Benzalkonium chloride as a preservative in nasal solutions: re-examining the data

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Abstract   Recent studies have suggested that benzalkonium chloride (BKC), an antimicrobial agent used as a preservative in nasal sprays, lacks deleterious effects on the nasal ciliated epithelium. Other data, including recent *in vivo* findings, suggest that BKC may, in fact, produce adverse clinical effects on human nasal tissue, including the aggravation of rhinitis medicamentosa. Toxic effects have also been reported. In light of the discrepancy between negative results and studies suggesting no safety concerns, we consider the possibility of problems in the design and methodology of some of the studies and in the interpretation of results. Clearly, further research is warranted to clarify the significance of conflicting findings. In the meantime, without conclusive data regarding BKC and the possibility of harmful effects, the use of nasal formulations without BKC might be a reasonable alternative. © 2001 Harcourt Publishers Ltd

INTRODUCTION

Benzalkonium chloride (BKC) is a quarternary ammonium compound commonly used to prevent bacterial contamination and to preserve pharmacological activity in topical aqueous drops and sprays. Although ophthalmic mucositis and contact dermatitis resulting from the presence of BKC in topical solutions have been documented (1,2), investigators have recently concluded that inclusion of the preservative BKC in nasal sprays does not yield any adverse biological effects *in vivo* (3,4). In studies conducted in both laboratory animals and perennial allergic rhinitis patients (3,4), the nasal mucosa was shown to be largely unaffected as assessed primarily by morphological indices. However, recent studies concerning the pathophysiology of rhinitis medicamentosa clearly indicate an adverse biological role of BKC *in vivo* (5–7). These data are consistent with the numerous in vitro studies characterizing the adverse effects of BKC on nasal mucociliary transport and nasal histology (8–15). Thus, the objective of this article is to reassess the hypothesis that BKC lacks deleterious effects *in vivo*.

STUDIES SHOWING NO DELETERIOUS EFFECTS

In vivo Animal Studies

In a study conducted in laboratory animals, Ainge *et al*. failed to show any deleterious effects of corticosteroid-containing nasal sprays on the nasal epithelium (3). The objective of the study was to examine the effect of repeated exposure of normal cynomolgus monkeys and rats to BKC-containing steroid formulations, namely, fluticasone propionate and beclomethasone dipropionate nasal sprays.

Cynomolgus monkeys were treated with fluticasone propionate (0·05% w/v) containing 0·02% BKC for 28 days ($8 \times 0\cdot1$ ml day^{-1} by intra-nasal spray). Rats were treated with beclomethasone dipropionate (0·2%) nasal spray containing 0·01% BKC for 28 days (1 h day^{-1} via inhalation). Control conditions included either a 5% glucose spray for monkeys or air inhalation for rats. At the conclusion of the treatment period, animals were sacrificed and the nasal mucosa examined by light microscopy. In addition, nasal mucosal tissues were sectioned for scanning and transmission electron microscopy to allow for counting of ciliary nasal cells and study of ciliary cell ultrastructure.

The number of ciliated cells per mm of turbinate was not different in either monkeys or rats when the group administered BKC-containing steroid was compared to control. The respiratory epithelia covering the turbinate

surface were also unchanged in treated animals. Likewise, no difference in nasal mucosal ultrastructure (i.e. nuclei, mitochondria, rough and smooth endoplasmic reticula, lysosomes, cytoplasmic vacuoles, microvilli, cilia and their basal bodies) was observed. In summary, no changes in morphological features were noted in the presence of BKC.

However, the significance of these negative findings may be limited for several reasons (16,17). First, the biopsies of the samples were restricted to the turbinate epithelium (i.e. right inferior turbinate, monkeys; intermediate turbinate, rats) and, therefore, the investigators may have failed to detect benzalkonium-induced structural changes in other regions of the nasal mucosa (17). Other studies have found BKC to be harmful to other regions of the nasal mucosa (16). Second, the studies were also conducted with inhaled sprays (i.e. metering/atomizing pumps, in monkeys; inhalation chamber using a glass concentric atomizer fed by a peristaltic pump, in rats), which may have lessened the insult accompanied by pressurized sprays. Ainge *et al.* (3) exposed the subjects to more BKC than typical decongestants usually contain. While the normal amount of sprays administered per nostril per day is 4, the monkeys received 8 sprays per day. The concentrations of BKC administered to the monkeys were lower than patients typically receive (0·01% vs. 0·02%). However, the nasal epithelium of the rats and monkeys was exposed to BKC continuously for 1h a day, which is much greater exposure than patients typically receive. The absence of morphological damage corroborates previous results revealing no damage to ciliated cells in patients receiving fluticasone propionate aqueous nasal spray or beclomethasone dipropionate aqueous nasal spray.

In contrast, different parts of the nasal bed may have varying reactions to BKC toxicity and different degrees of contact with the nasal spray (18). For example, the anterior area of the nasal septum, the tips of the inferior and middle turbinate, and the anterior aspect of a polyp are more directly challenged by the impact of the nasal spray and, therefore, are exposed to greater concentrations of preservative (18). Third, since adequate controls with BKC alone (i.e. 5% glucose, monkeys; air, rats) were not performed, it is possible that the corticosteroid masked the deleterious effects of BKC. Fourth, this study was strictly structural and did not consider possible adverse functional effects of BKC. Fifth, it is unclear as to whether the results of this animal trial are transferable to humans. In humans, the anterior parts of the nose are the areas where considerable deposition of inhaled substances occurs. Also, the lack of change in morphological features in the presence of BKC may be due to the pH of the solution (3). Van de Donk *et al.* (9) suggested that reducing the pH of BKC may significantly decrease ciliary movement of guinea pig trachea. In the study, the duration of ciliary movement decreased from

1·33 h to 1h when the BKC solution pH was reduced from 7·4 to 6·0.

In vivo **Human Studies**

BKC was also shown to be nontoxic to nasal mucosa in patients with perennial allergic rhinitis in a study by Braat *et al.* (4). The objective of this study was to examine the effects of BKC on the human nasal mucosa in a population of patients with perennial allergic rhinitis.

The study consisted of a single-center, double-blind trial of 22 patients with perennial allergic rhinitis who were also allergic to house dust mites (as confirmed by skin-prick test). After a 2-week placebo treatment period, patients were randomized to receive one of the following twice daily: a nasal spray of 200 μg fluticasone propionate containing 0·02% BKC, a placebo aqueous spray of 0·02% BKC, or placebo aqueous nasal spray.

Patients were assessed at 2 weekly intervals during treatment and 2 weeks after treatment by anterior rhinoscopy for turbinate swelling, crushing, bleeding, mucosa color and nasal secretions. Saccharine transport time was determined to assess mucociliary clearance. Prior to treatment and 6 weeks after treatment, two nasal biopsies were taken from each patient 2 cm behind the anterior tip of the inferior turbinate for microscopy. Light microscopy was performed to measure mucosal length and the number of ciliated epithelial cells. Scanning electron microscopy was conducted to determine the ratio of ciliated cells to nonciliated cells. Transmission electron microscopy was used to examine the ultrastructure of the ciliated epithelial cells.

An improvement was noted for mucosal color and secretions in the group of patients assigned fluticasone with BKC, but no change was noted with BKC alone. No difference was noted for any of the patient groups with reference to the ciliary transport times assessed at biweekly intervals by the saccharine clearance test. The number of ciliated cells varied widely but was also unchanged between patient groups. Decreased incidence of edema and inflammatory cell infiltration was noted in patients treated with fluticasone, while BKC appeared to have no effect on this parameter.

Despite the existence of some pathological features in all patients studied, transmission and scanning electron microscopy did not show BKC to have adverse effects. Common pathological features observed in biopsies included the presence of numerous cytoplasmic vacuoles, very electron-dense and swollen mitochondria, and swollen smooth endoplasmic reticulum. Importantly, the cilia appeared similar in all treatment groups, with the classic 9+2 configuration of microtubules present. Based on scanning electron microscopy, data was marred (i.e. varying number of ciliated cells) by residual

mucus. Furthermore, the surface area covered by cilia was unaffected by BKC.

As noted for the previous study, weaknesses limit the significance of the Braat study (16,17). First, the biopsies of samples were limited to the inferior turbinates and may not reflect the deposition and subsequent effects on other nasal regions. However, previous studies such as that by Newman et al. (19) demonstrated chief deposition in the anterior part of the nose in 10 normal subjects using nasal pump sprays. Within 30 min of administration, a mean 56% of the dose remained at the initial site of deposition, while the remainder (44%) of the dose cleared to the nasopharynx (19). Second, significant variation was observed in the patients studied under control conditions, particularly with regard to morphological data, such that any changes ascribed to BKC were difficult to detect. Third, the patient population under study was suffering from perennial allergic rhinitis, whereby protection afforded by excessive mucus production possibly obscured any adverse biological effects of BKC. This protective effect was observed in a study by Stanley et al. (11) who suggested that the lack effect of BKC on ciliary beat frequency or mucociliary clearance was the result of the effects of nasal mucus.

A third in vivo study by McMahon et al. attempted to determine the immediate and short-term effect of BKC in an intranasal corticosteroid spray on human nasal mucosa in vivo (20). In this study, the authors assessed mean saccharine clearance time, acoustic rhinometry, and ciliary beat frequency prior to and after 2 weeks of therapy in normal volunteers randomized to receive saline, fluticasone propionate aqueous nasal spray, or placebo. A 10-min exposure to BKC in 34 subjects led to a significant increase in the mean clearance time ($762 \cdot 7 \pm 459$ sec) versus that observed with saline alone (620 ± 437 sec). However, a double-blind multi-dose study comparing saline solution, fluticasone propionate plus BKC, and BKC alone, administered for 2 weeks at 2 puffs per day, showed no differences in saccharine clearance or in any of the variables tested.

The initial results of the study—that the BKC solution caused an immediate increase in mucociliary clearance time—were most likely due to ciliary dysfunction resulting from the application of BKC (20). Although the toxic effects of BKC were not apparent in the multidose study, both the initial test and the 2-week study included only normal individuals; patients suffering from rhinitis who had used a topical nasal treatment in the 2 weeks prior to the study were not tested. In patients with rhinitis, it is important to note that the capacity of the mucociliary defense system may be reduced as a result of the disease itself. Patients with rhinitis, therefore, may be more prone to the adverse effects of BKC than normal individuals (12). Recent studies suggest that the main determinant of nasal mucociliary clearance is ciliary beat frequency (21). In addition, Graf and Hallen (6) recorded

a potentiation of rhinitis medicamentosa in the presence of BKC. However, patients with rhinitis tend to produce more mucus, which can have a protective effect against BKC (11). Storaas et al. (22) showed that sustained administration of BKC to human nasal mucosa can generate tolerance without adverse effects. Using healthy subjects, Storaas et al. (22) administered isotonic saline and BKC ($0 \cdot 1$ mg ml^{-1}) acutely to nasal mucosa. At first the BKC induced immediate nasal smart or pain ($P < 0 \cdot 05$), but after repeated administrations over 10 days, patients developed tolerance.

STUDIES SHOWING DELETERIOUS EFFECTS

In vivo Animal Studies: Evidence of Squamous Cell Metaplasia

Using BKC as a preservative in corticosteroid-containing nasal sprays administered to rats, Berg et al. demonstrated that BKC may be potentially toxic to the nasal mucosa in vivo (16). In contrast to the study of Ainge et al. described above (3), the objective of this study was to examine the morphological effects of topical nasal steroids in the presence or absence of BKC in different parts of the nasal cavities originally covered by histiotypic respiratory ciliated mucosa (16).

The study was conducted in 30 rats divided into three groups and given one of the following: beclomethasone dipropionate aqueous nasal spray (plus 310 μg ml^{-1} BKC), flunisolide nasal spray (plus 220 μg ml^{-1} BKC), or budesonide (no BKC) aqueous nasal spray. In a separate experimental set-up, two groups of 10 animals were treated with either beclomethasone dipropionate or budesonide. The steroid solutions were administered by spray twice daily for 21 days into the right nostril, whereas the left nostril received saline delivered by a micro-pipette. At the conclusion of the treatment period, a careful morphological study was conducted by cutting the nose serially in frontal sections of 4 μm from the anterior and posterior parts of the nose.

The strengths of this study included the ability to: (a) use a rat animal model with respiratory mucosa devoid of respiratory tract disease with a similar structure, function, and metabolism to that found in humans; (b) control the dose and deposition of the drug; (c) investigate the nasal mucosa in total as opposed to focal biopsies; and (d) examine the effect of topical steroids with and without BKC versus controls in the same section (16).

In response to beclomethasone and flunisolide nasal sprays containing BKC, squamous cell metaplasia was evident primarily in the anterior portion of the nose. Characteristic of these tissues was a reduced epithelial cell height with some pleomorphism. In addition, cilia were rarely found and layers of mucus were absent.

Notably, animals treated with budesonide nasal spray that did not contain BKC exhibited no histological differences as compared to saline controls. A thick, pseudostratified epithelium was found on the lateral nasal wall and the septum. Individual cells were characterized as having a tall and columnar phenotype and typically displayed cilia on their apical surface covered with a layer of mucus. The submucosal layer was also unaffected by budesonide such that the connective tissue, vessels and submucosal glands appeared normal.

Based on these observations and other studies proving steroid compounds to be harmless to respiratory mucosa *in vitro* (23,24), the data suggest that the observed squamous cell hyperplasia was due to the inclusion of BKC in the steroidal nasal sprays.

In vivo Human Studies: Aggravation of Rhinitis Medicamentosa

Graf *et al*. showed adverse biological effects of BKC in defining the mechanism responsible for rhinitis medicamentosa found in response to overuse of vasoactive decongestants (5–7,24–26). Rhinitis medicamentosa is defined as a condition of nasal hyperreactivity, mucosal swelling, and tolerance that is also found in response to vasoactive decongestants as well as other drugs (24,27). In the case of decongestant-induced rhinitis medicamentosa, inclusion of BKC as a preservative has been shown to aggravate rhinitis medicamentosa primarily by increasing nasal swelling.

The effect of BKC as a preservative in a decongestant nasal spray was investigated in a randomized, double-blind, parallel study with 20 healthy volunteers (5). The principal aim of the study was to determine whether the use of BKC in oxymetazoline nasal spray exerts any effect on the development of rhinitis medicamentosa.

The subjects were divided into two groups that were treated with oxymetazoline ($0\cdot5\,\mathrm{mg\,ml^{-1}}$) nasal spray with or without 0.1 mg/ml BKC administered three times daily ($0\cdot1\,\mathrm{ml\,nostril^{-1}}$) for 30 days. Nasal mucosal swelling was determined by rhinostereometry, an optical, direct, non-invasive method via topographic measurement, without manipulating the nasal structures (28). Of note, this method allows one to distinguish between healthy patients and patients with vasomotor rhinitis who exhibit increased histamine sensitivity (26). Nasal stuffiness was estimated on a visual analogue scale (5).

In healthy volunteers treated with oxymetazoline in the presence of BKC for 30 days, a statistically significant increase in mucosal swelling ($1\cdot14$ mm) was found in both inferior conchae. Likewise, both morning and evening nasal stuffiness was elevated in healthy volunteers during 4 weeks of treatment with oxymetazoline and BKC (5). In fact, a significant increase was detected from the second week of drug treatment. Furthermore, elevated morning and evening stuffiness was significantly greater than that observed in patients treated with oxymetazoline alone as analysed by unpaired *t*-tests (5).

It should be noted that patients treated with oxymetazoline alone also exhibited significant increases in mucosal swelling and nasal stuffiness indicative of rhinitis medicamentosa—events that were aggravated by BKC. Thus, after 30 days of drug treatment, mucosal swelling and mean evening estimated nasal stuffiness were significantly greater in patients administered oxymetazoline with BKC versus oxymetazoline alone.

The detrimental effect of BKC is long-lived, as subsequent treatment of patients with the same combination of drugs led to reduced mucosal swelling in response to BKC after a 3-month period (7). In this study, the reexposure to drug treatment was limited to 10 days. Only those patients treated with the combination of oxymetazoline and BKC exhibited a significant increase in nasal stuffiness, apparent from the fourth day onward. Mucosal swelling was significantly increased after the 10-day reexposure in the oxymetazoline plus BKC group of patients.

In contrast, the patients on oxymetazoline alone failed to show a significant increase in mucosal swelling. The mucosal swelling found in the oxymetazoline plus BKC group was also significantly greater than that observed in the oxymetazoline alone group, as determined by an unpaired *t*-test. Thus, a nasal decongestant spray composed of vasoactive decongestant and BKC has a long-term adverse effect on the nasal mucosa (7).

A subsequent study by Graf and Hallen demonstrated that BKC alone causes mucosal swelling in healthy volunteers (6). The objective of this study was to clarify the effects of BKC since earlier studies implicating the preservative through its aggravation of rhinitis medicamentosa and its long-lasting adverse effect on the nasal mucosa were limited to inclusion of the preservative with oxymetazoline (5,7).

In this double-blind placebo-controlled study, 30 volunteers were randomized to receive 0·1 ml of either $0\cdot5\,\mathrm{mg\,ml^{-1}}$ oxymetazoline nasal spray without BKC, $0\cdot1\,\mathrm{mg\,ml^{-1}}$ BKC nasal spray alone, or placebo nasal spray consisting of an aqueous solution of sodium chloride, sodium phosphate, and ethylenediamine tetra-acetic acid. The study drugs were administered three times daily for 1 month. The variables studied included nasal mucosal swelling, symptom scores of nasal stuffiness, and nasal reactivity, as determined by histamine challenge.

BKC did not generate nasal stuffiness as shown in the earlier studies for oxymetazoline plus BKC (5). Instead, the patient group administered oxymetazoline alone showed elevated nasal stuffiness from day 14 onward to the completion of the study. Nasal reactivity, as assessed by histamine challenge, was significantly increased by BKC after 30 days. However, the increase in nasal reactivity was not as pronounced as that found in patients administered nasal sprays containing oxymetazoline alone.

Nevertheless, discrepancy between the recorded nasal mucosal swelling and the estimated stuffiness was found in the BKC group, in contrast to the oxymetazoline group. The explanation may be that after several days on oxymetazoline, the subjects adjust to breathing easily through the nose. When the decongestive effect disappears, subjects may experience nasal stuffiness without rebound swelling (i.e. a false or exaggerated sensation of nasal stuffiness). This may explain why some subjects find it hard to stop using the decongestants within the recommended time and why they ultimately develop rhinitis medicamentosa.

The most outstanding adverse biological event associated with the administration of BKC involved the swelling of the nasal mucosa after the completion of the treatment. At the end of treatment, a significant increase in mucosal swelling was found in the BKC group (0·67 mm) as compared to either the oxymetazoline group (0·29 mm) or the placebo group (0·09 mm).

Based on these findings, it was concluded that BKC aggravates decongestant-induced rhinitis medicamentosa via mucosal swelling (6). However, a possible confounding factor in this study is that oxymetazoline extends the duration of contact of BKC with the nasal mucosa (29). Combined with BKC, oxymetazoline is more likely to cause rhinitis medicamentosa (29).

SUMMARY

The objective of this article was to assess the deleterious effects of BKC *in vivo*. Previous studies concluding that BKC lacks *in vivo* effects may have been flawed, limiting the significance of their findings. Moreover, the data of Graf and colleagues, showing that *in vivo* administration of BKC aggravates rhinitis medicamentosa in response to vasoactive decongestant nasal sprays, support the contrary. In fact, Berg and colleagues also demonstrate adverse biological effects on rat respiratory mucosa associated with the use of benzalkonium-containing corticosteroid nasal sprays administered *in vivo* (16).

Further studies of BKC-containing corticosteroid nasal sprays in both normal volunteers and patients suffering from perennial rhinitis are warranted, particularly with functional correlates. Further study of the acute and chronic adverse biologic effects induced by BKC in patients with rhinitis medicamentosa is also warranted, as well as the role of pH in BKC toxicity. In lieu of these data, the use of formulations of corticosteroid-containing sprays now available that do not include BKC may avert any potential adverse biological effects.

Acknowledgements

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