

Effect of Mitomycin C Dissolved in Reversible Thermo-Setting Gel on the Outcome of Filtering Surgery in the Rabbit

Koji Ichien, Tetsuya Yamamoto, Akira Sawada, Yoshiaki Kitazawa, Reiko Shiraki and Mutsuyoshi Yoh

Department of Ophthalmology, Gifu University School of Medicine, Gifu, Japan

Purpose: Based on our previous report that showed enhanced transfer of mitomycin C to the sclera and the conjunctiva by dissolving the agent into a reversible thermo-setting gel, we conducted a study to investigate the efficacy of mitomycin C gel in the rabbit.

Methods: We subconjunctivally injected 0.1 mL of the mitomycin C gel solution containing several different amounts of the agent. Trephination was performed in the injected region 24 hours later. Intraocular pressure measurement, photography, and ultrasound biomicroscopic examination of the filtering bleb were conducted 1, 2, and 4 weeks postoperatively.

Results: The gel containing $3.0 \ \mu g$ or more mitomycin C significantly enhanced bleb formation and reduction in the intraocular pressure.

Conclusions: The reversible thermo-setting gel seems to help the filtering bleb to survive in the rabbit and deserves further investigation as a new method of mitomycin C application. **Jpn J Ophthalmol 2000;44:101–105** © 2000 Japanese Ophthalmological Society

Key Words: Filtering bleb, glaucoma filtering surgery, intraocular pressure, mitomycin C, reversible thermo-setting gel.

Introduction

The high success rate in controlling intraocular pressure (IOP) by adjunctive antiproliferative agents,^{1–7} especially mitomycin C, has changed the position of filtering surgery in the management of glaucoma. Mitomycin C is currently administered in the majority of cases intraoperatively, by applying the antiproliferative with a reservoir, such as a surgical sponge, for 3–5 minutes, followed by copious irrigation. This administration method, however, has several disadvantages that include variable target tissue concentrations,⁸ need of copious irrigation to eliminate the toxic effect of the antiproliferative agent,

possible penetration of the agent into the anterior chamber, and difficulty in determining dosage. It is known that only one sixth of the mitomycin C applied during trabeculectomy is released from surgical sponges to the target tissues in the clinical setting.⁹

To administer the minimum required dosage of mitomycin C, we have already reported on the effect of a reversible thermo-setting gel as a reservoir for mitomycin C and an increased concentration of the antiproliferative in the target ocular tissues when administered with the gel.¹⁰ The gel is composed of 1.4% methylcellulose, 3.5% citric acid, and 2% polyethylene glycol; it has gel form/solution form convertibility, depending on the temperature. In the previous experiment, we subconjunctivally injected 0.1 mL of the sterile mitomycin C solution containing 0.22 μ g, 2.9 μ g, or 28 μ g of the agent using a 30-gauge needle into the temporal superior quadrant of both eyes of 30 albino rabbits. The rabbits were sac-

Received: October 28, 1998

Correspondence and reprint requests to: Tetsuya YAMA-MOTO, MD, Department of Ophthalmology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu-shi, Gifu-ken 500-8705, Japan

rificed at predetermined time points after the mitomycin C gel injection. Mitomycin C concentrations in the conjunctiva and the sclera were measured using a high performance liquid chromatography (HPLC) method. In both ocular tissues, the mitomycin C concentration decreased over time and fell below the minimum detectable level of concentration 24 hours after the injection. The tissue concentration change after the 2.9-µg application with the gel was similar, in both the conjunctiva and the sclera, to that after the 200-µg application of mitomycin C with surgical sponges, which is practiced in clinical settings. Thus, the mitomycin C application using the thermo-setting gel at 1/70 of the dosage could maintain a target tissue concentration of the agent similar to the concentration attained in the method currently in clinical use.8

Based upon the above-mentioned pharmacokinetic analysis, we studied the effect of mitomycin C dissolved in the gel on IOP and the appearance of the filtering bleb in a filtering surgery model in the rabbit.

Materials and Methods

Thirty-six New Zealand albino rabbits weighing about 2.0 kg were used in the experiment. The animals were divided into six treatment groups, each of which comprised six rabbits. In three of the six treatment groups, we subconjunctivally injected 0.1 mL of sterile mitomycin C dissolved in a reversible thermo-setting gel (Wakamoto Pharmaceuticals, Tokyo) containing 0.3 μ g, 3.0 μ g, or 30 μ g of the agent, respectively, in the superotemporal quadrant of both eyes of each rabbit 24 hours before surgery (Figure 1).



Figure 1. Injection of thermo-setting gel containing mitomycin C.



Figure 2. Anterior segment photography 4 weeks after an injection of $30 \ \mu g$ of mitomycin C gel.

The remaining three groups served as the gel-treated group, 30-µg mitomycin C aqueous solution group, and untreated-no injection group, respectively. The mitomycin C-free thermo-setting gel was injected in the gel-treated group as done in the mitomycin C gel groups; the aqueous solution containing 30 µg mitomycin C was injected similarly in the 30-µg mitomycin C-aqueous solution group; no subconjunctival injection was done in the untreated-no injection group. Trephination was performed 24 hours later in the superotemporal region in all eyes by two of the authors (TY and KI), using a 1-mm-diameter trephine with a fornix-based conjunctival flap. Peripheral iridectomy was done, and the conjunctival wound was closed with an interrupted 10-0 nylon suture. After the surgery, 0.3% ofloxacin ophthalmic ointment was instilled in each eye. No cycloplegic or corticosteroid was given postoperatively.

Intraocular pressure IOP was measured with a pneumatonometer (Alcon Applanation Pneumatonograph[™]; Alcon, Ft. Worth, TX, USA) under topical anesthesia with oxybuprocaine before and 24 hours after subconjunctival injection, and 1, 2, and 4 weeks postoperatively. Slit-lamp examination was also conducted at the same time. Photography and ultrasound biomicroscopic examination of the filtering blebs were done 1, 2, and 4 weeks postoperatively under general anesthesia with 0.0875 mL of 0.05% ketamine and 0.0125 mL of 2% xylazine. The ultrasound biomicroscopic examination was conducted with an ultrasound biomicroscope (model 840; Humphrey Instruments, San Leandro, CA, USA). The measurement conditions were: 80 dB of the gain, 5 dB of the gain compensation, and 5.0×5.0 mm of the field of view. With an eye cup filled with ethyl-



Figure 3. Ultrasound biomicroscopic image of filtering bleb in same eye as in Figure 2. Height of bleb is indicated.

cellulose and physiological saline, the probe with a transducer scanned the entire region of the filtering bleb along the different meridians. During the scanning, three to six ultrasound biomicroscopic images for each eye were taken and stored in the hard drive installed in the apparatus. The bleb height was defined as the length of the longest line, from the surface of the sclera to the surface of the bleb, of the lines perpendicular to the sclera. All images were evaluated by one of us (KI) after the completion of the study.

The Wilcoxon signed-rank test was used to evaluate the difference between the preoperative and postoperative IOPs. Analysis of variance (ANOVA) with Fisher's protected least significant difference was applied to evaluate the change in bleb height. A change of P < .05 was considered statistically significant.

Results

Avascular cystic blebs were observed after the glaucoma-filtering surgery in all mitomycin C-treated groups (Figures 2, 3). The IOP significantly fell at 1 week postoperatively in the 30- μ g mitomycin C-gel group, at 2 and 4 weeks postoperatively in the 30- μ g mitomycin C-aqueous solution group and the 3- μ g mitomycin C gel group when compared with the baseline value (Figure 4). An intergroup comparison showed that the bleb was significantly higher at 2 weeks in the 30- μ g mitomycin C gel group, at 1 and 2 weeks in the 3- μ g mitomycin C gel group, and at 2 and 4 weeks in the 30- μ g mitomycin C gel group.





Figure 4. Changes in intraocular pressure. Circles with hatched lines: $30-\mu g$ mitomycin C-gel group; Squares with hatched lines: $3-\mu g$ mitomycin C-gel group; triangles with hatched lines: $0.3-\mu g$ mitomycin C-gel group; circles with solid lines: $30-\mu g$ mitomycin C-aqueous solution group; squares with solid lines: gel-treated group; triangles with solid lines: untreated- no-injection group. *P < .05, Wilcoxon signed-rank test.

Figure 5. Changes in bleb height. Circles with hatched lines: $30-\mu$ g mitomycin C-gel group; squares with hatched lines: $3-\mu$ g mitomycin C-gel group; triangles with hatched lines: $0.3-\mu$ g mitomycin C-gel group; circles with solid lines: $30-\mu$ g mitomycin C-aqueous solution group; squares with solid lines: gel-treated group; triangles with solid lines: untreated- no-injection group. *P < .05 (ANOVA, Fisher's PLSD).

Discussion

Excessive scarring at the site of surgery is the primary cause of failure of glaucoma filtering surgery.^{11,12} Intraoperative application of antimetabolites like mitomycin C and 5-fluorouracil or postoperative subconjunctival injection of the latter has gained popularity for suppressing the scarring process.^{1-7,13} The postoperative injection of 5-fluorouracil, however, must be done repeatedly, and is reported to frequently cause corneal epithelial defects.¹³ On the other hand, the present method of administering mitomycin C requires an excessive quantity of the agent and copious irrigation after application. In addition, mitomycin C itself has strong cytotoxicity;¹⁴⁻¹⁸ 6-12 months after a subconjunctival injection of the agent, it was shown that mitomycin C caused prolonged, dosedependent pathologic changes in the ciliary body in rabbits.¹⁸ These data suggest that the antiproliferative should be administered in the minimum required dosage. To achieve this goal, we investigated the effect of the thermo-setting gel as a reservoir for mitomycin C. Our previous study indicated that 3 µg or 30 µg of mitomycin C dissolved in the gel brought about a tissue concentration of the agent in the conjunctiva and the sclera similar to that with the conventional intraoperative application of 200 µg of mitomycin C. Hence, we chose the three dosages, ie, $0.3 \mu g$, $3 \mu g$, and $30 \mu g$, in the current experiment. The IOP dropped significantly with administration of both the 3-µg and 30-µg mitomycin C-gel formulations. Ultrasound biomicroscopy also demonstrated that the filtering bleb remained higher with the $3-\mu g$ and 30-µg mitomycin C-gel formulations. The bleb height is known to be closely correlated with bleb function.¹⁹ The results indicate that the markedly lower dose of mitomycin C dissolved in the gel has an antiproliferative activity similar to the conventional application of 200 µg of mitomycin C. Thus, it is possible to reduce the dosage of the agent by dissolving it in the reversible thermo-setting gel.

Another advantage of using the gel as a reservoir is to restrict the area of distribution of the agent. In the current experiment, the effect of $30-\mu g$ mitomycin C aqueous solution was about as good as with the thermo-setting gel in terms of IOP control and bleb formation. However, it is highly probable that the aqueous solution is distributed more extensively than the gel; thus, it may spread the cytotoxic effects of mitomycin C to ocular tissues beyond the targeted areas.

Disadvantages of the gel include some difficulty in preparation and a mild inflammatory reaction at the injection site. The toxic effect of the mitomycin gel has not yet been determined histologically. In an independent experiment, however, our group investigated the effect of the agent on aqueous flare in the rabbit eye. We found that the new method reduces aqueous flare significantly, when compared with a method that mimicked the current clinical dosing (Nishimura K et al; unpublished data, presented at ARVO 1998). Therefore, it is probably that the mitomycin C gel does not affect the anterior segment of the eye as much.

In conclusion, the reversible thermo-setting gel seems to facilitate filtration in the rabbit and deserves further investigation as a novel method of mitomycin C administration.

This paper was published in part in the Nippon Ganka Gakkai Zasshi (J Jpn Ophthalmol Soc) 1999;103:277–81. It appears here in a modified form after peer review and editing for the Japanese Journal of Ophthalmology. The authors thank Wakamoto Pharmaceuticals for providing the thermo-setting gel.

References

- 1. Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. Ophthalmology 1991;98:317–21.
- Kitazawa Y, Kawase K, Matsushita H, Minobe M. Trabeculectomy with mitomycin. A comparative study with fluorouracil. Arch Ophthalmol 1991;109:1693–8.
- Skuta GL, Beeson CC, Higginbotham EJ, Lichter PR. Intraoperative mitomycin versus postoperative 5-fluorouracil in high-risk glaucoma filtering surgery. Ophthalmology 1993; 116:72–8.
- 4. Mermoud A, Salmon JF, Murray ADN. Trabeculectomy with mitomycin C for refractory glaucoma in blacks. Am J Oph-thalmol 1993;116:72–3.
- Kitazawa Y, Suemori-Matsushita H, Yamamoto T, Kawase K. Low-dose and high-dose mitomycin C trabeculectomy as initial surgery in primary open-angle glaucoma. Ophthalmology 1993;100:1624–8.
- Yamamoto T, Ichien M, Suemori-Matsushita H, Kitazawa Y. Trabeculectomy with mitomycin C for normal-tension glaucoma. J Glaucoma 1995;4:158–63.
- Katz GJ, Higginbotham EJ, Lichter PR, et al. Mitomycin C versus 5-fluorouracil in high-risk glaucoma filtering surgery. Extended follow-up. Ophthalmology 1995;102:1263–9.
- Kawase K, Matsushita H, Yamamoto T, Kitazawa Y. Mitomycin concentration in rabbit and human ocular tissue after topical administration. Ophthalmology 1992;99:203–7.
- Yamamoto T, Kitazawa Y. Residual mitomycin C dosage in surgical sponges removed at the time of trabeculectomy. Am J Ophthalmol 1994;117:672–3.
- Ichien K, Yamamoto T, Kitazawa Y, Oguri A, Ando A, Kondo Y. Mitomycin C dissolved in a reversible thermo-setting gel: target tissue concentrations in the rabbit eye. Br J Ophthalmol 1997;81:72–5.
- Teng CC, Chi HH, Katzin HM. Histology and mechanism of filtering operations. Am J Ophthalmol 1959;47:16–34.
- 12. Addicks, EM, Quigley HA, Green WR, Robin AL. Histologic

characteristics of filtering blebs in glaucomatous eyes. Arch Ophthalmol 1983;101:795–8.

- 13. The Fluorouracil Filtering Surgery Study Group. Three-year follow-up of the fluorouracil filtering surgery study. Am J Ophthalmol 1993;115:82–92.
- 14. Kitazawa Y, Yamamoto T. The risk profile of mitomycin C glaucoma surgery. Curr Opin Ophthalmol 1994;5:105–9.
- 15. Fourman S. Scleritis after glaucoma filtering surgery with mitomycin C. Ophthalmology 1995;102:1569–71.
- 16. Nuyts RMMA, Felten PC, Pels E, et al. Histopathologic effects of mitomycin C after trabeculectomy in human glauco-

matous eyes with persistent hypotony. Am J Ophthalmol 1993;118:225-37.

- 17. Prata JA, Minckler DS, Baerveldt G, Lee PP, Heuer DK. Site of mitomycin C application during trabeculectomy. J Glaucoma 1994;3:296–301.
- Mietz H, Addicks K, Bloch W, Krieglstein GK. Long-term intraocular toxic effects of topical mitomycin C in rabbit. J Glaucoma 1996;5:325–33.
- Yamamoto T, Sakuma T, Kitazawa Y. An ultrasound biomicroscopic study of filtering blebs following mitomycin trabeculectomy. Ophthalmology 1995;102:1770–76.