-44.
32. Zuidema J, Merkus FWHM, van de Donk HJM, et al. Intranasal application of drugs and its influence on ciliary movement [abstract]. *Pharma Weekblad Sci Ed* 1983;5:38.
33. Zawadzka E, Stefanski P, Zgoda MM. Some physico-chemical properties of the micelle benzalkonium chloride on the interface in selected eye drops. *Acta Pol Pharmaceut* 1995;52:275-9.
34. Corrigan OI, Healy AM. Surfactants in pharmaceutical products and systems. In: Swarbrick, Boylan, editors. *Encyclopedia of pharmaceutical technology*. New York: Marcel Dekker; 1996. p. 295-332.
35. Scadding GK. Letter to the editor. *Clin Ther* 2000;22:893-4.
36. Berg OH, Bakken AM, Steinssvag SK, et al. Benzalkonium chloride interferes with energy production, secretion and morphology in human blood platelets. *Platelets* 2002.
37. XX. Wade A, Weller PJ. *Handbook of pharmaceutical excipients*. 2nd edition. Washington, DC: American Pharmaceutical Association; 1994. p. 27-9.