



## Effect of preservatives on ciliary beat frequency in human nasal epithelial cell culture: Single versus multiple exposure

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### Abstract

As preservatives may impair mucociliary clearance, we wanted to systematically study their time-dependent effect on the ciliary beat frequency (CBF) in human nasal epithelial cells (HNEC). CBF was determined using a high-speed digital imaging method. Five preservatives were selected including benzalkonium chloride, phenylethyl alcohol, methylparaben, propylparaben and chlorbutol. We were interested in the effect of these preservatives on CBF after single and repetitive exposure. Methylparaben (0.0033%), propylparaben (0.0017%) and chlorbutol (0.005%) did not impair CBF, neither after a single short-term exposure period, nor after a single long-term exposure period. Long-term exposure to benzalkonium chloride (0.001%), phenylethyl alcohol (0.125%) and a combination of methyl- and propylparaben (0.0033 and 0.0017%) significantly decreased CBF. After a short-term exposure period, CBF recovered for phenylethyl alcohol and the combination of methyl- and propylparaben. Benzalkonium chloride decreased CBF non-reversibly. For two compounds, the effect on CBF was evaluated after repetitive exposure during 15 min for 5 consecutive days. Benzalkonium chloride resulted in ciliostasis for all concentrations tested after 5 days. Phenylethyl alcohol revealed a concentration-dependent effect on CBF, but no ciliostasis was observed. In conclusion, methylparaben, propylparaben and chlorbutol can be considered as cilio-friendly. Repetitive exposure revealed a cumulative effect on CBF for benzalkonium chloride and phenylethyl alcohol.

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### 1. Introduction

The nasal route is widely used for the administration of drugs for both topical and systemic action (Kublik and Vidgren, 1998). Nasal drug delivery also holds promise for vaccination, while recent reports related to olfactory transport have created interesting perspectives for brain delivery (Westin et al., 2005). A prerequisite for nasally applied formulations is that drugs and additives in the dosage forms do not interfere with normal nasal functioning (Merkus et al., 1991). One of the most important local defense mechanisms of the respiratory tract is the mucociliary clearance (Jorissen et al., 2000), whose efficiency depends on the physiological control of the ciliated cells and on the rheological properties of the mucus blanket (Jorissen, 1998). The ciliary beat frequency (CBF) is

one of the basic functional ciliary parameters determining the mucociliary clearance (Boek et al., 1999; Jorissen et al., 2000). Several factors influencing the ciliary beat frequency have been described, including temperature, pH and osmolarity (Ingels et al., 1991).

Traditional multidose nasal sprays or drops require preservatives to prevent microbial contamination (Gibson Mark, 2001). However, the use of preservatives in nasal formulations remains controversial. Although benzalkonium chloride is by far the most used preservative in aqueous nasal formulations, several studies have revealed the impairment of the mucociliary clearance by benzalkonium chloride and other preservatives in vitro (Batts et al., 1990; Joki et al., 1996; Boek et al., 1999; Ricchelman et al., 2004; Hofmann et al., 2004; Arnitz et al., 2006). Some authors observed morphological changes in the nasal respiratory mucosa by application of benzalkonium chloride in vivo (Berg et al., 1997; Cho et al., 2000; Cureoglu et al., 2002; Lebe et al., 2004), illustrating its potentially toxic effect. Others state that the toxic effect of benzalkonium chlo-

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