Effect of Mitomycin in the Surgical Treatment of Tracheal Stenosis

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Objective: To assess the capacity of high and low doses of the antimotic drug mitomycin to prevent laryngeal stenosis in an animal model.

Methods: A prospective, randomized, double-blind, controlled study was carried out. End-to-end anastomosis was performed in 18 rabbits after tracheal annulus resection to produce inflammation. There were 3 treatment groups: topical saline (isotonic sodium chloride solution) and low-dose (0.2 mg/mL) and high-dose (0.5 mg/mL) topical mitomycin.

Results: A total of 107 procedures were performed: 54 surgical procedures, 35 fibrobronchoscopies, and 18 biopsies. The effect of mitomycin was dose related. In the high-dose mitomycin group, most rabbits progressed to stenosis with a percentage decrease in airway diameter that was significantly greater than in the other 2 groups (P<.001). The mean (SD) percentage of maximum stenosis in the high-dose group was 51% (22%). In the low-dose and saline groups, it was 18% (13%) and 16% (9%), respectively. No significant differences in tracheal stenosis between the low-dose mitomycin and saline groups were observed. Blinded histopathological analysis also showed no significant differences between the saline group and the low-dose mitomycin group. Compared with the other 2 groups, the high-dose mitomycin group had a significant increase in fibroproliferative tissue (P<.001).

Conclusion: These results suggest that topical mitomycin is not effective for avoiding tracheal stenosis and may provoke the opposite effect if the dose is not carefully selected.


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S EVERE LARYNGOTRACHEAL STE- nosis (LTS) has become more frequent because of the prolonged endotracheal intubation associated with mechanical ventilation and the development of reanimation measures in intensive care units.1-4 The stenosis process begins with mucosal edema and ulceration, which are often associated with local infection. Afterward, perichondritis and chondritis can damage tracheal annuli cartilage.2 Increased fibroblastic activity generates retractile fibrous tissue that causes stenosis and airway obstruction.3

Several treatments have been used with LTS: administration of local corticosteroids, use of antibiotics to prevent infection, use of stents, and surgical treatment.5-14 Several surgical techniques, including endoluminal resection of the scar with a laser or other devices, as well as simple resection with or without cartilage or bone grafts, have been proposed to help treat this problem.6-14 Use of endoscopic carbon dioxide laser treatment combined with rigid dilatation remains the preferred surgical treatment for LTS.15 Whichever surgical treatment is used, it is important to decrease inflammation and the risk of secondary infection to avoid fibroblastic activity and the retractile scar formation.

Mitomycin is an antimotic drug that inhibits in vitro fibroblast proliferation and can prevent the formation of scars and fibrosis in humans.3 The effects of this drug have been tested in surgical treatment for pterygium,16,17 upper urinary tract urothelial tumors,18 endoscopic sinus procedures,19 maxillary antrostomy,20 and dacryocystorhinostomy.21 In a small clinical study, mitomycin was applied topically to the tracheal scars of patients who underwent laser treatment for severe recurrent tracheal granulation.22 A recent retrospective study provides strong evidence that topical mitomycin is an effective adjuvant in the treatment of LTS in humans.23
Studies clarifying the optimal dose of mitomycin for avoiding LTS and titrated using the same experimental model are needed. Different doses of mitomycin, ranging from 0.2 mg/mL to 0.5 mg/mL, have been studied with variable results. The aim of this study was to determine the optimal dose of mitomycin for avoiding tracheal stenosis in a rabbit experimental model.

METHODS

POPULATION
A prospective, randomized, double-blinded, controlled study was conducted with 18 New Zealand white rabbits (weight range, 1500-2500 g). The rabbits were alive at the end of the experiment.

DESIGN
Rabbits were divided into 3 treatment groups: usual repair technique with topical application of saline (isotonic sodium chloride solution), usual repair technique with application of a low-dose of topical mitomycin (0.2 mg/mL), and usual repair technique with topical application of a high-dose of mitomycin (0.5 mg/mL). Between September 1, 2004, and September 1, 2005, each rabbit underwent 3 procedures and at least 2 fibrobronchoscopic examinations during a 3-month period to determine the degree of stenosis and to analyze the histological characteristics of the anastomosis. The animals received general anesthesia: intramuscular ketamine hydrochloride (20 mg/kg) and intramuscular xylazine (0.2 mg/kg). After each surgical procedure, the animals were treated with prophylactic antibiotics and analgesia according to a preestablished protocol: ciprofloxacin (5 mg/kg/d) for 5 days and ketoprofen (2 mg/kg/d) for 5 days.

For the first procedure, each rabbit underwent a circumferential resection of the fourth tracheal annulus to induce inflammation. End-to-end anastomosis was performed between the third and fifth annuli. One month later, each animal underwent another resection of the 2 adjacent tracheal annuli at the site of the previous procedure (third and fifth annuli). In addition, the trachea was repaired with another end-to-end anastomosis between the second and sixth tracheal annuli. In all rabbits, the repair technique for the trachea included termino-terminal anastomosis with multiple (×5) monofilament sutures (3-0 Prolene; Ethicon Inc, Piscataway, New Jersey). Before the second intervention, each rabbit was assigned randomly to 1 of the 3 defined groups. In each case, the blinded application of saline, low-dose mitomycin, or high-dose mitomycin took 5 minutes. After tracheal resection and before anastomosis, mitomycin or saline was applied with a soaked sterile sponge kept moist with a tuberculin syringe.

At 4 and 8 weeks postoperatively, all rabbits underwent rigid fibrobronchoscopy, which was recorded with a videocamera. From these recordings, 3 blinded observers (R.I.-S., S.Z.E., and D.J.P.) determined the percentage of stenosis in the anastomosis according to the Myer-Cotton classification system (grade I, 0%-50%; grade II, 51%-70%; grade III, 71%-99%; grade IV, no lumen). The mean of the 3 scores was computed for each rabbit. At 8 weeks, the anastomosis site was resected, and 1 blinded pathologist (S.G.B.) analyzed the samples. Fibroproliferative tissue (ie, collagen fibers and fibroblasts) and the inflammatory response, observed as inflammatory cellular infiltrates (ie, leukocytes, including neutrophils, monocytes, lymphocytes, and plasma cells), were graded by the pathologist on a semiquantitative scale. Cases showing no fibrosis or inflammation were scored 0; 1+, incipient fibrosis (scant and isolated collagen bundles) and mild inflammation of less than 10% of the affected area; 2+, moderate fibrosis (visible collagen bundles and fibroblasts) and less than 30% of cellular infiltrates in the affected area; 3+, intense inflammation (30%-60%) and fibrosis; and 4+, very strong fibrosis with evident luminal stenosis and diffuse inflammatory infiltrates of more than 60% of the affected area.

In cases of severe LTS, rabbits underwent a surgical procedure before the proposed observation period.

RESULTS
A total of 18 rabbits were used in this study. Over a period of 1 year, 54 surgical procedures, 35 fibrobronchoscopies, and 18 biopsies were performed. No tracheal stenosis was present during the second tracheal resection except for 2 cases. These 2 cases exhibit tracheal stenosis less than 20% (grade I Myer-Cotton stenosis).

In the high-dose mitomycin group, the percentage decrease in airway diameter was significantly higher than in the low-dose mitomycin and saline groups (P < .001).

Histopathological analysis of stenotic lesions at the completion of the study revealed similar changes, with no significant differences between the low-dose and saline groups. The amount of fibroproliferative tissue with fibrosis was greater in the high-dose mitomycin group (P < .001) (Table). Histological analysis revealed that the percentage decrease in airway diameter was also greater in the high-dose group (Figure 3). Pathologists confirmed that there were submucosal sutures at all anastomosis sites, which is evidence that the surgical technique was appropriate (Figure 4). In the high-dose

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mitomycin group, the stenotic area included inflamed fibrovascular connective tissue in the submucosal space with lymphoplasmocitary infiltration and neoformation of capillaries (granulation tissue) (Figure 5). Although this suggests an increased inflammatory process in the high-dose group, no statistical differences were found among the 3 groups (Table).

To rule out the possibility of an allergic reaction that could explain any inflammatory processes, a skin prick test was performed in the rabbit that showed the most fibrosis after treatment with mitomycin. No allergic reaction to mitomycin was found. One week afterward, this rabbit developed a necrotic ulcer on its skin.

**COMMENT**

Severe laryngotracheal stenosis is a diagnostic and therapeutic challenge. Topical application of mitomycin has been shown to reduce cicatricial scarring, and a single, low dose of mitomycin (0.1-0.2 mg/mL) has been shown to be effective in several studies.\(^5,20\) Still, results remain controversial. Some articles that report positive results with mitomycin were not controlled, blinded, prospective studies.\(^22-24\) In one randomized, double-blind, placebo-controlled trial, a low dose of topical mitomycin after laryngotracheal reconstruction had no significant effect.\(^25\)

We believe that the effect of mitomycin depends on the dose. In the ophthalmology literature, higher doses of topical mitomycin have been linked to a variety of toxic local reactions, such as severe secondary glaucoma, scleral calcification, corneal edema, corneal perforation, iritis, sudden-onset mature cataract, and scleral calcification.\(^26\)

A recently published rabbit study found that mitomycin at high doses (0.4 mg/mL and 10 mg/mL) did not prevent stenosis and that subglottic laser wounding and mitomycin treatment confer a significant risk of acute airway obstruction.\(^26\) Another retrospective study reported accumulation of fibrinous debris at the operative site in humans, resulting in partial airway obstruction and the need for emergency airway intervention, and concluded that caution should be exercised when topical mi-
tomycin is used to treat airway stenosis, especially in high
doses.27

In a pig model, there were no significant differences
in the amount of inflammatory tissue between animals
treated with a high dose of mitomycin (0.5 mg/mL) and
controls. The authors observed a mature subepithelial fi-
broproliferative response with deposition of fibroblasts was observed. The low-dose
mitomycin (0.2 mg/mL) (B) and saline (isotonic sodium chloride solution) (C) groups showed a relative decrease in granulation thickness between epithelium and
underlying cartilage without lumen stenosis.

Figure 3. Anastomosis sites in 3 samples from each group (hematoxylin-eosin, original magnification ×10). A. High-dose mitomycin (0.5 mg/mL) produced a
decreasing lumen at the anastomosis site. A significant subepithelial fibroproliferative response with deposition of fibroblasts was observed. The low-dose
mitomycin (0.2 mg/mL) (B) and saline (isotonic sodium chloride solution) (C) groups showed a relative decrease in granulation thickness between epithelium and
underlying cartilage without lumen stenosis.

In addition, one study19 of sinus procedures con-
cludes that mitomycin is not effective in reducing post-
operative frontal recess stenosis in primary and revision
procedures when used at a high dose (0.5 mg/mL).

We observed no allergic reaction to mitomycin in a
rabbit from our series that exhibited extensive stenosis
after topical treatment. However, 1 week after the al-

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Figure 4. Anastomosis sites. Arrows show sites of submucosal sutures
(hematoxylin-eosin, original magnification ×10).
lergy test, the rabbit developed a necrotic ulcer at the site of mitomycin exposure. This observation was in concordance with results of a study that found skin necrosis in rats that received a high dose (0.5 mg/mL) of intradermal mitomycin. Other studies in rabbits suggest that mitomycin could induce a dose-dependent toxic effect on the optic nerve, necrosis of tracheal bare cartilage rings with loss of support and a negative influence on airway healing, and conjunctival fibrosis. One possible explanation is that rabbits are more susceptible to the adverse effects of mitomycin. However, the rabbit is not the only animal that has exhibited adverse effects after mitomycin application. In rats, mitomycin can cause skin necrosis and inflammation of the intestinal mucosa. Furthermore, a study of humans reports that obstruction of the puncta lacrimalis can occur after topical treatment with 4-mg/mL mitomycin.

In our study, higher concentrations of mitomycin produced tracheal stenosis. A possible explanation for this phenomenon may be that mitomycin is an antimitotic agent that inhibits not only fibroblastic proliferation of in vitro tissue but also other important processes for the adequate repair of the tracheal lesions in vivo, such as mechanisms involved with blood supply to tissues. In all cases in our study, topical application of mitomycin at an anastomosis site provoked an immediate ischemic response in the involved tissues. Another important element to consider is that the antiproliferative mechanism of mitomycin may favor necrosis and tissue damage, delaying wound healing while not preventing inflammatory processes and allowing for the accumulation of fibrinous debris. In addition, there is also a subepithelial fibroproliferative response with deposition of fibroblasts. Furthermore, rabbits from the high-dose mitomycin group had significantly more fibroproliferative tissue and no inhibition of the granulatory reaction in a subepithelial location, compared with the other groups (Figure 4).

Further studies using animal models are needed to confirm that the effectiveness of mitomycin is related to dosage and to clarify the effect of mitomycin over time. Our
data suggest that caution should be used when using mitomycin in high doses.

CONCLUSIONS

In our animal model, we observed that the use of mitomycin to avoid LTS may not be effective and may even lead to severe adverse effects when the dose is not carefully selected. We observed that a high dose of mitomycin (0.5 mg/mL) produced more stenosis compared with the low-dose mitomycin group or the saline group. Conventional surgical treatment for tracheal stenosis without mitomycin is not less effective than using a low dose of mitomycin (0.2 mg/mL). These results should be considered in the use of mitomycin in clinical practice.

Submitted for Publication: March 7, 2007; final revision received June 29, 2007; accepted August 14, 2007.

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Financial Disclosure: None reported.

Funding/Support: This study was supported in part by a grant from the Sociedad Chilena de Otorrinolaringología, Medicina y Cirugía de Cabeza y Cuello.

Previous Presentation: This study was presented in part at the Congreso Hispano-Aleman de Otorrinolaringología y Patología Cervico-Facial; September 10, 2005; Lanzarote, Spain.

REFERENCES