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

Koji Ichien, Tetsuya Yamamoto, Yoshiaki Kitazawa, Akihiro Oguri, Hiroshi Ando, Yuji Kondo

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.

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 However, 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 al14 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 × 10⁻³%), 29 µg/ml (2.9 × 10⁻²%), and 280 µg/ml (2.8 × 10⁻¹%). 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...
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. The minimum MMC concentration detectable by this method was 5 × 10⁻³ µg/g.

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 (µg/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, 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, 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 1 Mitomycin C (MMC) concentration changes in ocular tissues (µg/g, mean (SD))

<table>
<thead>
<tr>
<th>Ocular tissue measured</th>
<th>MMC dosage applied (µg)</th>
<th>Hours after MMC application</th>
<th>Initial concentration (µg/g)</th>
<th>Time constant (h⁻¹)</th>
<th>Half life (h)</th>
<th>AUC† (µgh/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con junctiva</td>
<td>0.22</td>
<td>0.51 (0.18)</td>
<td>0.17 (0.18)</td>
<td>0.02 (0.02)</td>
<td>Trace*</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>3.62 (3.25)</td>
<td>1.50 (0.86)</td>
<td>0.18 (0.13)</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>62.15 (20.71)</td>
<td>21.83 (4.29)</td>
<td>5.31 (5.22)</td>
<td>0.18 (0.16)</td>
<td>Trace</td>
</tr>
<tr>
<td>Sclera</td>
<td>0.22</td>
<td>0.13 (0.14)</td>
<td>0.06 (0.07)</td>
<td>0.01 (0.01)</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>0.61 (0.48)</td>
<td>0.52 (0.45)</td>
<td>0.07 (0.05)</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>23.18 (6.80)</td>
<td>10.25 (3.41)</td>
<td>1.36 (0.93)</td>
<td>0.10 (0.10)</td>
<td>Trace</td>
</tr>
</tbody>
</table>

*Less than the minimum detectable mitomycin C concentration—that is, 5 × 10⁻³ µg/g.

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

<table>
<thead>
<tr>
<th>Method of MMC application</th>
<th>MMC dosage applied (µg)</th>
<th>Initial concentration (µg/g)</th>
<th>Time constant (h⁻¹)</th>
<th>Half life (h)</th>
<th>AUC† (µgh/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study with reversible thermostetting gel</td>
<td>0.22</td>
<td>1.23</td>
<td>2.40</td>
<td>0.29</td>
<td>0.40</td>
</tr>
<tr>
<td>Subconjunctival injection with sterile water*</td>
<td>2</td>
<td>1.05</td>
<td>2.28</td>
<td>0.31</td>
<td>0.29</td>
</tr>
<tr>
<td>Application with sponges for 5 minutes and copious irrigation*</td>
<td>200</td>
<td>158.0</td>
<td>3.92</td>
<td>0.18</td>
<td>23.49</td>
</tr>
</tbody>
</table>

*Numbers are quoted from or calculated using data that appeared elsewhere.†Area under the curve between 0.5 and 24 hours.

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

<table>
<thead>
<tr>
<th>Method of MMC application</th>
<th>MMC dosage applied (µg)</th>
<th>Initial concentration (µg/g)</th>
<th>Time constant (h⁻¹)</th>
<th>Half life (h)</th>
<th>AUC† (µgh/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study with reversible thermostetting gel</td>
<td>0.22</td>
<td>0.10</td>
<td>1.31</td>
<td>0.53</td>
<td>0.11</td>
</tr>
<tr>
<td>Subconjunctival injection with sterile water*</td>
<td>2</td>
<td>0.12</td>
<td>1.54</td>
<td>0.45</td>
<td>0.14</td>
</tr>
<tr>
<td>Application with sponges for 5 minutes and copious irrigation*</td>
<td>200</td>
<td>3.16</td>
<td>2.19</td>
<td>0.32</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*Numbers are quoted from or calculated using data that appeared elsewhere.†Area under the curve between 0.5 and 24 hours.
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becomes a sol at relatively low temperature

The reversible thermosetting gel we used

Discussion

our previous study in which no gel was used.

ting gel were higher than those in the eyes in

solution dissolved in the reversible thermostet-

as AUCs in the eyes treated with the MMC

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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

The reversible thermosetting gel we used

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The authors thank Wakamoto Pharmaceutical Co, Japan, for

providing and preparing the reversible thermosetting gel.

None of the authors has any proprietary interest in the develop-

ment and marketing of any products mentioned or of any

competing products.

1 Palmer SS. Mitomycin as adjunct chemotherapy with trab-


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

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

comparing with the same amount of the same

concentration of aqueous solution (unpub-

lished 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 subcon-

junctivally and therefore that the concentra-
tion 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 sub-

conjunctival injection of 0.1 ml of 2.9 × 10−6% MMC solution (MMC dosage, 2.9 µg) dis-

solved in the reversible thermosetting gel

allowed maintenance of an MMC concentra-
tion 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.13 The MMC concentration in the con-

junctiva 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 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 trans-

ferred to the target ocular tissues by injecting it

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 subcon-

junctival 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 topicaly applied or subconjunctivally

injected in glaucoma filtering surgery is to

avoid contact with ocular and periocular

tissues of concentrations of the agent high
eighth to cause adverse effects such as those

found after its use following pterygium

surgery.15 However, a great disadvantage of

the prevalent clinical method of MMC admin-

istration 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.17 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 thermoste-

gel. Although the effects of this method of

administering MMC on filtering bleb forma-

tion and complications must be further investi-

gated in animal filtration models and we have

to administer it preoperatively, a smaller

amount of MMC in gel may prove to be at least

effective as the current method.

The authors thank Wakamoto Pharmaceutical Co, Japan, for

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