MUCOCILIARY TRANSPORT PATHWAY ON LARYNGOTRACHEAL TRACT AND STENTED GLOTTIS IN GUINEA PIGS

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We investigated the laryngotracheal mucociliary transport pathway of guinea pigs *in vivo* and immediately postmortem. Only intraperitoneal anesthesia was used during the procedure to avoid the disturbance of mucociliary function. Resin particles were used as the marking substance. A microcolpohysteroscope was placed at different levels in the laryngotracheal region for observing the marking particles and recording the transport pattern. The tracheal mucociliary transport flow primarily moved along the posterior wall and both lateral walls in a zigzag trace. Upon reaching the subglottis, the resin particles stayed underneath the vocal cords, and a whirlpool phenomenon developed. The majority of the particles were shifted and directed onto the posterior glottic area. With a short delay, some resin particles crossed over the free edge of the vocal cords and turned posteriorly along the medial upper cordal margin. No mucociliary transport could be observed on the entire upper surface of the true vocal cords, which is covered by squamous epithelium. Occasionally, a few resin particles in the vicinity of the epiglottic root traveled along the aryepiglottic folds toward the posterior commissure. All streams of mucociliary transport finally joined together in the interarytenoid area. After leaving the glottis, the resin particles traveled to the hypopharynx and entered the esophagus through the motion of deglutition. The pattern of mucociliary clearance in the laryngotracheal region was not delayed by stenting.

KEY WORDS — glottis, guinea pig, laryngotrachea, mucociliary transport, stent.

INTRODUCTION

As air pollution increases, so do the number of cases of upper respiratory tract and lung disease. Foreign particles that our body breathes in, such as dust, smoke, and various contaminants, all accumulate in the airway secretions and might hinder the laryngotracheal mucociliary transport. Studying the flow

pattern of mucociliary transport will help to explain why certain areas of the laryngotracheal region are susceptible to diseases. For example, laryngeal tuberculosis is known to be spread directly to the posterior larynx by infectious airway secretions. This could be explained by the fact that the majority of the airway secretions passes through the posterior

Fig 1. Block diagram of experimental animal model for observation of mucociliary transport pathway. VTR — videotape recorder.

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Fig 2. Serial pictures (every 30 seconds, from A-C) of cervical trachea. Mucociliary transport pathway was traced in zigzag-flowing pattern (arrows).

commissure.¹ In addition, the foreign particles captured by airway secretions are carried by mucociliary transport, sent into the hypopharynx, and then swallowed. If an infectious substance stagnates on the mucus blanket and is not removed immediately, it will give chronic irritation locally. Thus, the pattern of transport of mucus in the laryngeal area might have

a strong correlation to some laryngeal diseases.

With an endolaryngeal microscopic viewing and recording device, the present experimental model was designed to observe the laryngotracheal mucociliary transport in vivo and immediately postmortem by a nonradioactive marker technique. In a pilot experiment in the guinea pig, we successfully observed the sphincteric action and cordal movement of the glottis during respiration.² The same procedure was used to investigate the mucociliary clearance of the laryngotracheal tract, and we found that laryngotracheal stenting did not disturb the mucociliary clearance.³ The purpose of this study was to elucidate the rheological characteristics of laryngotracheal secretions in vivo and with stenting.

MATERIALS AND METHODS

The experimental procedures were as described in a previous report.³ In brief, guinea pigs weighing between 300 and 460 g (average 350 g) were used. After overnight fasting, the guinea pigs were injected intraperitoneally with pentobarbital sodium (50 mg/mL), 25 mg/kg of body weight. The depth of anesthesia was maintained at the level of spontaneous breathing without a coughing or vomiting reflex. The room temperature was maintained at 26°C. The guinea pigs were placed in the supine position. A small pillow was placed under the guinea pig's shoulder to help in extending the neck. This in turn helped straighten the animal's airway from the oral cavity to the larynx. The next step was to shave off the fur around the animal's neck and to sterilize the skin. Two tracheal openings were fashioned. The upper tracheotomy was for the placement of resin particles. The second tracheotomy was made about 0.5 cm below the first, in order to prevent cyclic interference from the respiratory airflow. The transport pattern of the resin particles was observed by inserting a Hamou's microendoscope (4 mm in diameter) through the guinea pig's oral cavity and into its larynx. The light source was a 250-W xenon light bulb and an optical fiber. The endoscope was connected to a charge-coupled device (CCD) and a video camera

Fig 3. Tracheal specimen has been opened posteriorly. Photographs treated with "art" technique show increased contrast of resin particles. Flow pattern can be clearly seen (arrows). Dark trace demonstrates zigzag flow of particles (dark color) vertically along lateral wall. Transport stream was nearly absent anteriorly (open triangles). Arrowheads indicate chronological order of photos.

Fig 4. A) Immediate postmortem specimen demonstrates transport streams along cordal edge that join at posterior commissure. There is accumulation of resin particles at posterior commissure (arrowhead). B) Stream travels from posterior glottis to supraglottis and hypopharynx (arrow).

(Fig 1). The endoscopic laryngeal images were recorded by a video recorder (U-matic, $\frac{3}{4}$ inch, Sony V.0.9600). Simultaneously, an automatic focusing photo camera (F810, Nikon) was used to produce a

Fig 5. Summary of mucociliary flows in A) lateral and B) posteroanterior views of larynx. Tracheal mucociliary transport flow primarily moved along posterior wall and both lateral walls in zigzag trace (trace 1). Upon reaching subglottis, resin particles stayed underneath vocal cords, and whirlpool phenomenon developed (trace 2). Majority of particles were shifted and directed onto posterior glottic area (trace 3). Some resin particles crossed over free edge of cords and turned posteriorly along medial upper cordal margin (trace 4). Occasionally, some resin particles passed through anterior commissure (trace 5) and then, in vicinity of epiglottic root, traveled along aryepiglottic folds toward posterior commissure (trace 6). All streams of mucociliary transport finally joined together in interarytenoid area.

set of supplementary pictures as a reference. All recordings started from the moment the endoscope was inserted. The recorded images could be viewed via a monitor while the automatic photo camera was taking a picture every 30 seconds. The same procedures were performed on the animal with or without intralaryngeal stenting. The mucociliary clearing function at the levels of the trachea, subglottis, glottis, and supraglottis could be observed sequentially. The same recording techniques were also applied on several postmortem, opened laryngotracheal specimens.

RESULTS

The dark secretion on the image was a mixture of mucus and resin, and accordingly made it possible to detect the mucociliary transport pattern in the airway. We found that few streams crossed the midline. Almost no resin was noted along the anterior wall

Fig 6. Mucociliary transport also passes along superior and inferior edges (arrows) of vocal cords, reaching posterior commissure and joining main posterior tracheal stream, where they mix together.

Fig 7. A) Intralaryngeal stent (arrows) was inserted in larynx with its upper end above level of vocal cords, to ventricular folds. B) Major transport stream on posterior wall traveled from subglottis to glottic edge, accumulating at interarytenoid region. C) During swallowing action, larynx ascended and stent was elevated upward. Top of stent moved secretions with resin particles from glottis through posterior commissure. D) Dynamic cleansing function of larynx is assisted by action of stent.

up to the level of the subglottis or anterior commissure. Most of the mucus flowed in a zigzag trace on the posterior and lateral walls vertically (Fig 2). For demonstration of the tracheal mucociliary transport, we treated the video image with an "art" technique to augment the dark color of the resin particles by increasing the contrast against the background (Fig 3). The video images were developed into photographs for observing and comparing the transport pat-

tern of the particles and mucus on the glottis. Two photographs taken at intervals of 30 seconds are shown in Fig 4. The streams at both lower borders of the vocal cords turned sharply to the posterior direction, forming a "whirlpool" phenomenon, proceeded to the posterior commissure, and then joined the posterior subglottic flow at the interarytenoid region. The mucus streams crossed over the posterior glottis and traveled into the supraglottic area and then the hypopharynx (Fig 4).

A simplified sketch of the transport process of the mucus is shown in Fig 5. It depicts upward movement of the lateral transporting streams. Most of the streams traveled from anterior to posterior and proceeded directly to the posterior commissure. Eventually, all the resin particles were moved away from the laryngeal area and accumulated in the hypopharynx and pyriform sinus. A small amount of resin particles moved toward the anterior commissure and continued to move toward the border of the epiglottis.

There was rotatory mucociliary transport action (whirlpool) in a few small focal areas of the upper trachea near the subglottis. The speed of transit appeared to be low because of dehydration or the increasing viscosity of mucus in the region of the vocal cords. From the close-up images taken in the lower cordal region, we observed that the resin particles gathered around the anterolateral part subglottically.

Fig 8. Mucociliary transport pathway of postmortem subject revealed same pattern as in vivo. Stenting did not disturb transport flow posteriorly between stent and luminal wall (triangle).

The mucus accumulating in the lower region thus formed a whirlpool effect. The flows also passed along the superior and inferior (Fig 6) edges of the vocal cords to reach the posterior commissure, then joined the main stream along the posterior tracheal wall. The secretions composed of resin particles, mucus, and air bubbles stayed at the interarytenoid region and clearly were retained there longer than on the tracheal wall. None of the resin particles were observed to cross over the vocal cords and reach the upper surface at this time. Only after a short delay could the resin particles cross over the entire free edge of the vocal cords and reach the upper surface.

In the stented group, the intralaryngeal stent was inserted in the larynx of the live subject with its upper end reaching above the vocal cord level to the ventricular folds. The major transport stream on the posterior wall traveled from the subglottis and both edges of the glottis, accumulating at the interarytenoid region (Fig 7A,B). During the swallowing action, the larynx ascended and the stent was elevated, with the top end of the stent helping to evacuate secretions with resin particles from the glottis through the posterior commissure (Fig 7C,D). The findings in postmortem subjects revealed a similar transport pathway as *in vivo*. The secretion passed through the interarytenoid region to the supraglottic region (Fig 8).

DISCUSSION

Although knowledge of the mucociliary transport in the trachea and larynx has been gathered through animal research, we still have limited information on the cleansing ability of the tracheal and laryngeal mucociliary function in humans. In 1966, Ewert and Martensson⁴ observed the flow of mucus under the glottic area by placing a transconioscope through the cricothyroid membrane. Later, measuring the effectiveness of the mucociliary cleansing ability in humans was made easier and more accurate by introduction of a radioactive tracer and the use of gamma ray-emitting isotopes as labeling particles.⁵ However, the use of radioactive isotopes is troublesome, and its potential for causing tissue damage is still a significant concern.

The mechanical process of coughing out particles from the trachea and the laryngeal area is complex. Bridger and Proctor⁵ recall that Veale, in 1871, reported that voluntary efforts of coughing seem to contribute to expelling mucus only when the mucus is situated immediately beneath the epiglottis or just below or between the vocal cords. Actually, it is the mucociliary transport of mucus in the tracheal and laryngeal areas that renders the forceful cough effective in clearing out particles. Thus, the cleansing

function of mucociliary transport is crucial in clearing particles from the trachea and larynx. Although swallowing does not clear particles from the larynx, drinking liquid would effectively wash the particles away from the entrance of the pharynx and larynx.

The laryngeal mucociliary cleansing procedure was not as simple or symmetric as that described above. Serial histologic section of the laryngeal area revealed that the squamous epithelium of the vocal cords did not extend to the anterior commissure. In contrast, the ciliated epithelium continuously extended upward from the subglottis via the posterior commissure to the supraglottis. The difference in the covering epithelia and distributing vessels might be one of the major determinants for the transport pattern. We found that the zigzag transporting trace of the trachea mainly came from the posterior part and proceeded perpendicularly upward. It was seldom seen in the anterior part. Therefore, a vertical tracheal incision is preferred when performing a tracheotomy. It might avoid interference with the mucous transport. The anterior commissure is not an area in which the transported mucus tends to linger. Lack of mucociliary function does not explain the frequent occurrence of cancer in this area. In particular, the majority of the mucus passed through the posterior commissure. This could explain why most laryngeal tuberculosis occurs in the posterior commissure. The glottis seems to be the major barrier to the transport of airway secretions. From this conclusion, we can infer that inserting an endotracheolaryngeal T-tube over the vocal cords will not slow the mucous transport. Instead, it tends to reduce the period of lingering in the subglottic region and speeds up the transport process.³ According to the results of this experiment, an intraluminal stent with inward folding of the posterior wall will be useful in clinical practice from the point of view of laryngeal mucociliary transport. Many studies on the function of mucociliary transport were done on immediately postmortem subjects as in our experiments. These data were all gathered from an *in vitro* model. Unfortunately, we are unable to extrapolate the results to our experiment. Advances have been achieved in radioactive detectors and other types of gamma ray-emitting isotopes. Although it provides an easier and more accurate way to study mucociliary function under a relatively physiological state, we still have to be conservative about the harmful effects that radiation may cause.

In conclusion, the technique of using an endoscope to record rheological data in the airway from a living animal is satisfactory. Further study with this animal model will permit elucidation of the mucociliary function in the laryngotracheal region.

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