Abstract
Background: The preservatives benzalkonium chloride (BKC) and potassium sorbate (PS) are widely used for nasal drops and nasal spray. Recently, a lot of side effects that result from mucosal damage caused by BKC and PS was been reported. Methods: We investigated the toxicity after administration of BKC or PS on human nasal epithelial cells in vitro. Using primary human nasal epithelial cells, different concentrations of BKC, PS solution or phosphate buffered saline (control group) were cocultured with nasal epithelial cells for 15 mins. Then, the viability of the cells and the cell morphology was measured. Results: Nasal epithelial cell was more severe damage in clinical used or higher concentration BKC group than in control group. In addition, lysis of the nasal epithelial cells was found via EM in the same BKC groups. In contact, there was no significant cell damage in the PS group compared to control group, even the higher concentration than clinic used concentration. Conclusion: The clinical used concentration of BKC could lead to severe nasal epithelial cell damage.

Introduction
Local administration of medicine has been adapted to medical treatment for disease in nasal cavity and paranasal sinuses, including allergic rhinitis and sinusitis. Various kinds of preservatives have been used for preventing bacterial infection in local preparations of nasal spray. The preservatives should be used without altering the main pharmacologic actions and should not cause side effects in the body. Among these, benzalkonium chloride (BKC) and potassium sorbate (PS) are the preservatives we often find in use in daily lives for nasal drops, eye drops, cosmetics, and food preparations. Several studies have demonstrated that BKC may induce alteration to the nasal mucosa in vitro and in vivo. But there were some reported demonstrated that BKC did not induced nasal damage. The actual effects of BKC on the nose are still controversial. However, studies of the influence of PS on nasal mucosa have been insufficiently performed. In 2004, Hofmann T et al. found that the isolated preservative PS did not have negative influence on ciliary beat frequency in vitro. But in vivo, they demonstrated that even a low-concentration solution of PS can lead to nasal lesion, including intraepithelial glandular formation, inflammatory cell infiltration, vascular hyperplasia, and edematous change. In this study, we investigated the different concentrations of BKC and PS influenced the primary human nasal epithelial cell via measurement of cell viability and cell morphology.

Effects of Preservatives in Nasal Spray on Human Nasal Epithelial Cells
Ching-Yin Ho, Meng-Chuan Wu, and An-Hang Yang
Department of Otolaryngology and Pathology, Veterans General Hospital-Taipei

Fig. 1. Trypan blue method for cytotoxicity of BKC and PS. Primary human nasal epithelial cells were incubated with various concentration of PBS, BKC, or PS for 15 mins. Cell viability was evaluated by Trypan blue method. Data are means ± SE from three independent experiments. *: P < 0.05 compared with PBS; a: P < 0.05 compared with 0.1% BKC (A) and 1% PS (B); b: P < 0.05 compared with 0.001% BKC.

Fig. 2. MTT for cytotoxicity of BKC and PS. Primary human nasal epithelial cells were incubated with various concentration of PBS, BKC, or PS for 15 mins. Cell viability was evaluated by MTT assay. Data are means ± SE from three independent experiments. *: P < 0.05 compared with PBS; a: P < 0.05 compared with 0.1% BKC (A) and 1% PS (B); b: P < 0.05 compared with 0.01% BKC.

Fig. 3. Morphological appearances of primary nasal epithelial cells after incubation with various concentration of PBS, BKC, or PS for 15 mins. Upper panel: treated with PBS. The cells are intact and have good cell-cell contact. Middle panel: treated with 0.1% PS for 15 min. The cell morphology did not change compared with PBS-treated cells. Lower panel: treated with 0.01% BKC for 15 min. The cell was rounding and loss cell-cell contact.

Morphological appearances of primary nasal epithelial cells measured by transmission electron microscopy. Cells of control group (A) and 0.1% PS-treated group (B) showed microvilli (arrow) both of which are specific characters of respiratory epithelial cells. Nasal epithelial cells incubated with 0.01% BKC (C and D) for 15 minutes demonstrated the losing of microvilli, the lysis of cell membrane (arrow) and destruction of keratin intermediate filaments (arrowhead). Scale bar as figure showing (2 um in A, B and D; 5 um in C).