Histologic Effect of Mitomycin C on Strabismus Surgery in the Rabbit

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PURPOSE. To evaluate the efficiency of mitomycin C (MMC) in limiting the postoperative inflammatory response and scarring after strabismus surgery.

METHODS. A prospective, two-stage, masked, controlled trial was conducted. In the first stage, the inflammatory response at the extraocular muscle reattachment site was increased after inferior rectus recession in eight rabbits. In the second stage, MMC (0.4 mg/ml) was applied during surgery to the eyes of 22 rabbits with inferior rectus recession. As a control, contralateral eyes were treated with saline solution. Seven weeks later, exenteration was performed, and the sites of muscle reattachment were processed for histologic examinations. The sums of the areas of the granulomas in the extraocular muscle reattachment sites of control and treated eyes were compared.

RESULTS. There was no significant inhibitory effect of MMC on the inflammatory response of treated eyes compared with that of control eyes.

CONCLUSIONS. The intraoperative use of MMC (0.4 mg/ml) was not effective in controlling the postoperative inflammatory response in rabbit eyes after extraocular muscle surgery. These data do not support the hypothesis that MMC reduces postoperative adhesions after strabismus surgery.


Mechanical contributions to the success of strabismus surgery include the absence of excessive fibrous tissue. Recently, the usefulness of mitomycin C (MMC), an antimetabolic agent, has been tested experimentally to reduce postoperative adhesions in strabismus surgery. Cruz reported reduced formation of adhesions and postoperative scarring when MMC was used during surgery. In contrast, Brooks et al. found no significant reduction in scarring and restriction of motility with MMC. Similarly, Ohtsuki et al. found MMC to be ineffective in preventing muscle migration, a supposed result of vigorous healing response.

The objective of this study was to further examine the influence of MMC on postoperative inflammatory response and scarring in strabismus surgery. This was done by performing a semiquantitative histologic analysis of the effect of MMC after inferior rectus (IR) recession in rabbits.

MATERIALS AND METHODS

Experimental Design
This was a prospective, two-stage, masked, controlled animal trial. In the first stage, inflammatory response surrounding the muscle insertion site was enhanced to allow a better evaluation of the supposed inhibitory effect of MMC on fibrosis, and in the second stage the effect of MMC was examined.

Animals
Thirty New Zealand White rabbits (2–3 kg) were used. Each rabbit was anesthetized with an intramuscular injection of ketamine HCl (40 mg/kg, Ketalar; Parke-Davis, Guarulhos, Brazil) and xylazine (4 mg/kg, Rompun; Bayer, São Paulo, Brazil) before surgery and orbit exenteration, which were followed by euthanasia. The procedures followed the recommendations of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the policies in the Guidelines for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, rev. 1985).

First Stage: Increasing Inflammatory Response
Eight rabbits had IR muscle of both eyes recessed 2 mm, as described elsewhere. In one randomly chosen eye of each pair (treated eye), the procedure included cauterization of a 1-cm² area of the muscle’s inferior surface and the underlying sclera; the other eye served as a control. Seven weeks later, the orbits were exenterated with care, to ensure that the IR junction was not disrupted, and the eyes were then processed for histologic analysis. The extent of the inflammatory response was accessed histologically followed by comparison of the treated and control eyes.

Second Stage: MMC Testing
Twenty-two rabbits underwent 2-mm recession with cauterization in both eyes as described for the treated eyes in the first stage. After cauterization, a sponge soaked in MMC (0.4 mg/ml, Mitocin; B-MS, São Paulo, Brazil) was placed for 3 minutes.
between the muscle and the sclera in a randomly chosen eye in each pair. The area was then irrigated with 200 ml normal saline. A sponge soaked in balanced salt solution was placed on the contralateral eye as a control. The orbits were exenterated 7 weeks later, as described for the first stage.

**Histologic Preparation and Quantification of Inflammatory Response**

The eyes were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Three consecutive sagittal sections for each eye were cut perpendicularly to the line of the postsurgical muscle insertion. The sections were stained with Masson trichrome, and the intensity of the inflammatory response was semiquantified by summing the area of each granuloma. The area (A) of the granuloma was estimated using the formula for the area of an ellipse: $A = \pi (a \cdot b)$, where $a$ is major diameter and $b$ is minor diameter.

The diameters of the granulomas were measured using a light optical system supplied with a graduated eyepiece micrometer and ×10 or ×40 objective (for final magnification of 100 or 400).

**Statistical Analysis**

Group differences in total granuloma area were compared using the Mann–Whitney test with $P < 0.05$ indicating significance.

**RESULTS**

**First Stage**

Histologic analysis revealed a significant difference ($P = 0.01$) in the total areas of granulomas between the control eyes and those treated with cauterezation (Table 1). The data for rabbit 6 were not considered, because the animal developed an ocular infection.

**Second Stage**

Table 2 shows the total area of the granulomas for 19 rabbits. Although a few MMC-treated eyes unexpectedly showed a more prominent inflammatory response than the control eyes (Fig. 1), there was no significant difference ($P = 0.23$) in areas

![Figure 1: Histologic appearance of the IR muscle in a control eye (A) and in an eye treated with 0.4 mg/ml MMC (B). Compared with the control, the treated eye shows a more prominent inflammatory response, with more fibrous tissue around the suture materials (*asterisks*). Masson trichrome; original magnification, ×64.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932910/)
of granulomas. Data from two rabbits that died during this stage and one that developed an ocular infection were excluded from the final analysis.

**DISCUSSION**

This study was designed to examine further the tissue effects of MMC in rabbits after extraocular muscle surgery, particularly with regard to the effect of this compound on scarring. We have created a new way to quantify the inflammatory response of the muscle-tendon–scleral interface. We showed that cautery to the muscle and sclera increases the area of the subsequent granuloma at the site of muscle reattachment. This provides a new animal model for measuring that inflammation.

Previous studies using similar animal models have emphasized the clinical effects of this antimetabolite, which were evaluated indirectly based on tensile strength or directly by dissection exploration of postoperative adhesions. The general histologic characteristics described for the muscle insertion sites agreed with the clinical findings in these investigations, although the histologic analysis was only qualitative with no statistical evaluation.

In the present study, an initial qualitative histologic analysis 7 weeks after surgery unexpectedly revealed a more prominent granulomatous inflammation in the MMC-treated eyes of a few animals, compared with the controls (Fig. 1). However, there was no significant difference between areas of granulomas in treated and control eyes. This finding highlights the importance of using a sensitive histologic method for evaluating the tissue reaction.

Although there were no prominent signals of fibrosis present in the examined sections, fibrosis is a common and important complication in granulomatous inflammation. This suggests that the amount of resultant fibrosis would be proportional to the severity of the previous inflammation.

Thus, contrary to other reports, and according to this new animal model for measuring granulomatous inflammation, our data do not support the hypothesis that MMC would be effective in reducing postoperative scarring after strabismus procedure in rabbits. However, this study could not offer conclusions about other inflammatory interactions. The scar tissue of strabismus surgery involves conjunctiva, Tenon’s fascia, muscle-tendon, sclera, orbital fat, and probably other factors.

Clearly, more animal studies are needed to investigate other aspects of tissue adherence and to establish or refute the usefulness of MMC in extraocular muscle surgery.

**Acknowledgments**

The authors thank everyone in the Faculty of Medical Science, particularly from the departments of Experimental Surgery, Anatomic Pathology, and Statistics, for their contributions.

**References**