Introduction

Tracheal stenosis has been studied in New Zealand White rabbits since 1981 when Natvig and Olving studied differing tracheostomy techniques and the development of tracheal stenosis. Since that time several other studies have used New Zealand White rabbits as a model for tracheal stenosis. The New Zealand white rabbit is a suitable model because the rabbit larynx corresponds remarkably to that of man and approximates the size and complexity of the human neonate.

Unfortunately there are several models of inducing stenosis that have never been compared. Current methods to induce tracheal stenosis in the rabbit model are somewhat costly and time consuming; using laser technology and/or an open approach to control for depth of injury. In spite of these methods, a consistent degree of airway obstruction has not been demonstrated. We hypothesized that a comparable, quick and simple endoscopic method could be equally effective at inducing tracheal stenosis in a rabbit model. This model would make further tracheal stenosis studies more accessible, less time consuming and available to larger numbers of research institutions. Our study attempts to analyze open and endoscopic methods of inducing tracheal stenosis.

Methods

This study was approved by the Georgetown University Animal Care and Use Committee (GUCAC) and was compliant with all committee regulations. Fifteen adult New Zealand White rabbits were used in the experiment, with 6 opened to the group and 9 assigned to the endoscopic group.

Endoscopic procedure

The 9 animals in the endoscopic group were anesthetized with 25 mg/kg of intramuscular ketamine and 0.5 mg/kg of medetomidine. Spontaneous respiration was maintained throughout the procedure. Rabbits were then placed supine on the operating table with a Bair Hugger (Arizant, Eden Prairie, MN) warming blanket kept at 34°C. With a Wia-Hippe size 0 laryngoscope, the larynx was visualized and assessed for any abnormalities. A size 3.5 Storz bronchoscope (outer diameter 5.5 mm) was inserted into the trachea. The telescope was then removed from the bronchoscope and a 5.0 mm diameter nylon brush (Key Surgical, Eden Prairie, MN) was passed from the front of the bronchoscope 4 times. The bronchoscope was then rotated 90 degrees to direct the beveled edge of the bronchoscope to the next quadrant; this procedure was repeated until all 4 quadrants were brushed with a total of 16 brush strokes. The rabbits then received 50 mL of lactated ringer's solution subcutaneously and a small area of dorsal skin was shaved to prepare the skin for a Duragesic (Alza Corporation, Mountain View, CA) transdermal patch (dose 12.5 mcg/hr). The rabbits then received 0.5 mg/kg of intramuscular atipamezole to induce emergence from anesthesia.

Open Procedure

The 6 rabbits in the open group received the same preoperative, intraoperative, and postoperative care as the rabbits in the endoscopic group. Prior to incision, the anterior cervical fur was shaved to expose the skin. The skin was then prepared with a povidone-iodine (Betadine, Purdue Pharma LP, Stamford, CT). A vertical midline incision was made, and the underlying strap muscles were dissected bluntly to expose the trachea. An incision was made from the cricothyroid membrane to the sixth tracheal ring. The posterior and lateral portions of tracheal rings 3, 4 and 5 were brushed with a 5.0 mm diameter nylon brush (Key Surgical, Eden Prairie, MN) while blood was suctioned from the airway. A total of ten brush strokes was performed per rabbit. The tracheal rings were placed into anatomical position, and the skin and subcutaneous tissues were closed with 5.0 nylon interrupted sutures.

Endoscopic Evaluation

At 2 and 3 weeks postoperatively, all rabbits were examined endoscopically under the same anesthetic conditions to evaluate the degree of stenosis induced from the procedures (Figure 1). Tracheal stenosis was measured as a percentage of luminal narrowing by a consensus of 3 individuals viewing the procedure live on a television monitor and graded as a percentage of narrowing of the entire airway.

Discussion

The majority of methods to induce tracheal stenosis in animal models use an open technique in a variety of ways, including: scalpel, curette, hydrochloric acid, silver nitrate, electrocautery, Bard Parker blade, diode laser, or carbon dioxide laser.

We chose to model our open group after a method described by Nakagishi et al. They produced an injury from a tracheotomy with a 5.5 mm nylon brush with a total of 10 brush strokes. The differences between their method and ours include: the use of Japanese White rabbits; the use of antibiotics; and the measurement of stenosis, in which sections of each trachea were photographed and analyzed at multiple days after injury (days 9-28). Their results were quite different. In 8 animals, the degree of injury ranged from 29 to 87% versus only 1 animal achieving any degree of notable stenosis (40%) in our group. The mortality rate in their group was 37.5% (3/8), all due to airway obstruction as a direct result of their injury. In our group, the mortality rate was 16.6% (1/6) and was a result of antibiotic use, not airway obstruction.

It is difficult to conclude with certainty why our results differed so significantly. However, it is likely that the vigor with which the brush was applied is the main factor that determined the resultant injury. Depth of injury has been shown previously to be more closely correlated with the degree of tracheal stenosis. The much greater degree of stenosis and higher mortality rate in the Nakagishi group are evidence that their depth of injury was greater.

Nevertheless, our objective was to develop a reliable and faster method of stenosis induction that avoided the need for an open incision, wound care, and antibiotics. In developing our endoscopic technique, we reduced our operative time from 30 minutes in the first 2 animals to 5 minutes in the remaining 7. This difference is important, as both animals with an operative time of 40 minutes had significant laryngeal edema after the procedure due to prolonged instrumentation, and they both expired within 48 hours. Compared to the 45 minutes required to perform the open procedure, 5 minutes is a significant reduction in operative time. In addition to the better mortality rates, this also decreased time, increased efficiency, and reduced costs.

The ability to achieve highly consistent degrees of stenosis, however, remains difficult. Despite our success inducing stenosis in all animals by 2 and 3 weeks, our stenosis ranged widely from 10 to 80%. It has been proposed that the use of lasers may provide a controlled depth of injury and therefore result in a predictable degree of stenosis. While these injuries provide predictable histologic changes, the ability to achieve a predictable overall degree of stenosis has not been clearly demonstrated. Although still unable to obtain highly consistent degrees of stenosis, we still demonstrate an extremely rapid and reliable method to induce tracheal injury and resultant stenosis.

Results

Only 1 animal in the tracheotomy group had noticeable stenosis (40%) at 2 weeks. This stenosis remained stable at 3 weeks.

In the endoscopic group, all animals had a degree of stenosis at 2 weeks, ranging from 10-80% (mean 43%, median 40%). At 3 weeks, only 1 animal had a reduction of stenosis, decreasing from 70 to 50%. The mean stenosis at 3 weeks was 40% (median 40%, range 10-80%).

Table 1. Open Versus Endoscopic Induction of Tracheal Stenosis

<table>
<thead>
<tr>
<th>Method of induction</th>
<th>Number of animals</th>
<th>Mortality</th>
<th>Animals achieving stenosis</th>
<th>Range of Stenosis</th>
<th>Time of procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>6</td>
<td>1</td>
<td>1 of 5</td>
<td>0-40%</td>
<td>45 minutes</td>
</tr>
<tr>
<td>Endoscopic</td>
<td>9</td>
<td>2</td>
<td>7 of 7</td>
<td>10-80%</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

The overall mortality rate was 20% (3/15). There was 1 mortality in the tracheotomy group and 2 mortalities in the endoscopic group. In the tracheotomy group, the mortality occurred secondary to antibiotic-induced gastroenteritis. In the endoscopic group, the 2 mortalities occurred in the immediate postoperative period secondary to laryngeal edema. These 2 animals were the first to undergo the procedure, which lasted approximately 30 minutes. After refining our technique, succeeding procedures lasted an average of 5 minutes and there were no mortalities. The average duration of the tracheotomy procedure was 45 minutes (Table 1).