Effects of Saline Solution on Ciliary Movement in Human Nasal Epithelium in vitro

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ABSTRACT

Background and Objectives Osmolarity is known to affect ciliary beat frequency (CBF) however, little is known about the effects of saline spray or irrigation on ciliary activity in human nasal epithelium. The aim of this study was to assess whether CBF is affected by hypertonic, isotonic or hypotonic saline solution in vitro and whether histologic changes are associated with the alteration of ciliary movement. Materials and Methods Normal turbinate mucosa was exposed to five different concentrations including 0.06%, 0.12%, 0.9%, 3.0%, and 7.0% of phosphate-buffered saline solutions. CBF was measured up to 60 minutes after exposure to each concentration of saline solution and histologic changes were examined by transmission electron microscopy. Results Isotonic and hypotonic saline solutions produced no ciliary slowing however, ciliostasis was observed within a few minutes in 3.0% or 7.0% saline solution. Immediately after complete ciliostasis in the hypertonic solutions, the solutions were replaced with 0.12% or 0.9% saline solution in order to observe the reversibility of CBF. Only 0.12% hypotonic saline induced the recovery of ciliary movement. The ultrastructural changes demonstrated that the ciliary slowing might be attributed to epithelial damage by fluid transport toward the surrounding medium. Conclusion Our results suggest that isotonic and hypotonic saline solutions may be more appropriate for nasal irrigation than hypertonic saline solutions.

KEY WORDS· Saline solution · Ciliary beat frequency.

INTRODUCTION

Otolaryngologists have frequently recommended nasal drops or nasal irrigations in the treatment of patients with acute and chronic rhinosinusitis and in the post-operative care of patients who underwent nasal or sinus surgery. Recently hypertonic saline has been applied in attempts to increase mucociliary clearance, because hypertonic saline is known to improve mucociliary transport in various infectious diseases of the respiratory tract though its mechanism of action has not been fully understood. Ciliary movement in vitro mainly depends on the properties of the surrounding medium, such as its pH, ionic strength and viscosity. The previous several studies showed that either hypotonic or hypertonic solution in the aspect of ciliary movement in the human nasal epithelium may derange the milieu of mucus blanket of the respiratory epithelium. This study aimed to assess whether CBF is affected in vitro by the use of hypertonic, isotonic or hypotonic saline solutions in human nasal turbinate mucosa and...
whether histologic changes are associated with the alteration of ciliary movement.

**MATERIALS AND METHODS**

**Subjects and preparation of ciliated epithelial cells**

Normal respiratory epithelia were taken from the midportion of the inferior turbinates in eight male adults during septoplasty operation with informed consents. They had no history of smoking, previous nasal surgery, prolonged use of topical medication or respiratory infection within one month before surgery, or of chronic nasal diseases such as allergic rhinitis or chronic sinusitis. The turbinate mucosa specimen was washed in a sterile normal saline solution, and immediately placed in a pre-warmed culture medium, Dulbecco’s modified Eagle Medium-Ham’s nutrient (DMEM-F12) Gibco BRL, Grand Island, NY). The turinate mucosa from each subject was divided into six small pieces and placed into 37°C, 5% CO₂ incubator for 60 minutes. Each piece of turinate mucosa was placed in five different concentrations of phosphate - buffered saline solutions including 0.06%, 0.12%, 0.9%, 3.0%, 7.0% and DMEM-F12. The temperature in the culture dish was maintained at 37°C throughout the experiment.

**Measurement of ciliary beat frequency**

CBF was measured using the video-computerized analysis technique at 1, 5, 10, 20, 40, and 60 minutes after addition of five different concentrations of saline solutions. Baseline CBF was measured in the nasal epithelium immersed in DMEM-F12. Then the medium was replaced with various concentrations of saline solutions. Control specimens were exposed to DMEM-F12 for 60 minutes. When the ciliated cells stopped beating in hypertonic solutions, the saline solutions were immediately replaced with 0.12% hypotonic or 0.9% isotonic saline solution to observe whether CBF recovers. CBF was measured in the three most actively beating areas per each specimen. The mean of the three values was taken as the CBF in the specimen studied.

**Histologic examination**

For transmission electron microscopy, the specimens were prepared by prefixation, postfixation, dehydration, replacement with propylene oxide, and embedding in epon. They were sliced 1 μm thick and stained with alkaline toluidine blue for examination under the light microscope, in order to precisely determine the area to be examined under the transmission electron microscope. The samples were sliced to a thickness of 30 to 50 nm, placed on a copper grid, and stained with lead citrate and uranyl acetate. The specimens were observed under a transmission electron microscope (ORVAL MT 6000, Dupont, Wilmington, Del). The electron microscopic findings, including the shape of each epithelial cell, changes in the nucleus or cytoplasmic organelles and ciliary ultrastructure, were observed under magnification × 3,000.

The mean of CBF in each concentration was analyzed using repeated measure ANOVA at a statistical significance of 0.05.

**RESULTS**

**CBF after exposure to different concentrations of saline solutions**

Table 1 shows CBF according to the duration of exposure to different concentrations of saline solutions.

<table>
<thead>
<tr>
<th>Concentration of saline (%)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06</td>
<td>11.31±0.59</td>
<td>11.49±0.56</td>
<td>11.49±0.50</td>
<td>11.19±0.47</td>
<td>10.90±0.33</td>
<td>10.83±0.39</td>
<td>10.78±0.36</td>
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<tr>
<td>0.12</td>
<td>11.73±0.78</td>
<td>11.72±0.62</td>
<td>11.60±0.65</td>
<td>11.49±0.56</td>
<td>11.34±0.64</td>
<td>11.13±0.55</td>
<td>11.01±0.57</td>
</tr>
<tr>
<td>0.90</td>
<td>11.95±1.15</td>
<td>12.00±0.97</td>
<td>12.01±1.03</td>
<td>11.90±0.83</td>
<td>11.60±0.86</td>
<td>11.54±0.87</td>
<td>11.30±0.82</td>
</tr>
<tr>
<td>3.00</td>
<td>11.07±0.83</td>
<td>6.16±1.43*</td>
<td>0.47±0.75*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td>7.00</td>
<td>11.12±0.93</td>
<td>0.54±1.01*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
</tbody>
</table>

Data are mean ciliary beat frequency ± standard deviation.
Statistical difference was assessed by repeated measure ANOVA, *p<0.05
In 3.0% hypertonic saline solution, CBF markedly decreased at 1 minute and ciliary movement completely stopped in all specimens at 10 minutes. In 7.0% hypertonic saline solution, CBF decreased immediately after addition of the saline solution and complete ciliostasis was observed in all specimens at 5 minutes. However, there was no change in CBF of all the specimens up to 60 minutes in 0.9% saline solution. In hypotonic saline solution of 0.06% or 0.12%, no significant difference in CBF was observed during 60 minutes when compared to the baseline CBF. When the medium was replaced with hypotonic saline solution (0.12%) after complete ciliostasis in 3.0% or 7.0% hypertonic saline, CBF increased significantly at 5 minutes, and restored to the baseline frequency at 10 minutes in all the specimens. However, we could not observe the recovery of ciliary movement when we replaced the hypertonic saline with physiologic saline solution (0.9%) (Table 2).

**Transmission electron microscopic findings**

The shape of each epithelial cell, the nucleus, the cytoplasmic organelles and ciliary ultrastructure were well preserved in control solution and 0.06%, 0.12%, and 0.9% saline solutions. However, in the specimens with ciliostasis after exposure to hypertonic saline solutions, intercellular tight junction and desmosome were disrupted and the intercellular spaces were widened. In addition, the ciliated cells and their nuclei were contracted and the margin of ciliated cells was irregular (Fig 1). However, there were no changes in the ultrastructure of cilia.

**DISCUSSION**

In this study, we used phosphate-buffered saline solutions, because pH of plain 0.06%, 0.12%, 0.9%, 3.0%, and 7.0% saline solutions is lower than 7.0. It has been reported that pH higher than 7.0 decreases rat tracheal CBF. The significantly lower CBFs were observed below pH 7.0 in human bronchial mucosa. Plain physiologic saline has been known to have a negative effect on CBF in vitro. The concentrations of hypotonic (0.06%, 0.12%) and hypertonic (3.0%, 7.0%) saline solutions used in this study were determined from the several in vivo reports. Hypertonic saline solutions (3.0%, 7.0%) showed ciliostasis within a few minutes, while hypotonic solutions (0.06%, 0.12%) did not alter ciliary movement for 60 minutes. These results indicate that hypertonic saline may be more harmful to ciliary movement than hypotonic saline. In transmission electron microscopic examination we could observe disrupted intercellular tight junction and desmosome, widened intercellular spaces, contracted ciliated cells, and irregular margin of ciliated cells. The histologic findings demonstrate that the decrease of ciliary movement might be attributed to epithelial damage by fluid transport toward the surrounding medium because high osmolarity results in epithelial shrinkage and the distortion of cytoplasm and organelles. In this study these functional changes were reversible when a hypertonic saline

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Table 2. Effects of isotonic (0.90%) and hypotonic (0.12%) saline solutions on the recovery of altered ciliary activity of the human nasal ciliated epithelial cells

<table>
<thead>
<tr>
<th>Concentration of saline (%)</th>
<th>Recovery time (min)</th>
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<tbody>
<tr>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>0.90</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are mean ciliary beat frequency ± standard deviation. Statistical difference was assessed by repeated measure ANOVA. * p<0.05

Fig 1. A transmission electron micrograph of the human nasal epithelial cells after 5-minute exposure to 7.0% saline solution. The intercellular spaces are widened and the ciliated cells and their nuclei (arrowhead) are contracted. The margin of ciliated cells is irregular (original magnification × 3,000. Scale 2 μm).
solution was immediately replaced with a hypotonic one. Electron microscopic findings demonstrate that the functional reversibility might be attributed to the recovery of volume of the ciliated cells.

The previous studies\(^9\)\(^{10}\) have shown that 0.45% NaCl solution decreases CBF of chicken cilia by 50% after 1 hour in comparison with the baseline CBF, and that CBF decreases as osmolarity deviates from the iso-osmolarity (0.9%). These experimental values are in contrast to our results that there were no changes in CBF in hypotonic saline solutions. The reason for this discrepancy is not known and probably related to the use of a different experimental specimen and methodology.

Nasal irrigation has been performed for the clearance of secretions, debris and intranasal crusts. Nasal irrigation with physiologic saline has been recommended in rhinitis and rhinosinusitis for many decades. Recently, hypertonic saline solutions have been used in order to improve mucociliary clearance in patients with cystic fibrosis, \(^7\) \(^8\) \(^15\) asthma, \(^6\) or chronic sinusitis. \(^4\) \(^6\) Although its mechanism of action has not been fully established, hypertonic saline is believed to improve the rheologic properties of the mucus. \(^7\) \(^8\) \(^17\) However, little is known about the effects of saline solution on CBF in human nasal epithelium and there have been different reports on the effects of hypertonic saline on ciliary movement in vivo and in vitro conditions. While hypertonic saline increases or maintains CBF in vivo, \(^8\) \(^18\) it markedly decreases ciliary movement in vitro. \(^9\) \(^10\) \(^13\) It is difficult to compare nebulization or irrigation in vivo with immersion used in vitro, \(^10\) because it is likely that compensatory transepithelial water transport mechanism \(^18\) and various ciliostimulatory mediators \(^7\) may occur in vivo when mucus milieu is disturbed.

In this study, isotonic and hypotonic saline solutions did not induce changes in CBF, while hypertonic saline solution induced complete ciliostasis. The histologic changes, including disrupted intercel-llular junction and desmosome and widened intercellular space, were observed in the specimens with exposure to hypertonic saline solutions. From the results of this study it is suggested that isotonic or hypotonic solutions might be more appropriate for nasal irrigation than hypertonic solutions.

**Acknowledgement**

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**References**
