Cartilage Regeneration Using Slow Release of Bone Morphogenetic Protein-2 from a Gelatin Sponge to Treat Experimental Canine Tracheomalacia: A Preliminary Report

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We investigated whether saber sheath-type tracheomalacia could be treated by the slow release of bone morphogenetic protein (BMP)-2 from a gelatin sponge. A 1 cm gap was made in the middle portion of each of 10 consecutive tracheal cartilage rings in the canine cervix (control group, n = 3), then a gelatin sponge containing 12 μg of BMP-2 solution was implanted in the gap (12 μg group, n = 3). In another group (120 μg + P group, n = 3), the implanted gelatin sponge contained 120 μg of BMP-2 solution, and the gap was covered with periosteum. All of the control dogs developed saber sheath-type tracheomalacia, whereas tracheomalacia was not observed in the 12 μg and 120 μg + P groups. In the 12 μg group, fibrous cartilage was observed at the ends of the cartilage stumps. In the 120 μg + P group, newly formed bone and cartilage were observed to form a bridge between the cartilage stumps. The regeneration of cartilage or bone induced by the slow release of BMP-2 from a gelatin sponge might be useful for treatment of tracheomalacia. 


Tracheomalacia is a condition characterized by the collapse of the internal lumen of the trachea, and affected patients suffer severe coughing attacks that are often misdiagnosed as asthma. Tracheomalacia is classified into two subtypes based on the form of tracheal collapse. One is the crescent moon type, in which the membranous portion of the trachea protrudes into the lumen when the patient performs forced expiration. This type is characterized by shortness of the anteroposterior axis of the trachea. The other is the saber sheath-type, in which the cartilage portion of the trachea shows folding into the lumen upon forced expiration. This type is characterized by shortness of the anteroposterior axis of the trachea. The literature, the pathogenesis of the saber sheath-type involves interruption of the tracheal cartilage, which reduces the mechanical strength of the tracheal wall. Therefore, if the interrupted tracheal cartilage could be regenerated to regain its continuity, saber sheath-type tracheomalacia might be treatable.

It is well known that bone morphogenetic protein (BMP)-2 can transform immature mesenchymal cells into mature osteocytes or chondrocytes. Slow release of BMP-2 from biodegradable materials promotes the regeneration of chondrocytes in articular cartilage defects. Moreover, we have already reported that BMP-2 can induce chondrocyte neogenesis in ectopic areas such as the subcutis.

In the current study, we examined whether BMP-2 released slowly from a gelatin sponge could induce regeneration of tracheal cartilage and be used to treat saber sheath-type tracheomalacia in dogs.

Materials and Methods

Preparation of Gelatin Sponge for Slow Release of Bone Morphogenetic Protein-2

A 5 wt% aqueous solution of gelatin with an isoelectric point of 5.0 (Nitta Gelatin Co., Osaka, Japan) containing 0.05 wt% glutaraldehyde (Wako Pure Chemical Industries, Osaka,
Macroscopic appearance of the gelatin sponge. The material was hard and friable when dry but converted to a gel form when bone morphogenetic protein-2 solution was soaked into it (top, dry status; bottom, wet status containing BMP-2 solution).

Japan) was cast in a Teflon mold, then stored at 4°C for 12 h for completion of the cross-linking reaction. The specimens were then immersed in 100 mM aqueous glycine (Wako) solution at 37°C for 1 h to block any residual aldehyde groups, followed by washing with distilled water. Subsequently, the specimens were freeze dried and sterilized by ethylene oxide gas. The sterilized gelatin sponge plates were trimmed aseptically to approximately 1 X 5 cm (Figure 2, top). Just before implantation, 12 or 120 μg of aqueous BMP-2 solution was soaked into the gelatin sponge (Figure 2, bottom).14-11

Operative Procedures

Tracheomalacia models (control group, n = 3). A 1 cm length was resected from the middle portion of each of 10 consecutive tracheal cartilage rings in the canine cervix, conserving the tracheal mucosa (Figure 3).15

Implantation of a gelatin sponge containing 12 μg of BMP-2 (12 μg group, n = 3). The gelatin sponge containing 12 μg of BMP-2 was implanted into the tracheal cartilage defect in the tracheomalacia models. The implanted prepara-

Operative findings in the control group. A 1 cm length of cartilage was resected from 10 sequential tracheal rings without damaging the tracheal mucosa. The tracheal wall lost its mechanical strength in the region of the cartilage gap. Arrows show the cartilage defect.

Operative findings in the 12 μg group. Gelatin sponge containing 12 μg of slowly releasing bone morphogenetic protein-2 was implanted in the cartilage gap (arrow) and then fixed by sutures (arrowheads) at the implant site.

Operative findings in the 120 μg + P group. After resection of the tracheal cartilage, the gelatin sponge containing slowly releasing bone morphogenetic protein-2 was implanted and covered with periosteum harvested from a rib of the same dog (arrow). The periosteum was then sutured to the tracheal wall (arrowheads).
tion was fixed by 2-0 silk sutures to prevent dislodgement (Figure 4).

Implantation of a gelatin sponge containing 120 µg of BMP-2 (120 /Hg + P group, n = 3). The gelatin sponge containing 120 tig of BMP-2 was implanted into the tracheal cartilage defect in the tracheomalacia models. Subsequently, the right 5th rib bone was resected and the periosteum was harvested as a free graft. The periosteum, fixed by 4-0 Prolene sutures, was used to cover the implant site to avoid dislodgment of the gelatin sponge and promote the regeneration of host tissue (Figure 5).

Table 1. Cartilage Regeneration Using a BPM-2 Slow Releasing Gelatin Sponge to Treat Experimental Canine Tracheomalacia

<table>
<thead>
<tr>
<th>Dog Numbers</th>
<th>BMP-2 (µg)</th>
<th>Survival Time (months)</th>
<th>Dead/Alive</th>
<th>Stridor</th>
<th>Malacia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>-</td>
<td>14</td>
<td>Alive</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>4</td>
<td>Alive</td>
<td>+</td>
<td>+</td>
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<tr>
<td>2</td>
<td>-</td>
<td>1</td>
<td>Dead</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12 µg group</td>
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<td>11</td>
<td>Alive</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>2</td>
<td>Alive</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>1</td>
<td>Dead</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120 µg + P group</td>
<td>120</td>
<td>13</td>
<td>Alive</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
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<td>12</td>
<td>Dead</td>
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<tr>
<td>3</td>
<td>120</td>
<td>1</td>
<td>Dead</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 6. Endoscopic findings at 1 month after surgery in the control group. The tracheal lumen collapsed during coughing (top, at rest; bottom, during forced expiration).

Figure 7. Endoscopic findings at 1 month after surgery in the 12 /µg group. The tracheal lumen was maintained during coughing but showed stenosis at the implant site (top, at rest; bottom, during forced expiration).
Figure 8. Endoscopic findings at 1 month after surgery in the 12 μg + P group. The tracheal lumen was maintained during coughing, and no stenosis was observed (top, at rest; bottom, during forced expiration).

Figure 9. Macroscopic appearance of the internal lumen of the trachea in the control group at 1 month. The resected portion of the tracheal cartilage was not supported by solid tissue in the control group (arrows: cartilageal defects).

Figure 10. Macroscopic appearance of the internal lumen of the trachea in the 12 μg and 120 μg + P groups at 1 month. In these groups, the resected portion of the tracheal cartilage was fixed by the solid tissue (top, 12 μg group; bottom, 120 μg + P group) (arrows: cartilageal defects).

All the animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH publication No. 86-23, revised 1985).

Evaluation of Tracheomalacia

To evaluate the degree of tracheomalacia, we performed bronchoscopic examination of each dog once a month. The experimental dogs maintained spontaneous breathing under general anesthesia with intramuscularly administered pentobarbital sodium. Coughing attacks were induced by injection of saline solution into the tracheal lumen through the fiberscope or by mechanical stimulation of the tracheal wall with the fiberscope itself. Photographs were taken at the moment of collapse of the tracheal lumen during the coughing attacks.

Microscopic Evaluation

One dog in each group was killed 1 month after surgery, and the trachea was removed from each of them. The membranous portion was incised longitudinally, and the internal lumen was examined macroscopically. Transverse sections of the tracheal cartilage defect or the implant site were then prepared. Thin
slice sections were made, and light microscopic examination was performed.

Results

Except for the four that were killed, all of the experimental dogs were alive at the time of writing (Table 1). Stridor was observed in the control group when the dogs barked in excitement, but this was not evident in the 12 μg group or the 120 μg + P group. Neither wheezing nor stridor was observed in any of the dogs when they stayed calm.

Endoscopic Findings

In the control group at 1 month, all of the three dogs showed saber sheath-type tracheomalacia upon expiration during coughing attacks. The tracheal lumen in this group showed 90% collapse or more (Figure 6). In the 12 μg group at 1 month, tracheal stenosis was observed, but there was no luminal collapse, even during coughing (Figure 7). In the 120 μg + P group, neither stenosis nor luminal collapse of the trachea was observed, even during coughing (Figure 8).

Macroscopic Findings

In the control group, it was easy to open the lumen after longitudinal incision of the membranous portion of the trachea because the region of the cartilage defect was filled with soft granulomatous tissue with insufficient mechanical strength to support it (Figure 9). In the 12 μg and 120 μg + P groups, the area of defective cartilage was firmly fixed with solid tissue, making it difficult to open the lumen even when forceps were used (Figure 10).

Microscopic Findings

In the control group, the thick submucosal layer, consisting of fibroblasts and collagen bundles, was revealed. Neither cartilage nor chondrocytes regenerated, even near the cartilage stumps. No shrinkage of the distance between the two cartilage stumps was observed (Figure 11), whereas in the 12 μg group, the distance between the stumps was severely reduced. The submucosal layer between the two stumps was thick. Immature cartilage, which continued to the perichondrium of the host cartilage, was observed (Figure 12). In the 120 μg + P group, the gap between the cartilage stumps was...
bridged by newly formed bone, and the distance between the stumps was not reduced. Immature cartilage was observed at the stumps, which seemed to have regenerated from the perichondrium. This regenerated immature cartilage was connected to the newly formed bone and host cartilage stumps (Figure 13).

Discussion

In the current study, a 1 cm length was resected from each of 10 consecutive tracheal cartilage rings in the control group, and the dogs showed grade III saber sheath-type tracheomalacia as defined by Johnson et al., that is, more than 90% collapse of the tracheal lumen during coughing attacks. Therefore, this method was suitable for use as a tracheomalacia model.

We have already succeeded in promoting cartilage regeneration with the slow release of BMP-2 from a gelatin sponge. In the current study, we attempted to use this approach to treat saber sheath-type tracheomalacia. Gelatin has a wide range of clinical uses, such as a surgical hemostat or adhesive agent. It does not cause problematic side effects; it causes only a minimal foreign body reaction because of its biodegradability, and its medical safety is well established. However, gelatin alone does not have the ability to induce cartilage regeneration, and therefore, we used gelatin sponge as a vehicle for the slow release of BMP-2.

Endoscopic and macroscopic examination of the 12 μg and 120 μg + P groups showed that the tracheal lumen did not collapse during coughing attacks because the tracheal walls were fixed by solid tissue. Although the mechanical strength of the trachea was not quantified in this study, our findings in this canine model revealed that reinforcement of the tracheal wall induced by slow release of BMP-2 from a gelatin sponge could be helpful for treatment of saber sheath-type tracheomalacia even in a clinical setting.

In the 12 μg group, the macro- and microscopic findings showed cartilage regeneration near the cartilage stumps, although the gap between the cartilage stumps and the distance between them were reduced. We speculated that this gap shortening might be due to granulation tissue, which had invaded the gap before it was filled by regenerated cartilage. In general, granulation plays an important role in wound healing, especially in the wound contraction phase, and this might involve fibroblasts or myofibroblasts. In spite of the incomplete cartilage regeneration, the shrinkage of the gap and thick granulation tissue would have maintained the tracheal lumen without collapse during coughing attacks in this group. Moreover, the regenerated cartilage differed from native cartilage in that it was fibrous, which has lower mechanical strength than the normal hyaline cartilage of the trachea. Regeneration of the cartilage in the 12 μg group was slow and limited to near the stumps. We conclude that 12 μg of BMP-2 released slowly from gelatin sponge is itself inadequate for treatment of tracheomalacia.

In the 120 μg + P group, the macro- and microscopic findings showed that newly formed bone tissue formed a bridge between the ends of the cartilage stumps, preventing collapse of the tracheal wall during coughing. We speculated that this bone tissue regenerated because of the periosteum covering it and the increased dosage of BMP-2. It is also possible that the periosteum prevented the invasion of granulomatous tissue into the gap, allowing it to be filled with the newly formed bone. Development of bone tissue in the cartilage gap would not be disadvantageous for the treatment of tracheomalacia because bone has enough mechanical strength to maintain the internal lumen of the trachea. We conclude that implantation of a gelatin sponge slowly releasing 120 μg BMP-2, together with a periosteum covering, could be used for clinical treatment of tracheomalacia.

We are now evaluating the long-term results in the dogs of all the present study groups.

References


Figure 13. Microscopic appearance of the implant site in the 120 μg + P group. The length of the cartilage gap is maintained, and newly formed bone is evident in the gap between the cartilage stumps. Transition of newly formed cartilage from newly formed bone is observed at the ends of the cartilage stumps (arrows) (Masson's trichrome stain: top, ×90; bottom, ×150).