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 or 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 1935[1]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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0194-5998/$30.00

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10.1016/j.otohns.2003.07.005

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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 al[18] 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 al[19] in 1999 that concluded that oxymeta-

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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).

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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% thiomerisol 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.

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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 ($P > 0.05$, 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 ($P < 0.06$), all showed decreased ET from PT compared with EOT; qualitative an LM: no significant difference in ET between all 3 treatment groups ($P = 0.06$), 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 ($P > 0.05$)
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 ($P > 0.053$); all treatments showed a significant decrease in CBF ($P \leq 0.013$); SCT tended to increase but not significantly ($P \geq 0.20$); 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 ($P < 0.01$); BKC significantly increased fucose secretion ($P < 0.05$); 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 ($P = 0.79$); histamine increased nasal symptoms before and after BKC ($P < 0.01$); histamine increased α_2-MG before and after 10 d BKC ($P < 0.01$) 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-

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