Prevention of Anterior Glottic Stenosis After Bilateral Vocal Fold Stripping With Mitomycin C

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Objective: To examine the effects of immediate application of topical mitomycin C (MMC) on the prevention of anterior glottic stenosis (AGS) after microsurgical stripping of both vocal folds, including the anterior commissure, in a canine model.

Design: Prospective randomized experimental study.

Interventions: Twelve canine larynges were injured by a stripping procedure of both entire membranous vocal folds. The dogs were randomly divided into 2 groups for treatment with 1.0 mg/mL of MMC or with isotonic sodium chloride solution (control) for 5 minutes immediately after surgery. Three and 6 weeks after surgery, the glottic webs were lysed and repeatedly treated with MMC or isotonic sodium chloride solution. The glottic wound healing and AGS formation were examined every week. Ten weeks after the initial surgery, all larynges were collected and examined histologically.

Results: The stripping procedure induced AGS, affecting 58% to 86% (mean, 72%) of the length from the anterior commissure to the vocal process in the control group. The application of MMC at the time of initial surgery significantly lowered the incidence and extent of the web formation (P=.004). The AGS lesions were resolved by web lysis and treatment with MMC, without significant local adverse effects. Histological staining for collagen and elastin revealed that MMC treatment did not induce excessive fibrotic or atrophic changes in the lamina propria of the vocal folds.

Conclusion: Bilateral stripping of the membranous vocal folds induces significant AGS, which can be minimized by use of MMC at initial surgery.


ANTERIOR GLOTTIC STENOSIS (AGS) or web is the formation of a bridge of scar tissue covered by epithelium between the vocal folds and involving the anterior commissure. Anterior glottic stenosis commonly occurs secondary to laryngeal injury resulting from laryngeal surgery. Unintended injury from endoscopic or microsurgical manipulation involving both vocal folds simultaneously can induce anterior glottic scarring and adhesions that disturb the delicate workings of the vocal folds. Excessive AGS causes significant impairment of phonation, ventilation, and airway protection. Therefore, aggressive endolaryngeal microsurgical management of lesions involving the anterior glottic commissure has often been avoided or discouraged. When required, it has been recommended that bilateral surgical interventions be separated into 2 unilateral procedures staged at least 2 weeks apart. Nonetheless, reports have suggested that endolaryngeal microsurgery can be successfully performed for treatment of benign or malignant lesions involving the anterior glottic commissure.

The conventional treatments of AGS have included intraläsional injection, endolaryngeal procedures, and keel insertion via laryngofissure. Recently, topical mitomycin C (MMC) has been used to prevent restenosis after the lysis of anterior glottic webs. Mitomycin C is an antineoplastic agent that is known to inhibit proliferation of fibroblasts and tumor growth. Its proven antifibrotic effect has led to several experimental and clinical trials. Mitomycin C has been widely used for prevention of stenosis or adhesion after otolaryngological surgical treatments. In a previous experiment, MMC was used only to prevent recurrence after the lysis of anterior glottic webs that were originally induced by laser injury of the vocal folds. These clinical and experimental studies of MMC suggest that MMC can be used for prevention of AGS following microsurgical removal of lesions involving
both anterior vocal folds. However, to our knowledge, there have been no reports of experiments in which MMC was applied at the time of initial surgery to prevent the development of AGS.

In this study, we first developed a canine model in which AGS is induced by bilateral microsurgical stripping of the epithelium from the entire length of both membranous vocal folds. We then used this model to examine the effects of MMC in preventing AGS after surgery.

### METHODS

#### EXPERIMENTAL DESIGN

Twelve male beagles aged 1½ to 2 years and weighing 10 to 12 kg each were used in this study. The animals were anesthetized by intravenous administration of propofol, 6 mg/kg of body weight. Maintenance anesthesia was achieved with a continuous infusion of propofol at 0.4 mg/kg per minute, with additional small boluses of 1 mg/kg if anesthesia appeared inadequate. Direct laryngoscopy was performed using a Kleinsasser laryngoscope modified for exposure of the anterior commissure (8590J; Karl Storz GmbH & Co, Tuttingen, Germany) and an operating microscope (Carl Zeiss, Göttingen, Germany) to visualize the canine glottis. Microforceps and microscissors (Karl Storz GmbH & Co) were used to remove the entire epithelium and a layer of the lamina propria from both sides of the membranous vocal folds, from the anterior commissure to the vocal process, and from the upper lateral margin of the true vocal folds to a point 2 mm below the edge of the vocal folds.7

After the vocal folds were stripped, the anterior glottic area of the lesions was immediately treated for 5 minutes by application of a cottonoid pledget soaked in a solution of 1.0 mg/mL of MMC in sterile water or soaked in isotonic sodium chloride solution (control) (6 in each group) (Table 1). After treatment with MMC, the glottis was washed with an isotonic sodium chloride solution–soaked cottonoid pledget. The selected MMC concentration was expected to be sufficient to achieve significant effects, while avoiding necrosis at the site of local treatment, based on reports in the literature.8-10 The depth and extent of the vocal fold stripping were identical in all the animals, and the treatment with MMC or isotonic sodium chloride solution was randomized at the time of the initial intervention.

At weekly intervals from the original surgery until the end of the experiment, each animal was anesthetized with propofol, and the vocal folds were examined and photographed. Three weeks after the initial injury, the animals that had developed AGS underwent web lysis using microscissors and were treated for 5 minutes with 1.0 mg/mL of MMC (dogs 1-9) or with isotonic sodium chloride solution (dogs 10-12) (Table 2). Treatment with MMC or isotonic sodium chloride solution after the first web lysis was also randomized in the 6 animals initially treated with isotonic sodium chloride solution that had developed significant AGS. Six weeks after the initial surgery, all residual AGS lesions were relased and treated for 5 minutes with 1.0 mg/mL of MMC (dogs 5, 6, and 8-12) (Table 3).

All animals were housed in an approved animal care facility with water ad libitum and regular canine food until their humane killing 10 weeks after the original injury. All experiments were performed with the approval of the Animal Experiment Committee of the Clinical Research Institute at Chungnam National University Hospital.

#### GROSS AND HISTOLOGICAL EXAMINATIONS

All animals were examined under anesthesia each week. The entire glottis was evaluated for the presence of mucosal scarring, granulation tissues, and AGS using a 40-cm-long, 5-mm-diameter, 0° telescope (Karl Storz GmbH & Co). The extent of the web formation was determined by dividing the anterior to posterior length of the web by the entire length of the membranous vocal folds from the anterior commissure to the vocal process. The glottis of each animal was documented with telescopc photographs taken after careful examination.

At the end of the 10-week follow-up, each animal was humanely killed with a lethal dose of sodium pentobarbital.
The larynges were excised and placed in 10% formaldehyde for 24 hours. The samples were processed using standard alcohol dehydration and paraffin embedding procedures. The whole vocal folds, including the anterior commissure, were sectioned at 5-µm thickness at the level of the free margins and stained with hematoxylin-eosin. Masson trichrome and van Gieson stains were used to assess the collagen and elastin contents, respectively, and the levels were graded from 0 to 3+ in the lamina propria. All preinjury vocal fold samples collected at the time of initial surgery were processed and stained as control specimens for comparison with the injured larynges collected when the animals were killed. The anterior commissure region of each glottis was compared between the treatment groups and the preinjured larynges. The telescopic and histological examinations and the scoring of the larynges were performed in a blinded fashion with regard to which animals received MMC treatment after the initial surgery.

### Statistical Analysis

The extent of AGS formation after microsurgical stripping of the vocal folds and the extent of recurrence after web lysis were compared between the MMC-treated group and the control group using the Mann-Whitney test (SPSS 11.0 for Windows; SPSS Inc, Chicago, Ill). The degree of mucosal scarring or granulation and scar tissue formation on the vocal folds and the histological grades of collagen deposition or elastin content were compared between the groups using the Fisher exact test. P<.05 was considered significant.

### Results

#### MMC Treatment After Vocal Fold Stripping

All of the animals survived all procedures during the experimental period of 10 weeks, and all of the surgical wounds healed without hemorrhage or infection. Microsurgical stripping of the epithelium from the bilateral vocal folds induced AGS in all animals within 2 weeks after wounding when only isotonic sodium chloride solution was applied immediately after stripping. Three weeks after surgery, the AGS lesions in the control group affected 58% to 86% (mean, 72%) of the length of the vocal folds from the anterior commissure to the vocal process (Table 1 and Figure 1A). In contrast, the animals treated with MMC at initial surgery had smaller webs affecting 7% to 57% (mean, 31%) of the length of the vocal folds, which was significantly less than the extent of web formation in the control group (P =.004). All of the control group had extensive anterior glottic webs, but one half of the MMC-treated group had webs involving less than 50% of the membranous vocal folds, and the other half developed only very small glottic webs (Figure 1B). On endoscopic examination, the anterior glottic movement was decreased in animals with severe AGS. In addition, the formation of granulation and scar tissue on the vocal fold mucosa was found more frequently in the control group (4 of 6 animals) at 3 weeks after the initial surgery compared with the MMC-treated group (2 of 6 animals), but this difference was not statistically significant (P =.56). However, wound healing in the injured vocal folds seemed to be delayed in the MMC-treated group.

#### MMC Treatment After Lysis of Glottal Webs

Three weeks after the initial surgery, the dogs that had developed AGS were treated by surgical lysis of the lesions and immediate application of MMC or isotonic sodium chloride solution. Among the dogs that had initially been treated with only isotonic sodium chloride solution after the original surgery (dogs 7-12), anterior glottic webs involving a mean of 42% of the vocal fold length had reformed 3 weeks after web lysis in all of the animals treated with only isotonic sodium chloride solution at the time of lysis (dogs 10-12) (Figure 1C), and webs involving a mean of 23% of the vocal fold length had reformed in 2 of the 3 animals treated with MMC (dogs 7-9), revealing no significant differences between the treatments (P =.28) (Table 2). However, the extent of the web reformation after lysis was smaller than the extent of the webs before lysis. In the group treated with MMC after the initial surgery (dogs 1-6), the recurrence of anterior glottic webs after lysis and reapplication of MMC was less prominent; no webs reformed in 4 of the 6 animals, yielding a significantly lower rate of recurrence compared with the group initially treated with isotonic sodium chloride solution (dogs 7-12) (P =.02).

Ten weeks after initial surgery, all of the injured vocal folds had healed well, without severe scarring, granulation tissues, or webs. Small webs were found in 3 of the 6 animals that were treated with isotonic sodium chloride solution after the initial surgery. No glottic webs were found in the group initially treated with MMC (Figure 1D). On gross examination, no significant atrophic changes in the vocal folds were found in the animals treated repeatedly with MMC. Although the MMC appeared to induce delayed wound healing in the injured vocal folds, there were no significant local adverse effects, such as necrosis or infection. No limitation of vocal fold movement was found in any of the ani-
HISTOLOGICAL EXAMINATION

Histological examination 10 weeks after the initial surgery revealed that the vocal folds had mild collagen deposition and fibroblast proliferation, although the collagen content was not prominently increased in the wounded vocal folds of the animals initially treated with MMC. When present, collagen fibers were found in the lamina propria of the vocal folds, particularly around the anterior glottis and the anterior commissure on Masson trichrome staining (Figure 2A). However, in the animals treated with isotonic sodium chloride solution after the initial surgery, irregular, thickened epithelium was found in some of the vocal folds and was accompanied by collagen deposition, especially in the superficial layer of the lamina propria (Figure 2B). Nonetheless, the overall grade of collagen content in the control group was not significantly different from that in the MMC-treated group and the preinjury vocal folds (P = .21 and P = .46, respectively). Elastin fibers were observed in the lamina propria of the vocal folds, with particularly dense elastin staining seen in the superficial layer (Figure 2C). However, the degree of elastin staining in the injured vocal folds and anterior commissure was not significantly different from that in the preinjury vocal folds (P = .59) (Figure 2D).

COMMENT

Bilateral stripping of the entire length of the membranous vocal folds induced significant AGS in this study. This finding supports previous observations that surgical manipulation of both anterior vocal folds can lead to postoperative AGS. Our animal model of AGS was created to simulate such clinical situations. The extent and depth of injury to the glottis that we used in the model were suggested by a study of endolaryngeal microsur-
surgery for laryngeal cancer, in which subligamental cordectomy (type II) was performed on both sides according to a proposed classification of endoscopic cordectomy of the European Laryngological Society. The results of that study showed that a single microsurgical stripping of the entire length of the membranous vocal folds induced more severe AGS than had been induced in a previous experiment using repeated laser vaporization of the mucosa in the same area.7

The present study also focused on the prevention of AGS following bilateral endolaryngeal surgery of the anterior vocal folds. The conventional methods used to prevent AGS4-6 are listed in the introduction. In this study, we applied MMC to the injured anterior glottis immediately after bilateral vocal cord stripping. The application of MMC significantly lowered the incidence and extent of AGS. In addition, MMC had a preventive effect on restenosis after lysis of anterior glottic webs. All of the animals initially treated with MMC were AGS-free after web lysis and the reapplication of MMC.

A previous study12 showed that injured canine vocal folds became atrophic 4 weeks after MMC treatment owing to decreased connective tissue response to the surgical injury. The atrophy of the vibratory surface resulted in diminished mucosal wave vibration in the vocal folds after treatment with MMC. However, another experiment showed that although MMC delayed the healing of surgical wounds in rats up to the fourth week following treatment, the degree of fibrosis was comparable in the treated and untreated wounds after 12 weeks.13 This latter finding was supported by the gross and histological observations in the present study, in which the injured vocal folds showed no significant atrophy and acceptable amounts of scarring. Instead, MMC appeared to prevent severe, broad scarring of the vocal fold lamina propria, which is attributable to abundant and disorganized collagen deposition that is a characteristic feature of surgically injured vocal folds.14 In addition, no significant local adverse effects were induced by use of 1.0 mg/mL of MMC, a dose that may be considered as being in the upper range of typical clinical doses of MMC.8-10 The concentration used in this study was less than the 10 mg/mL of MMC concentration reported by Spector et al7 but is comparable to the concentrations used clinically. The safe use of MMC is also supported by a recent report showing that the use of MMC at concentrations

Figure 2. Photomicrographs of the canine vocal folds that underwent stripping and web lysis. Ten weeks after the initial wounding, the vocal folds had healed without significant web formation or fibrosis (A, Masson trichrome stain, original magnification ×15). However, thickening and irregularity of the vocal fold epithelium were sometimes found, accompanied by collagen deposition in the lamina propria, in the animals not treated with mitomycin C at the initial surgery (B, Masson trichrome stain, original magnification ×40). The degree of elastin staining in the lamina propria of the vocal folds was not significantly altered 10 weeks after injury (C, van Gieson stain, original magnification ×40) compared with the preinjury vocal fold tissue samples (D, van Gieson stain, original magnification ×200).
in excess of 0.4 mg/mL did not alter the protein concentrations in the extracellular matrix or the apoptosis rate. The present study thus confirms that MMC at a typical clinical dose can be effectively and safely used to treat the anterior vocal folds at the time of initial surgery.

In conclusion, we suggest that MMC can be used to minimize the development of AGS in the injured anterior glottis when applied at the end of endolaryngeal microsurgery involving the bilateral anterior vocal folds. Prophylactic use of the drug may be advocated in clinical settings for surgical management of lesions involving the anterior glottic commissure, although further randomized clinical studies will be required.

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REFERENCES


