

Original Investigation

Concentration Accuracy of Compounded Mitomycin C for Ophthalmic Surgery

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IMPORTANCE Ophthalmologists rely on accurate concentrations of mitomycin C (MMC) to prevent scarring with trabeculectomy surgery. To our knowledge, the concentration accuracy and variability of compounded MMC are unknown.

OBJECTIVE To determine whether the measured concentration differs from the expected concentration of 0.4 mg/mL of MMC used in ophthalmic surgery.

DESIGN, SETTING, AND PARTICIPANTS Laboratory experimental investigation conducted in July 2013. We acquired 60 samples of 0.4 mg/mL of MMC from a spectrum of common compounding and storage techniques (refrigeration, freezing, and immediately compounded dry powder) and a variety of pharmacies (an academic hospital, a community hospital, and an independent Pharmacy Compounding Accreditation Board–accredited pharmacy). We used C18 reversed-phase high-performance liquid chromatography to measure the MMC concentration of all samples. We used pure MMC (Medisca Inc) to generate calibration curves and sulfanilamide as an internal standard.

MAIN OUTCOMES AND MEASURES We calculated MMC concentration using a calibration curve (range, 0.3–0.5 mg/mL) generated by dividing MMC peak area by internal standard peak area and plotting the area ratio against the calibrant concentrations. We compared the measured concentration against the expected 0.4 mg/mL concentration for all samples.

RESULTS Measurement of MMC using the high-performance liquid chromatography method demonstrated acceptable accuracy (92%–100%), precision (2%–6% coefficient of variation), and linearity (mean correlation coefficient of $r^2 = 0.99$). The measured MMC concentration determined using the high-performance liquid chromatography method for all samples was 12.5% lower than the expected 0.4 mg/mL value (mean [SD], 0.35 [0.04] mg/mL; 95% CI, 0.34–0.36; $P < .001$) with a wide concentration range between 0.26 and 0.46 mg/mL.

CONCLUSIONS AND RELEVANCE Common compounding and storage techniques for MMC resulted in a lower accuracy and wider range of concentration than expected. These differences in concentration may result from compounding techniques and/or MMC degradation. Variability in MMC concentration could cause inconsistency in glaucoma surgical results, but the clinical relevance of such findings on glaucoma surgery outcomes remains unknown.

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Ophthalmologists often use compounded mitomycin C (MMC) during trabeculectomy surgery to prevent subconjunctival scarring and fibrosis. Sponges may be soaked in compounded MMC solution and applied to the episclera, or MMC may be directly injected into the sub-Tenon space.^{1,2} The concentration of MMC used in trabeculectomy surgery can influence the degree of postoperative bleb filtration and resultant intraocular pressure. The concentration is directly proportional to therapeutic effect and indirectly proportional to complications such as bleb leaks and ischemia of the bleb.³ For these reasons, ophthalmologists adjust the concentration of MMC, usually 0.2 to 0.4 mg/mL, based on risk factors for scarring and overfiltration such as race/ethnicity, previous surgery, and age.⁴

We are unaware of any prior studies evaluating the concentration accuracy and variability of compounded MMC used in ophthalmic surgery. The goal of this study was to determine whether the measured concentration differs from the expected concentration of 0.4 mg/mL of MMC across a wide spectrum of samples. We used C18 reversed-phase high-performance liquid chromatography (HPLC) and precisely generated calibration curves to evaluate the concentration of 60 MMC samples from multiple common sources. Clinicians and researchers may be interested in these results to decrease the variability of trabeculectomy outcomes.

Methods

Preparation of MMC and Other Chemical Solutions

Buffer salts, sulfanilamide (internal standard), and HPLC solvents were obtained from Sigma-Aldrich. Mitomycin C was obtained as pure powder from Medisca Inc to construct calibration curves. Tested samples of MMC used either pharmaceutical-grade MMC powder from Accord Healthcare Inc or Mobius Therapeutics (Mitosol).

We acquired 60 samples of 0.4 mg/mL of MMC from a variety of pharmacies (an academic hospital, a community hospital, and an independent Pharmacy Compounding Accreditation Board-accredited pharmacy) that used several different common compounding and storage methods. Samples of MMC ($n = 12$ per storage method) were stored either refrigerated (-4°C) for 1 week, refrigerated for 2 weeks, frozen (-20°C) for 23 days, shipped on ice and then refrigerated for 1 week, or freshly made from dry powder (Mitosol). The compounding and storage techniques were practiced in accordance with the protocols in respective hospital settings. We selected refrigeration and freezing storage time points based on half the US Pharmacopeia National Formulary chapter 797 low-risk chemical storage guidelines: 2 weeks and 45 days, respectively.⁵ These storage times were consistent with published pharmacy survey data describing ophthalmic MMC use.⁶

The Institutional Review Board of Legacy Health designated this study as exempt from review because no human participants or animals were involved.

Preparation of HPLC Calibrators and Samples

We generated MMC calibration curves (0.3-0.5 mg/mL) using dilutions of a pure MMC, 1 mg/mL, stock solution in sterile

At a Glance

- We aimed to determine whether the measured concentration differs from the expected concentration of 0.4 mg/mL of mitomycin C (MMC) used in ophthalmic surgery.
- We acquired 60 samples of 0.4 mg/mL of MMC from a variety of pharmacies using several different common compounding and storage techniques.
- We used C18 reversed-phase high-performance liquid chromatography to measure the MMC concentration of all samples.
- The measured mean (SD) MMC concentration was 0.35 (0.04) mg/mL, with a wide range between 0.26 and 0.46 mg/mL.
- The clinical relevance of these findings on glaucoma surgery outcomes is unknown.

water (Medisca Inc). For the internal standard, a 1-mg/mL solution of sulphanilamide was used. Twenty microlites of the internal standard was added to each 200 μL aliquot of pure MMC calibrant or compounded MMC sample. After removal from storage or immediate compounding, all 60 MMC samples were tested with HPLC within 24 hours of reaching room temperature. Six additional MMC samples, kept at room temperature for 24 hours, were subject to repeat analysis to assess sample stability.

HPLC Analysis

The MMC concentration of each sample was measured with HPLC. In brief, samples were analyzed with C18 reversed-phase chromatography with a Kromasil C18, 100- \AA , 3.5- μm , 2.1 \times 150-mm column (Sigma-Aldrich) using an Agilent 1200 series degasser (model G1379B), HPLC pumps (model G1312A), a diode array detector (model G1365B), and an autosampler (model G1329A). The injection volume was 10 μL . Mitomycin C was resolved from degradants generated within 35 minutes using an isocratic mobile phase consisting of 10mM ammonium acetate buffer, pH 6.5, and methanol (75:25) at a flow rate of 0.1 mL/min with a column temperature of 30 $^{\circ}\text{C}$.⁷ Mitomycin C and breakdown products were detected using absorbance at 210nm. Sample and data analyses were performed using ChemStation software (Agilent Technologies).

Method Performance

To calculate measured MMC concentrations, 5 replicate 5-point calibration curves were generated by performing least-squares linear regression for peak area ratios (MMC divided by internal standard peak area) plotted against specified calibrant concentration. The analyte and internal standard peaks were baseline integrated.

The lower limit of quantification was determined as the lowest concentration for which the signal-to-noise ratio was greater than 5, and the reproducibility of calculated concentrations was less than or equal to 20% coefficient of variation. The accuracy and precision for measured MMC were determined by analyzing samples at nominal MMC concentrations of 0.3, 0.4, and 0.5 mg/mL. The method precision was established by determining the coefficient of variation for calculated MMC values from 5 replicate samples analyzed on the

Figure 1. Representative High-Performance Liquid Chromatography of Mitomycin C Sample

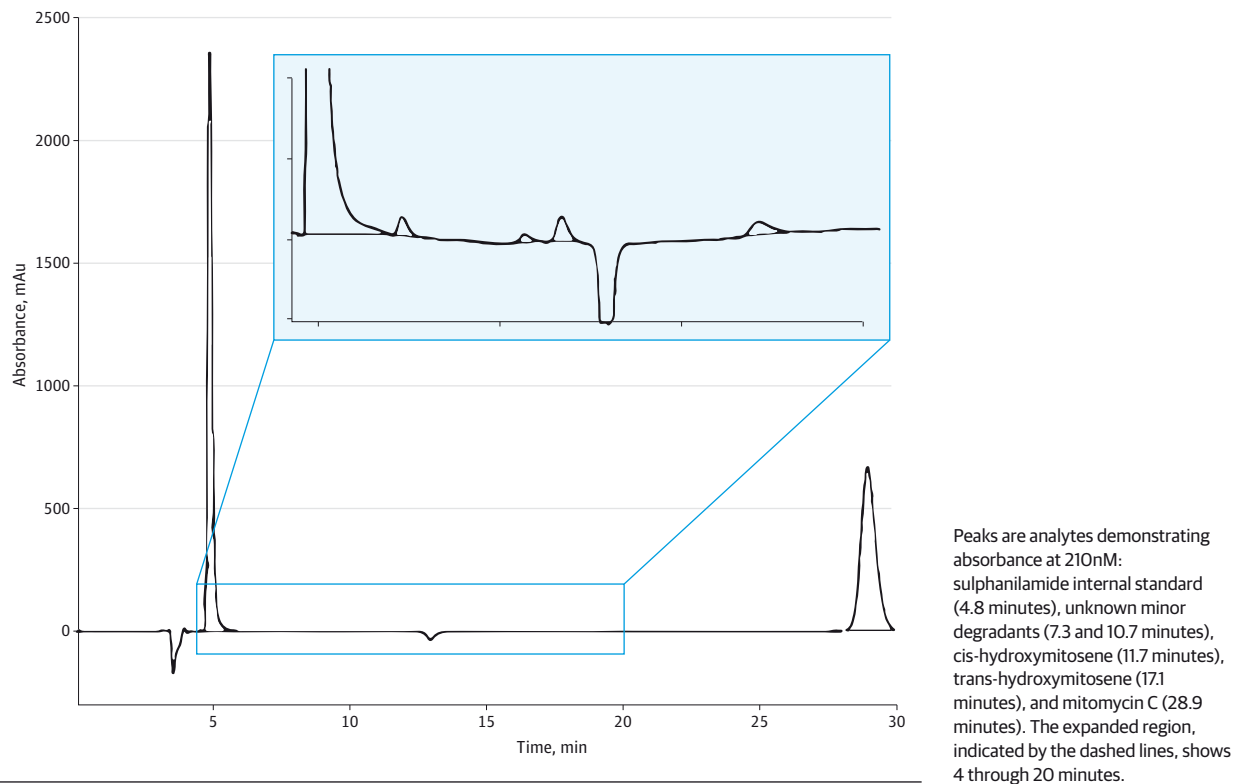


Table. Calculated Concentration of the Calibration Curve Standards for MMC

Nominal MMC concentration, mg/mL	Intraday (n = 3)			Interday (n = 5)		
	Precision		Accuracy, %	Precision		Accuracy, %
	Mean (SD)	CV, %		Mean (SD)	CV, %	
0.3	0.27 (0.01)	6.1	92.0	0.29 (0.01)	4.2	98.0
0.4	0.37 (0.02)	5.2	92.9	0.40 (0.02)	4.1	100.5
0.5	0.47 (0.00)	1.9	95.1	0.49 (0.01)	2.7	98.6

Abbreviations: CV, coefficient of variation; MMC, mitomycin C.

same day for intraday precision and over 3 days for the interday precision. Accuracy was established by comparing the mean calculated MMC value to the nominal value and was expressed as percentage recovery.

Statistical Methods

Statistical analysis was carried out with GraphPad Prism software, version 6.02 and SPSS software, version 21.0 (IBM). All data were represented as mean (SD). We used a 1-way analysis of variance with a Ryan-Einot-Gabriel-Welsh-Q test for post hoc analysis to compare MMC concentration between groups, and *F* ratios to compare differences in variability between groups. We compared the measured concentration of all 60 samples against the expected 0.4 mg/mL concentration using a single-sample *t* test and also used single-sample *t* tests to compare the measured concentration of MMC in each group against the expected 0.4 mg/mL concentration. A *P* value of less than .01 (using a

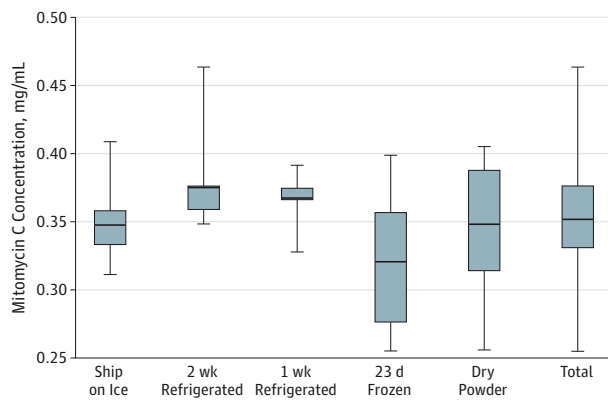
Bonferroni correction of 0.05/5 = number of groups) was considered significant.

Results

HPLC Analysis

Under the HPLC conditions used, MMC eluted with a retention time of around 30 minutes (Figure 1 is a representative HPLC chromatogram). Calibration curves for MMC (normalized to internal standard) demonstrated acceptable linearity and a mean correlation coefficient of $r^2 = 0.99$ across the range of 0.3 to 0.5 mg/mL (with acceptable intrarun and interrun accuracy and precision data for 5 replicate samples at 0.3, 0.4, and 0.5 mg/mL; Table). Repeat measurements ($n = 4$) of 6 MMC samples kept at room temperature over 24 hours to assess stability demonstrated little variability in measured concentration (mean coefficient of variation, 1.4%; range 0.7%-2.6%). For

Figure 2. Box Plots of Measured Concentrations of Expected 0.4 mg/mL of Mitomycin C Using a Variety of Storage Techniques and Sources



Shaded box areas indicate 25th and 75th percentiles. Error bars show concentration ranges of the 60 samples (12 samples per storage method).

example, a representative MMC sample analyzed 4 times over 24 hours with HPLC yielded concentration measurements of 0.314, 0.311, 0.312, and 0.309 mg/mL.

Measured Concentration of MMC

Figure 2 shows that the measured concentration of all samples was 12.5% less than the expected concentration of 0.4 mg/mL (mean [SD], 0.35 [0.04] mg/mL; 95% CI, 0.34-0.36; range, 0.26-0.46 mg/mL; $P < .001$). Samples from all storage types, except 2-week refrigeration (mean [SD], 0.38 [0.03]), had significantly lower measured mean (SD) concentrations than the expected value: dry powder, 0.35 (0.05) mg/mL ($P = .003$); shipment on ice, 0.35 (0.03) mg/mL ($P < .001$); 1-week refrigerated, 0.37 (0.02) mg/mL ($P < .001$); and 23 days frozen, 0.32 (0.05) mg/mL ($P < .001$). A 1-way analysis of variance showed a significant difference in concentration between storage types ($F_{4,55} = 3.85$; $P = .008$). A post hoc Ryan-Einot-Gabriel-Welsch-Q test revealed that samples from the 3-week frozen group had significantly lower concentrations than samples that were stored in the refrigerator for 1 week and 2 weeks. No other storage types differed from each other in average concentration. We found no significant differences in the variability of concentrations between storage types ($P = .04$ - $.56$). Therefore, the different compounding and storage techniques resulted in similar high variability of measured MMC concentrations.

Discussion

We describe here characterization of the measured concentration of 0.4 mg/mL of MMC solutions across a wide spectrum of compounded samples. Across all samples, the mean measured concentration of MMC was significantly lower than the expected concentration. The measured concentration exhibited a wide range between 0.26 and 0.46 mg/mL. The clinical relevance of these findings on glaucoma surgery outcomes remains unknown. However, improving the accuracy

and variability of compounded MMC concentration may enhance trabeculectomy outcomes.

Reproducible calibration curves and precise MMC measurements indicate that the HPLC method was unlikely to be the source of the high variability in the measured MMC concentrations. Other explanations of the inaccuracy and imprecision of measured concentrations may include MMC degradation or compounding technique. We previously reported that our MMC samples degraded between 2% and 7%.⁷ Pharmacy guidelines suggest that a stable concentration is less than 10% degradation of the active component at the time of use.^{6,8-10} If the MMC samples had been compounded at exactly 0.4 mg/mL, degradation would theoretically decrease the concentration to between 0.37 and 0.39 mg/mL, which is still considerably higher than the mean concentration measured in this study.

Mitomycin C compounding techniques could have also contributed to the variability in MMC concentration. Pharmacies typically compound MMC powder with sterile water in large quantities and then store individual doses refrigerated or frozen. For example, a 5-mg vial of MMC can produce up to 25 doses. To compound a 0.4-mg/mL concentration, 12.5 mL of water is mixed in the 5-mg vial and then separated into individual 0.5-mL syringes, which requires technical expertise. Accord Healthcare reports up to a 3% overfill in their 5-mg vial of MMC (oral communication; October 7, 2013). This potential 0.15-mg overfill would be dispersed among the 25 doses. Mobius Therapeutics reports $\pm 6\%$ variability in each individual 0.2-mg vial of MMC (written communication; June 30, 2015). At 0.4-mg/mL concentration, these 0.2-mg dry powder kits require injecting 0.5 mL of water from an attachable 1-mL syringe. The imprecision of the fluid meniscus at the kit's demarcation line indicating 0.5 mL of water may contribute to the kit's concentration variability. It is possible the dry powder kit may provide more consistent concentration measurements using the entire 1 mL of water at 0.2 mg/mL, but we tested only the 0.4-mg/mL concentration.

One limitation of this study was that although we requested 12 consecutive MMC samples from each pharmacy, we could not verify whether all samples derived from the same compounded batch of MMC. As previously mentioned, a 5-mg vial of MMC can produce up to 25 compounded syringes. It is possible that the MMC concentration variability is higher between batches than within a batch, so our results may have shown even higher variability if we had sourced each sample from a different batch of MMC. This discrepancy would not affect the 0.2-mg dry powder kits, which are always compounded as single syringes.

Dose-response studies have suggested that the concentration of MMC is proportional to its therapeutic effect.^{3,11-15} However, these studies used larger differences in MMC as compared with the concentration difference found in our study (0.35 vs expected 0.4 mg/mL). For example, Robin et al³ compared 0.2 mg/mL vs 0.4 mg/mL of MMC, and Lee et al¹⁴ investigated 0.1, 0.2, and 0.4 mg/mL. They found increased success rates of intraocular pressure control with the higher MMC concentrations, and these studies compared similar magnitude differences to our range in MMC concentration (0.26-0.46 mg/mL). In contrast, other studies have not supported a correlation between

MMC concentration and resultant intraocular pressure.^{16,17} Overall, we do not know the clinical relevance of the mean (SD) concentration (0.35 [0.04] mg/mL) or concentration range (0.26-0.46 mg/mL) in MMC found in the current study.

We did not find a statistical difference in variability between MMC sources (Figure 2); however, our study was not designed or powered to detect such a difference. The frozen samples exhibited the lowest concentration values and widest range (Figure 2). Repeat freeze-thaw cycles have been reported to cause small concentration changes in other solutes.^{18,19} Other explanations for the variability are discussed in previous paragraphs of this section.

Conclusions

The results of this study suggest that MMC concentration variability is greater than expected. Reducing concentration variability may help improve outcomes of trabeculectomy surgery. Future HPLC analysis of MMC solutions could be useful to compare different MMC sources at multiple concentrations, including diluted MMC concentrations used in sub-Tenon injections, which are increasingly performed in trabeculectomy, bleb needling, and minimally invasive ab interno subconjunctival stent surgery.

ARTICLE INFORMATION

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Study concept and design: Kinast, Mansberger, Barker.

Acquisition, analysis, or interpretation of data:

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