

Mitomycin C dissolved in a reversible thermosetting gel: target tissue concentrations in the rabbit eye

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Abstract

Aims—To determine whether a new, reversible thermosetting gel enhances mitomycin C transfer to target ocular tissues in the rabbit eye.

Methods—A 0.1 ml solution of mitomycin C containing 0.22 µg, 2.9 µg, or 28 µg of the agent dissolved in a reversible thermosetting gel consisting of methylcellulose, citric acid, and polyethylene glycol was injected subconjunctivally in 30 New Zealand albino rabbits. Scleral and conjunctival tissues were excised at 0.5, 1, 2, 4, or 24 hours after the injection and mitomycin C concentrations in these tissues were determined by high performance liquid chromatography. The concentration over time was approximated to a single exponential curve, and initial mitomycin C concentrations, time constants, and half life values were determined. Finally, the areas under the curves (AUCs) between 0.5 and 24 hours were calculated.

Results—The mitomycin C concentrations in the target tissues were dose dependent and decreased rapidly over 24 hours. Both the initial mitomycin C concentrations as well as AUCs in these eyes treated with mitomycin C, dissolved in a reversible thermosetting gel, were higher than those in eyes treated similarly in a previous study in which the gel was not used.

Conclusion—Applied subconjunctivally in the rabbit eye, mitomycin C dissolved in the reversible thermosetting gel enhanced transfer of the agent to the sclera and the conjunctiva.

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Mitomycin C (MMC) has been demonstrated to be an effective adjunct in glaucoma filtering surgery.¹⁻⁷ However, this antiproliferative agent, a potent inhibitor of DNA synthesis, can cause severe adverse effects if the dosage is too high. For example, scleromalacia and scleral perforation have been reported following MMC application after pterygium surgery.⁸⁻¹⁰ Such scleral complications have not been reported clinically except for five cases of moderate scleritis that developed following a 3 minute application of 0.05% solution of the agent during inferior trabeculectomy.¹¹ How-

ever, histopathological changes of the sclera such as disorganisation of collagen were reported in human trabeculectomised eyes.¹²

As an adjunct to glaucoma filtering surgery, MMC is typically applied intraoperatively with surgical sponges saturated with 0.04% of the agent, followed by copious irrigation. The amount of MMC transferred to target ocular tissues by this method varies considerably and in any case is only a small fraction of that applied. Yamamoto and Kitazawa¹³ reported that in clinical settings a mean of 83.2% (SD 18.0%) of the MMC applied during trabeculectomy remained unreleased in the surgical sponges. Kawase *et al*¹⁴ demonstrated in rabbits that copious irrigation of the wound immediately after MMC administration reduced its concentration in the sclera and the conjunctiva to as little as one tenth of that remaining in these tissues without such irrigation.

Given its narrow safety margin and low therapeutic index, reducing MMC's adverse effects while preserving its pharmacological activity calls for carefully quantified administration of the minimum required dosage. We tested such a method involving the use of a reversible thermosetting gel, recently developed in Japan. Comprised of 1.4% methylcellulose, 3.53% citric acid, and 2% polyethylene glycol, this gel changes from a gel to a sol depending on the temperature.¹⁵ The viscosity is 26.2 cp/s at 30°C, 42.0 cp/s at 32°C, and over 100 cp/s at 34°C. The pH is 7.8. We conducted the current study in order to determine whether this gel enhances MMC transfer to target ocular tissues in the rabbit eye. If it does, the amount of MMC needed to facilitate filtration might be reduced, thereby decreasing the risk of adverse reactions.

Materials and methods

Three concentrations of MMC in a reversible thermosetting gel (Wakamoto Pharmaceutical Co Ltd, Tokyo, Japan), measured by high performance liquid chromatography (HPLC), were provided by the manufacturer: 2.2 µg/ml ($2.2 \times 10^{-4}\%$), 29 µg/ml ($2.9 \times 10^{-3}\%$), and 280 µg/ml ($2.8 \times 10^{-2}\%$). Immediately before the experiment, to facilitate liquidisation, a predetermined volume of cold sterile water was added to the MMC preparation, which was then placed on ice cold water.

Thirty New Zealand albino rabbits weighing about 2 kg were used. All of the experiments were conducted in accordance with the ARVO

Table 1 Mitomycin C (MMC) concentration changes in ocular tissues ($\mu\text{g/g}$, mean (SD))

Ocular tissue measured	MMC dosage applied (μg)	Hours after MMC application				
		0.5	1	2	4	24
Conjunctiva	0.22	0.51 (0.18)	0.17 (0.18)	0.02 (0.02)	Trace*	Trace
	2.9	3.62 (3.25)	1.50 (0.86)	0.18 (0.13)	Trace	Trace
	28	62.15 (20.71)	21.83 (4.29)	5.31 (5.22)	0.18 (0.16)	Trace
Sclera	0.22	0.13 (0.14)	0.06 (0.07)	0.01 (0.01)	Trace	Trace
	2.9	0.61 (0.48)	0.52 (0.45)	0.07 (0.05)	Trace	Trace
	28	23.18 (6.80)	10.25 (3.41)	1.36 (0.93)	0.10 (0.10)	Trace

*Less than the minimum detectable mitomycin C concentration—that is, $5 \times 10^{-3} \mu\text{g/g}$.¹⁴

Table 2 Pharmacokinetic analyses of mitomycin C (MMC) concentration changes in the conjunctiva

Method of MMC application	MMC dosage applied (μg)	Initial concentration ($\mu\text{g/g}$)	Time constant (h^{-1})	Half life (h)	AUC \dagger ($\mu\text{g h/g}$)
Current study with reversible thermosetting gel	0.22	1.23	2.40	0.29	0.40
	2.9	5.26	1.78	0.39	3.2
	28	140.28	1.80	0.39	57.0
Subconjunctival injection with sterile water*	2	1.05	2.28	0.31	0.29
	20	5.76	2.33	0.30	1.03
	200	158.0	3.92	0.18	23.49
Application with sponges for 5 minutes and copious irrigation*	200	12.6	2.33		
				0.30	5.18

*Numbers are quoted from or calculated using data that appeared elsewhere.¹⁴

\dagger Area under the curve between 0.5 and 24 hours.

Resolution on the Use of Animals in Research. After administering topical anaesthesia with 0.4% oxybuprocaine, we subconjunctivally injected 0.1 ml (0.22 μg , 2.9 μg , or 28 μg) of the sterile MMC solution using a 30 gauge needle into the temporal superior quadrant of both eyes of the rabbits; the same dose of MMC was used in both eyes of each rabbit. The needle was inserted 10 mm away from the limbus and the injection raised about 8×8 mm sized circular area of the conjunctiva. The rabbits were killed by injection of an excessive amount of 5% pentobarbitone at 0.5, 1, 2, 4, or 24 hours after the MMC injections. Then we excised a section of conjunctiva and sclera measuring 10×10 mm from each injected site. Aqueous and vitreous specimens were not obtained. Four eyes of two rabbits were used for each designated time point and each drug concentration. The excised tissues were immediately frozen at -80°C and MMC concentrations were measured using the same HPLC method we have described elsewhere.¹⁴ The minimum MMC concentration detectable by this method was $5 \times 10^{-3} \mu\text{g/g}$.¹⁴

The concentration over time was approximated to the single exponential curve of $C_t = C_0 \exp(-at)$, where C_t was the MMC concentration ($\mu\text{g/g}$) at time t , C_0 the MMC concentration at time 0, a the time constant of MMC disappearance (h^{-1}), and t the time after

MMC administration. Initial MMC concentrations, time constants and half life values were determined using this equation. Additionally, the areas under the curves (AUCs) between 0.5 and 24 hours were calculated.

As a control, we used data from our previous experiments,¹⁴ in which three different concentrations of MMC solutions were applied subconjunctivally, and 0.2 mg MMC was applied with surgical sponges and the ocular surface copiously irrigated 5 minutes later. For the current and previous experiments,¹⁴ the same standard MMC solution prepared just before the HPLC was used for calibration.

Results

The concentrations of MMC in the target tissues are shown in Table 1 and in Figure 1A and B. The concentration at each time point correlated with the dosage injected: the greater the dose, the greater the concentration and vice versa.

The initial MMC concentrations, time constants, and half life values calculated using the exponential equation and AUCs are listed in Tables 2 and 3. The time constants ranged from 1.78 to 2.40 h^{-1} for the conjunctiva, and 1.31 to 2.03 h^{-1} for the sclera. The half life values ranged from 0.29 to 0.39 hours for the conjunctiva, and 0.28 to 0.34 hours for the sclera. Correcting for the different dosages

Table 3 Pharmacokinetic analyses of mitomycin C (MMC) concentration changes in the sclera

Method of MMC application	MMC dosage applied (μg)	Initial concentration ($\mu\text{g/g}$)	Time constant (h^{-1})	Half life (h)	AUC \dagger ($\mu\text{g h/g}$)
Current study with reversible thermosetting gel	0.22	0.10	1.31	0.53	0.11
	2.9	0.56	1.49	0.47	0.80
	28	66.68	2.03	0.34	22.42
Subconjunctival injection with sterile water*	2	0.12	1.54	0.45	0.14
	20	0.62	1.82	0.38	0.14
	200	20.0	3.41	0.20	4.23
Application with sponges for 5 minutes and copious irrigation*	200	3.16	2.19	0.32	0.67

*Numbers are quoted from or calculated using data that appeared elsewhere.¹⁴

\dagger Area under the curve between 0.5 and 24 hours.

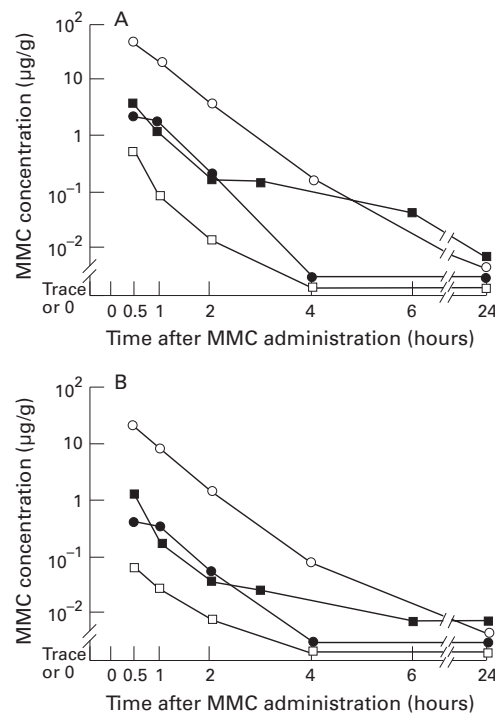


Figure 1 Mitomycin C (MMC) concentrations in the conjunctiva (A) and in the sclera (B) after a single subconjunctival injection of the agent dissolved in a reversible thermosetting gel. The open squares show the 0.22 µg dose; the closed circles the 2.9 µg dose, and the open circle the 28 µg dose. The closed squares show the tissue concentrations following an application of 200 µg of the agent with sponges for 5 minutes and copious irrigation.¹⁴

used, the initial MMC concentrations as well as AUCs in the eyes treated with the MMC solution dissolved in the reversible thermosetting gel were higher than those in the eyes in our previous study in which no gel was used.

Discussion

The reversible thermosetting gel we used becomes a sol at relatively low temperature and turns into a gel as the temperature nears body temperature. The constituents—methylcellulose, citric acid, and polyethylene glycol—have been proved to be non-toxic to ocular tissues.¹⁶ The compound is now being tested in several clinical trials in Japan as a potential solvent for timolol maleate, and it has already been shown that once daily instillation of 0.5% timolol maleate prepared with the gel is as effective as twice daily use of 0.5% timolol aqueous solution (unpublished data). An animal study revealed that the AUCs of tissue concentration of timolol in the cornea, the aqueous, and the iris and the ciliary body increased by 1.8 to 2.2 times with 50 µl of 0.25% timolol maleate prepared with the gel compared with the same amount of the same concentration of aqueous solution (unpublished data). These favourable effects possibly derive from the increased drug–corneal contact time made possible by the gel. That is, it is likely that subconjunctivally injected MMC dissolved in the gel remains longer subconjunctivally and therefore that the concentration of MMC in the target tissues is higher than

when MMC dissolved in saline or water is injected subconjunctivally.

The current study demonstrated that a subconjunctival injection of 0.1 ml of $2.9 \times 10^{-3}\%$ MMC solution (MMC dosage, 2.9 µg) dissolved in the reversible thermosetting gel allowed maintenance of an MMC concentration in the sclera and conjunctiva during the first 24 hours at a level similar to that found in our previous study in eyes in which 0.2 mg of the agent were applied with surgical sponges and the wound copiously irrigated 5 minutes later.¹⁴ The MMC concentration in the conjunctiva detected in the current study was higher than that in the sclera. This finding is compatible with that in our previous study¹⁴ where MMC aqueous solution was used and in another study using 5-fluorouracil,¹⁷ although it is noted that a portion of MMC injected still remained unreleased. The AUCs of the eyes treated using the gel solution were 62% and 119% of those of the eyes in our previous study in the conjunctiva and sclera, respectively, even though the MMC dosage we used in the current study was only 1.45% of that used in that earlier study. Thus, the MMC dose transferred to the target ocular tissues by injecting it in a gel solution was about 69 times (200 µg versus 2.9 µg) greater than that transferred by the usual current clinical method. Moreover, compared with MMC application via subconjunctival injections with sterile water,¹⁴ the tissue concentrations as well as the AUCs were some 10 times higher.

One, though not the only, reason why MMC is not topically applied or subconjunctivally injected in glaucoma filtering surgery is to avoid contact with ocular and periocular tissues of concentrations of the agent high enough to cause adverse effects such as those found after its use following pterygium surgery.^{8–10} However, a great disadvantage of the prevalent clinical method of MMC administration is that far greater dosages than are actually needed in the target ocular tissues must be administered. Also, the dose of MMC released to the ocular tissues is highly variable when it is applied intraoperatively, because the amount of the agent that remains unreleased is quite variable.¹³ In the current study, tissue MMC concentration was as variable as that following injection of aqueous MMC solution in the previous experiment.¹⁴

We showed that a smaller amount of MMC is needed to maintain the tissue concentration of the agent by dissolving it into a thermosetting gel. Although the effects of this method of administering MMC on filtering bleb formation and complications must be further investigated in animal filtration models and we have to administer it preoperatively, a smaller amount of MMC in gel may prove to be as least as effective as the current method.

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1 Palmer SS. Mitomycin as adjunct chemotherapy with trabeculectomy. *Ophthalmology* 1991;98:317–21.

- 2 Kitazawa Y, Kawase K, Matsushita H, Minobe M. Trabeculectomy with mitomycin. A comparative study with fluorouracil. *Arch Ophthalmol* 1991;**109**:1693–8.
- 3 Skuta GL, Beeson CC, Higginbotham EJ, Lichter PR, Musch DC, Bergstrom TJ, *et al*. Intraoperative mitomycin versus postoperative 5-fluorouracil in high-risk glaucoma filtering surgery. *Ophthalmology* 1992;**99**:438–44.
- 4 Mermoud A, Salmon JF, Murray ADN. Trabeculectomy with mitomycin C for refractory glaucoma in blacks. *Am J Ophthalmol* 1993;**116**:72–8.
- 5 Kitazawa Y, Suemori-Matsushita H, Yamamoto T, Kawase K. Low-dose and high-dose mitomycin trabeculectomy as an initial surgery in primary open-angle glaucoma. *Ophthalmology* 1993;**100**:1624–8.
- 6 Yamamoto T, Ichien M, Suemori-Matsushita H, Kitazawa Y. Trabeculectomy with mitomycin C for normal-tension glaucoma. *J Glaucoma* 1995;**4**:158–63.
- 7 Katz GJ, Higginbotham EJ, Lichter PR, Skuta GL, Musch DC, Bergstrom TJ, *et al*. Mitomycin C versus 5-fluorouracil in high-risk glaucoma filtering surgery. Extended follow-up. *Ophthalmology* 1995;**102**:1263–9.
- 8 Hayasaka S, Noda S, Yamamoto Y, Setogawa T. Postoperative instillation of low-dose mitomycin C in the treatment of primary pterygium. *Am J Ophthalmol* 1988;**106**:715–8.
- 9 Rubinfeld RS, Pfister RR, Stein RM, Foster CS, Martin NF, Stoleru S, *et al*. Serious complications of topical mitomycin-C after pterygium surgery. *Ophthalmology* 1992;**99**:1647–54.
- 10 Kitazawa Y, Yamamoto T. The risk profile of mitomycin C in glaucoma surgery. *Curr Opin Ophthalmol* 1994;**5**:105–9.
- 11 Fourman S. Scleritis after glaucoma filtering surgery with mitomycin C. *Ophthalmology* 1995;**102**:1569–71.
- 12 Nuyts RMMA, Felten PC, Pels E, Langerhorst CT, Geijssen HC, Crossniklaus HE, *et al*. Histopathologic effects of mitomycin C after trabeculectomy in human glaucomatous eyes with persistent hypotony. *Am J Ophthalmol* 1994;**118**:225–37.
- 13 Yamamoto T, Kitazawa Y. Residual mitomycin C dosage in surgical sponges removed at the time of trabeculectomy. *Am J Ophthalmol* 1994;**117**:672–3.
- 14 Kawase K, Matsushita H, Yamamoto T, Kitazawa Y. Mitomycin concentration in rabbit and human ocular tissues after topical administration. *Ophthalmology* 1992;**99**:203–7.
- 15 Kondo Y, Kono Y, Okada K, Yamamoto T, Misao T, Katagiri Y, *et al*. Novel timolol ophthalmic solution dissolved in reversible thermosetting gel: safety and hypotensive effect in normal volunteers. *Atarashii Ganka (J Eye)* 1995;**12**:1289–93.
- 16 Grant WM. *Toxicology of the eye*. 3rd ed. Springfield: Charles C Thomas, 1986.
- 17 Kondo M, Araie M. Concentration change of fluorouracil in the external segment of the eye after subconjunctival injection. *Arch Ophthalmol* 1988;**106**:1718–21.