Ciliary Function, Cell Viability, and in Vitro Effect of Ribavirin on Nasal Epithelial Cells in Acute Rhinorrhea*

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Nasal epithelial (NE) cells were collected from the nasopharynx of 25 individuals with symptomatic colds and 27 healthy volunteers (controls), and ciliary beat frequency (CBF) was assessed by microscopy employing video motion analysis techniques. Baseline CBF was statistically significantly elevated in the group with colds compared to the control group (14.6 ± 1.5 Hz [mean ± SD] vs 13.8 ± 0.9 Hz; p = 0.02). After four days of incubation in culture, there was a significant decrease in the CBF in both groups, with a change from baseline of 1.9 Hz for the cold group, compared to 1.0 Hz for the control group (p = 0.0001). The in vitro addition of ribavirin at 500µg/ml to NE cells from individuals with colds preserved the viability of the cells and maintained the CBF at baseline values. Twenty-four (96 percent) of 25 ribavirin-treated specimens from the cold group survived for four days in culture, compared with 17 (68 percent) of 25 untreated cold specimens. In addition, the ribavirin-treated cells had a mean CBF of 14.2 ± 1.3 Hz, compared with 12.7 ± 1.9 Hz for the untreated cell samples (p = 0.0005). Ribavirin had no effect on NE cells from the control group. These results suggest that ribavirin in a concentration of 500µg/ml may have some benefit in the treatment of acute rhinorrhea.

Materials and Methods

Specimens

Patients studied were all employees of St. Joseph's Hospital and gave written consent to a protocol which was approved by the St. Joseph's Hospital Ethics Committee. The mean age of all subjects was 33 ± 9 yr (range, 16 to 50 yr). Acute rhinorrhea was defined as acute nasal discharge with or without nasal congestion and in the absence of any clinical features of allergic rhinitis and with or without virus isolated from nasal specimens. Nasal epithelial cells from healthy volunteers or individuals with acute rhinorrhea were collected from the nasal septum at the level of the inferior turbinate using a small bronchial brush.11 If necessary, multiple specimens were taken and pooled to obtain sufficient cells for analysis. In the group with rhinitis, the cells were harvested within 48 h of the appearance of symptoms. Cells were placed into 5 ml of minimal essential medium, pH 7.4, containing Earle's salts and 10 percent fetal bovine serum (growth medium). Samples were shaken to disperse the cells through the medium and then were divided into equal aliquots for testing.

Virus isolation

Nasal epithelial specimens were disrupted on a vortex mixer with glass beads and inoculated onto preformed cell cultures in tubes for virus isolation. Cultures included primary green-monkey kidney (GMK), rhesus monkey kidney (RMK), continuous Hep-2, human diploid foreskin fibroblasts, and WI-38 cells. Tubes were incubated at 37°C on a rotating drum in a humidified atmosphere containing 5 percent CO2, and examined daily for 10 days for viral cytopathic effect (CPE). WI-38 cells were rotated at 33°C for isolation of acid-labile rhinoviruses causing a typical granular CPE.11

Measurement of Cell Viability

Cell viability was expressed as the percentage of viable cells assessed by the ability of cells to exclude trypan blue dye.

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(Chest 1992; 102:284-87)
Table 1—Cell Viability and Ciliary Function of NE Cells Collected from Healthy Individuals

<table>
<thead>
<tr>
<th>Day</th>
<th>No. of Viable Specimens*</th>
<th>CBF, Hz†</th>
<th>p Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>14.5±0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>14.6±1.1</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>13.7±1.0</td>
<td>0.027</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>13.0±2.2</td>
<td>0.039</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>12.7±0.7</td>
<td>0.002</td>
</tr>
<tr>
<td>11</td>
<td>3</td>
<td>12.7±1.0</td>
<td>0.035</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>NT§</td>
<td></td>
</tr>
</tbody>
</table>

*NE specimens from first 8 of 27 healthy individuals lacking any cold symptoms recruited for study. Specimens were stored at 33°C between measurements.
†CBF was determined by slow-motion video analysis and was expressed as mean ± 1 SD.
§Significantly different from day 0 by paired t-test comparing surviving matched specimens only.
NT, Not tested due to absence of viable cells.

Ciliary Beat Frequency

Using a sterile pipette, a 0.5-ml aliquot of the NE specimen was withdrawn and transferred to a depression slide. The slide was then placed on a heated (37°C) stage of a photomicroscope (Leitz). The CBF of the NE cells was measured immediately after collection of the specimen and at various times up to 13 days in culture using a slow-motion video analyzer (Sony) connected to the microscope. Determinations were made by averaging the number of cycles counted per minute obtained from 15 different beating sites for each specimen, with the results expressed as a mean frequency in Hertz (± 1 SD). Sites containing clusters of cells with clear borders were chosen for analysis.

Statistical Analysis

Mean values for CBF were compared using Student's t-test for paired and unpaired samples. The Wilcoxon rank sum test was used to compare baseline CBF for the two groups of subjects.

RESULTS

We have examined the cell viability, ciliary function, and in vitro effect of ribavirin on NE cells from individuals with acute rhinorrhea and have compared the results to those of healthy individuals.

Virus Isolation

Virus was isolated from only four of 25 individuals

\[
\text{Controls (n=27)}
\]

\[
\begin{array}{ccccccc}
10.80 & 12.00 & 13.20 & 14.40 & 15.60 & 16.80 \\
\end{array}
\]

\[
\text{Colds (n=25)}
\]

CILIARY BEAT FREQUENCY (CBF) - Hz

Figure 1. Distribution of CBF of NE cells obtained from individuals with colds (n = 25) and healthy controls (n = 27). Median CBF for each group (circled plus signs) was 13.8 Hz for controls and 14.7 Hz for cold group.

Table 2—Effect of Ribavirin on CBF of NE Cells Collected from Individuals with Acute Rhinitis or Healthy Controls

<table>
<thead>
<tr>
<th>Day and Treatment</th>
<th>CBF, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Day 0 (baseline)</td>
<td></td>
</tr>
<tr>
<td>No drug</td>
<td>13.8±0.9</td>
</tr>
<tr>
<td>(n = 27)†</td>
<td></td>
</tr>
<tr>
<td>Drug*</td>
<td>NT§</td>
</tr>
<tr>
<td>Day 4</td>
<td></td>
</tr>
<tr>
<td>No drug</td>
<td>12.8±1.3</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 17)</td>
</tr>
<tr>
<td>12.7±0.7</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Drug*</td>
<td>11.8±1.2‡</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 24)</td>
</tr>
</tbody>
</table>

*Ribavirin concentration, 500μg/ml.
†n, No. of cultures containing viable cells.
‡p = 0.02 for normal vs rhinorrhea at baseline.
§NT, Not tested.
‖Only 6 of 27 normal specimens were treated with ribavirin.
§p = 0.08 for day 4 drug vs no drug (n = 6 normal specimens).
**p = 0.0005 for day 4 drug vs no drug (n = 17 matched pairs of subjects with rhinorrhea).

with acute rhinorrhea and none of 27 healthy controls. Rhinovirus was isolated from NE scrapings of two individuals with rhinorrhea, and respiratory syncytial virus (RSV) was isolated from an additional two individuals.

Ciliary Function of Epithelial Cells from Individuals with Rhinorrhea and Healthy Controls

Nasal epithelial cells from healthy individuals did not divide in culture and did not establish a continuous cell line; however, cell viability was maintained for at least 11 days in culture, as judged by the exclusion of trypan blue dye and the maintenance of ciliary beating. Epithelial cells obtained from eight healthy individuals remained viable with a normal CBF (mean ± SD) of 14.6±1.1 Hz for the first two days in cultures (Table 1). After four days, cultures from seven to eight individuals contained viable cells, and the CBF showed a small but significant drop from the value on day 2 to 13.7±1.0 Hz (p = 0.027). By 11 days, only
three of eight cultures had viable cells, and these specimens maintained a CBF of $12.7 \pm 1.0$ Hz. On day 14, no viable cells were detected in any cultures.

Epithelial cells from individuals with acute rhinorrhea showed a slightly higher baseline CBF on day 0 ($14.6 \pm 1.5$ Hz) compared with a group of 27 controls ($13.8 \pm 0.9$ Hz) (Table 2). This difference was statistically significant ($p = 0.023$; Wilcoxon test). The distributions of CBFs for both groups is illustrated in Figure 1. After four days in culture, epithelial cells from individuals with acute rhinorrhea had a significant drop in CBF to $12.7 \pm 1.9$ Hz, a value similar to that seen with cells from healthy individuals ($12.8 \pm 1.3$ Hz) (Table 2). The decrease in CBF was greater for the group with rhinorrhea than for the control group ($1.9$ Hz vs $1.0$ Hz; $p = 0.0001$).

**In Vitro Effect of Ribavirin on Ciliary Function**

The addition of ribavirin to cultures of epithelial cells from healthy individuals had no effect on either cell viability or ciliary function. Six out of six cultures were viable on day 4, and the CBF of cultures treated with ribavirin was not statistically different from cultures with no ribavirin ($12.7 \pm 0.7$ vs $11.8 \pm 1.2$ Hz; $p = 0.08$; Table 2). In contrast, the addition of ribavirin to cultures from individuals with rhinorrhea had a marked effect. Eight (32 percent) of 25 cultures without ribavirin added were dead on day 4, compared with only one (4 percent) of 25 with drug added. The CBF of the 24 viable cultures that received ribavirin was significantly higher ($14.2 \pm 1.3$ Hz) on day 4 than the 17 cultures that did not receive the drug ($12.7 \pm 1.9$ Hz; Table 2). Comparing the CBF of the 17 of 25 viable cultures that received ribavirin with that of their matched untreated cultures indicated that the CBF was significantly greater in the treated group ($14.2 \pm 1.3$ Hz vs $12.7 \pm 1.9$ Hz; $p = 0.0005$).

**Discussion**

We have examined NE cells collected from individuals with cold symptoms (i.e., acute rhinorrhea), measuring ciliary function and response to the antiviral drug, ribavirin. The CBF of NE cells collected from individuals with acute rhinorrhea was statistically significantly increased ($p = 0.02$) compared to healthy control individuals. The values, though, are considered to be within the normal range. After 4 days in culture, the CBF of NE cells from the group with acute rhinorrhea showed a significantly greater decrease in CBF compared to the controls. Ribavirin, at a concentration of 500 $\mu$g/ml, appeared to increase the viability of NE cells taken from the “cold” group. The drug prevented the decrease in CBF seen in cells from these same individuals after 4 days in culture, whereas the CBF of normal specimens was unaffected by ribavirin. In a recent report from Han et al., a decrease in CBF of normal NE cells was observed when the cells were immersed in ribavirin at concentrations greater than 40 mg/ml. Our specimens were tested at 0.5 mg/ml, a concentration of ribavirin which we believed would be representative of that received when inhaling an aerosol generated from the standard dose used in human treatment (20 mg/ml for 14 to 20 h). Han and co-workers saw no adverse effect of the drug on NE cells brushed after nasal inhalation of an aerosol produced from a 60-mg/ml solution of ribavirin, suggesting that the concentration of ribavirin delivered in vivo was much less than that tested in their in vitro experiment; however, higher concentrations of ribavirin (0.9 mg/ml) measured in respiratory secretions have recently been reported following only 2 h of treatment using a dose of 60 mg/ml.

Reductions in both nasal mucociliary transport (NMCT) function and CBF have been observed in subjects with a naturally occurring (common) cold or a cold resulting from a controlled exposure to a single rhinovirus. An elevated CBF has also been measured early after infection with rhinovirus, as a result of the local production of leukocyte endogenous pyrogen. Sakakura et al demonstrated a maximally decreased NMCT 3 days after infection, possibly due to an increase in the secretion load or perhaps a reduction in CBF. In the study by Pedersen et al., both the CBF and NMCT rate were reduced in individuals with natural colds, and these measurements remained subnormal even 2 mo later, suggesting that the return to normal frequency is a slow process; however, nine normal subjects infected with RV9, RV12, and influenza B exhibited decreases in nasal mucociliary clearance (NMCC) only, and not CBF, 4 days after the appearance of symptoms. This variability in CBF observations during a cold may be due, in part, to the timing of the measurements after the appearance of symptoms. In addition, the uncertainty as to the extent of loss of viable ciliated cells from the epithelial surface, initially with the infection and during recovery, can make the CBF determination more difficult. That our absolute results differ from those of Pedersen et al may also be explained by the differences in temperature at which we perform our CBF analysis (37°C vs room temperature) or by differences in the populations. More work will be required to elucidate the precise effect of various cold viruses on CBF.

The increase observed in the number of viable NE cell specimens from our infected subjects when treated with ribavirin for 4 days, compared to those not treated, is encouraging. This protective effect of ribavirin on NE cell ciliary function from infected individuals suggests that this drug could play a role in preventing degradation of the nasal epithelium and perhaps help maintain normal NMCT during the
common cold. The success of ribavirin in treating lower respiratory tract infections remains to be demonstrated in vivo in subjects with acute rhinitis due to rhinovirus infection.

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REFERENCES

17. Pedersen M, Sakakura Y, Winther B, Brofeldt S, Mygind N. Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. Eur J Respir Dis 1983; 64(suppl 128):355-64