Inhalation mitomycin-C in the management of laryngeal fibrosis: rationale, benefits, and pitfalls

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Abstract

This study has pursued the development of animal model to assess the efficacy and safety of administrating mitomycin-C (MMC) by inhalation route for management of laryngeal fibrosis with the potential of human application. Glottic trauma was created in 10 mongrel dogs aiming at scaring and web formation. Eight dogs were randomized to receive a single daily dose of inhalation MMC using a mixture of 1 cm\(^3\) of 0.5 mg/ml MMC and 2 cm\(^3\) of normal saline. The remaining two dogs were left untreated and assigned as controls. A blood sample was withdrawn from the MMC group before trauma, on the 14th, and 28th day. Direct microlaryngoscopy (DML) was performed in all animals on the 14th and 28th day to allow clinical evaluation and photodocumentation. Immediately after 28 days, all animals were painlessly euthanized. The larynx, trachea, lung, liver, kidney, and spleen were harvested and studied for possible pathologic changes. Vocal granuloma and glottic webbing were documented in the controls. The inhalation MMC group demonstrated significant inhibition of fibrosis and scar formation. No local or systemic toxic effects were documented. Our study submits the technique of inhalation MMC as a simple, non-invasive adjuvant to the therapeutic tool for the management of laryngeal stenosis.

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1. Introduction

Mitomycin-C (MMC) is an antibiotic derived from the bacterium streptomyces caesipitosus. It has traditionally been used as a systemic chemotherapeutic agent for the
management of disseminated adenocarcinoma, as well as transitional cell tumors of the genitourinary tract [1]. In addition to this antitumor activity, MMC was proved to exert antiproliferative activity by inhibiting fibroblasts [2]. Recently MMC has gained wide acceptance as an adjuvant agent in the management of condition in which fibrosis and lesions reformation is problematic [1–3]. The main experience with the role of topical application of MMC in the management of fibrosis has evolved in the field of ophthalmology where it has been successfully used after pterygium surgery [4,5], in maintaining patency after glaucoma filtration surgery and dacryocystorhinostomy [6], and prevention of scarring after strabismus surgery [7]. In the field of otolaryngology topical application of MMC to the injured airway, mucosa proved to be effective in preventing fibrous tissue and scar formation in animals [8–10] and in human [11,12]. However, topical use has the disadvantage of requiring repeated application that should be conducted under general anesthesia that might not be feasible every time. Based on the simplicity and efficacy of aerosol therapy, this study has pursued the development of an animal model with the objective of assessing the efficacy of inhalation MMC in the prevention of fibrosis and scar formation in injured canine larynx, also to study and address local and systemic toxic effects that might follow the possible absorption of inhaled MMC to the circulation.

2. Material and methods

2.1. Design

Ten adult healthy mongrel dogs weighing from 15 to 20 kg were included in this randomized, prospective study. The animals were anesthetized with a mixture of atropine (0.05 mg/kg), butorphanol (0.05 mg/kg), and xylazine (0.275 mg/kg), and underwent suspension direct microlaryngoscopy (DML). A microlaryngosurgical trauma was created in all animals by removing the mucosa of the anterior commissure and the anterior part of both vocal cords aiming at inducing fibrous tissue webbing and scarring. Eight dogs were randomized to receive a single daily dose of inhalation MMC through an electronic micro-nebulizer using a mixture of 1 cm³ of 0.5 mg/ml MMC and 2 cm³ of normal saline. The remaining two dogs were left untreated and assigned as controls. A blood sample for complete blood picture (CBC), liver function tests, and renal function tests were withdrawn from experimental MMC group before trauma, on the 14th, and 28th day after initial trauma. DML was performed in all animals on the 14th and 28th day to allow clinical evaluation and photodocumentation. On the 28th day, and with the animal still under anesthesia, all animals were painlessly euthanized. The larynx, trachea, lung, liver, kidney, and spleen were harvested and studied histopathologically for possible pathologic changes.

2.2. Histopathology

The specimens were decalcified, sectioned by a rotary microtome (4 um), and stained with hematoxylin and eosin (H&E) and Masson’s trichrome. The H&E sections were used
in the analysis of the inflammatory infiltrate. The trichrome stain was used in the analysis of the connective tissue components where it stains fibroblasts a deep pink, and collagen blue.

3. Results

All animals survived the designed experimental period, and all wounds healed without infection.

3.1. Photodocumentation

Fig. 1 includes representative endoscopic photos of one control dog before and at time of trauma, at 14th and 28th day post-trauma. A definite scar and web formation is evident. Similar analysis photographs in one of the inhalation MMC group demonstrated smooth healing with minimal atrophic changes along the vocal cord with no webbing (Fig. 2).

3.2. Histologic examination

On gross examination after sacrifice, the controls showed vocal granuloma with webbing of anterior commissure (Fig. 3I). The eight larynges exposed to inhaled MMC demonstrated no obvious lesion development, although the area of injury could be identified on close examination (Fig. 3II).

Microscopic evaluation of controls sections revealed near total replacement of the subepithelial tissue with granulation. Characteristic fine, irregular collagen fibers and fibroblasts were seen throughout the submucosa, and were defined by blue and deep pink

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Fig. 1. Endoscopic photodocumentation of clinical course of control animal: I—canine larynx before trauma, II—initial glottic trauma, III—day 14; progressive scarring and fibrosis, IV—day 28; well-defined webbing.
Fig. 2. Endoscopic photodocumentation of clinical course of MMC animal: I—canine larynx before trauma, II—initial glottic trauma, III—day 14; trauma site is identified, IV—day 28; smooth healing.

colour respectively on Masson’s trichrome-stained slides (Fig. 3III). Sections from inhalation MMC group demonstrated definite decrease in fibroblasts collagen fibers with preservation of submucosal glands and the underlying cartilage (Fig. 3IV).

Fig. 3. Gross and microscopic histopathologic study of control and MMC animals: I—web in control dog (W), II—smooth healing in MMC inhalation dog, III—Mason’s trichrome stain of control animal demonstrating near total replacement of submucosa layer by fibrous tissue (FT), IV—Mason’s trachoma stain of MMC animal demonstrating minimal fibrous tissue (FT) with preservation of submucosal glands (G) and underlying cartilage (C).
3.3. Toxic effects of inhaled MMC

No local ulcerations or infections were detected in the MMC group. Blood study: comparison of blood samples withdrawn 14 and 28 days after MMC inhalation revealed comparable CBC, liver function tests, and renal function tests to the pre-inhalation data without significant changes. Only two of the eight dogs demonstrated mild reactive lymphocytosis.

Histologic study of the trachea, lung, liver, kidney, and spleen of the eight dogs in the inhalation MMC group revealed normal findings with no pathologic changes.

4. Discussion

In 1963, Kunitomo and Mori [13] presented the first clinical use of MMC in the prevention and treatment of scar formation in pterygium surgery. Many studies since that time have attempted to investigate the role of mitomycin-C in the management fibrosis and scarring. In the field of otolaryngology, mitomycin-C has been successfully implicated as a topical adjuvant tool in the management of airway stenosis in animals. Recently, our team has documented the efficacy of endoscopic topical application of MMC in the management of laser-induced laryngeal fibrosis in human [12]. However, we have faced the problem of requiring repeated application under general anesthesia. The rationale for administrating MMC through inhalation route in the current study was based on the efficacy and simplicity of aerosol therapy. Literature review revealed that no studies exploring the use of MMC by inhalation route have been previously published. The goal in this first reported use of inhalation MMC was to ascertain whether MMC would reduce fibrosis in an area in which development of scar tissue can lead to significant postoperative complications, and to address the possible local and systemic toxic complications of MMC. We did not perform conventional micro flaps surgery with preservation of the mucosal cover, as the effect of MMC on wound healing might not be as easily appreciated with a lesser injury. The current study demonstrated a notable difference in the gross and microscopic appearance of animals’ glottis with definite inhibition of fibrosis in the MMC group. All glottic trauma in inhalation MMC group healed smoothly compared with the controls. The only unpleasant finding was the ability of identifying the site of trauma on close observation compared to the controls. This might be explained by the minimal atrophic changes of vocal mucosa produced by MMC, consistent with the histological finding of decreasing connective tissue components within the lamina propria. This finding is in accordance with the report of Garrett et al. [14] who demonstrated atrophy of vocal cord treated with topical MMC with negative consequence on the vocal fold vibratory pattern. In the systemic use of mitomycin-C as a chemotherapeutic agent, myelosuppression has been the primary documented toxicity [15]. Other reported toxic effects include interstitial pulmonary fibrosis, hemolytic uraemic syndrome and hepatic disorders [16]. Although acute leukemia was reported in 5% of patients who had been treated with alkylating agents, mainly melphalan [17], MMC has not been accused of inducing this carcinogenic effect. In the current study, we were concerned about the possible absorption of MMC to the...
circulation that would induce the aforementioned toxicity. This study demonstrated promising result as regard the safety of inhalation MMC, as neither local adverse tissue reaction nor systemic toxic effect impairing animals’ health had been detected. These data were confirmed by normal laboratory findings and normal histopathologic sections of systemic organs in animals receiving inhalation MMC. The only exception was the detection of a relative reactive lymphocytosis.

5. Conclusion

In this study, inhalation MMC was proved to be a simple non-invasive effective tool that can be applied without the need for general anesthesia. We submit this technique as an adjunctive tool to the traditionally available therapeutic modalities for the management of laryngotracheal stenosis with the potential of human application. Although no local or systemic toxic effects of MMC were encountered in this experimental study, more randomized trials with prolonged follow-up period should be conducted to evaluate titration to dose response levels and to assess exposure duration, prior to human application.

References

