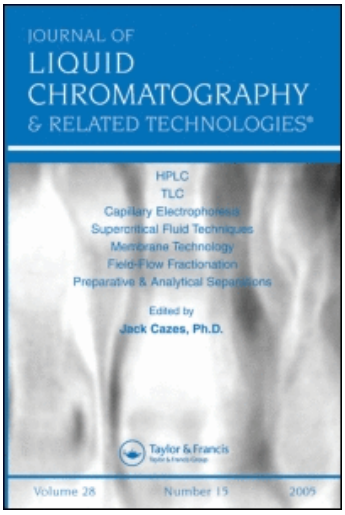

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DETERMINATION OF MITOMYCIN C IN RABBIT OCULAR TISSUE AFTER TOPICAL ADMINISTRATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high-performance liquid chromatographic method is described for the determination of mitomycin C (MMC) in rabbit ocular tissues. The optimial conditions were 20 mM sodium phosphate buffer, pH 7.0 - methanol (70:30) in isocratic elution (flow rate 1mL/min). The detection limit of MMC of this method is 0.02 μg/g for conjunctive and sciera or 0.02 μg/mL for aqueous humor. The effect of pH value and temperature on the stability of MMC was investigated. Acetonitrile was used for extracting MMC from conjunctiva and sclera, and ethyl acetate for aqueous humor. Recoveries ranged from 49% to 92% over a wide range of concentrations (0.1-400 μg/g). The half-life of MMC, after cellulose sponge administration, is 0.58 hours for the conjunctiva and 0.45 hours for the sclera. The peak concentration of MMC in aqueous humor is 1 hour.

INTRODUCTION

Mitomycin C (MMC), an antibiotic-antimetabolite, is regarded as the prototype bioreductive drug in clinical use due to its requirement for activation of cytotoxic metabolites by reductive enzymes. It is known to have a potential antiproliferative effect on cultured rabbit subconjunctival fibroblasts.[1] Glaucoma filtration surgery may fail as a result of fibroblast proliferation at the sclerostomy site, with subsequent obstruction of aqueous humor drainage.[2,3] Antimetabolites with antifibroblastic proliferative activity have been used to modify the normal wound healing response and increase surgical success ratest.[4,5] MMC has gained popularity as the adjunctive drug of choice. The intraoperative cellulose sponge administration of this agent has been successful in improving the outcome of glaucoma filtration surgery in the eyes, with a poor surgical prognosis.[6]

Suresh[7] and Kawase[8] described the method of measuring MMC in aqueous and vitreous humour using normal phase HPLC. Reversed phase HPLC methods to measure MMC concentration in tumour tissue have been previously reported.[9-11] To clarify the pharmacokinetics of MMC in the eye, we developed the present HPLC methods for determining MMC concentration in ocular tissues after cellulose sponge administration, and studied the stability of MMC at different pH values. We also investigated the concentration changes of MMC after a single application in rabbit ocular tissue under different time intervals.

MATERIALS

Reagents

Methanol, acetonitrile were HPLC reagent grade. Ethyl acetate, sodium dihydrogen phosphate, sodium hydrogen phosphate and sodium chloride were analytical reagent grade. All of the above chemicals were purchased from Shanghai Reagent Factory (China). Mitomycin C was obtained from Kywa, Hakko Kegyo (Tokyo, Japan). Water was deionized and double-distilled in a quartz glass still. All other chemicals were of analytical reagent grade.

Apparatus

The chromatographic system consisted of a Shimadzu (Japan) model LC-6A liquid chromatograph, including an SCL-6A system controller, an LC-6A pump, an SIL-6A autosampling injector, a CTO-6A column oven, an SPD-6A UV-VIS spectrophotometric detector and a C-R3A data processing

unit. The analytical column was Zorbax ODS, 250 x 4.0mm, 5 μm (DuPont Instruments, USA). An ultrasonicator (Shanghai, China) was used for extracting the sample and degassing the mobile phase. The extracts of MMC were evaporated in a vacuum desiccator (Shanghai, China) at 40 °C.

METHODS

Chromatographic Conditions

Separations were carried out on a 250 x 4.0mm Zorbax ODS reversed-phase column (DuPont Instruments). The mobile phase consisted of 20 mM sodium phosphate buffer (pH7.0)-methanol (70:30). Elution was performed isocratically at a flow rate of 1 mL/min; the column was maintained at a constant temperature of 40 °C.

The mobile phase components were filtered before using (0.45μm filter, Waters Millipore, USA) and desgassed with an ultrasonicator. The UV-VIS detector was used for monitoring MMC operating at wavelength 360 nm. The standard solution of 0.2 μg/mL of MMC was prepared with sodium phosphate buffer, pH 7.0.

Treatment of Rabbits

Healthy, pigmented rabbits, weighing between 2.0 and 3.0 Kg, were anesthetized with an intramuscular injection of 5% pentobartal sodium 0.44mL/Kg before surgery and incising the conjunctiva 5mm posterior to where the limbus was performed. A 0.4 mg/mL solution of Mitomycin C was prepared by dissolving 2 mg Mitomycin C powder in 5mL of 0.9% sodium chloride solution. Rabbits received Mitomycin C in each eye via cellulose sponge administration. A sponge, mearsuring 3 x 3 x 3 mm, was saturated with Mitomycin C solution (0.4 mg/mL). Sponges were applied directly between the conjunctiva and the sclera in the operated eyes. After thoroughly rinsing with 0.9% sodium chloride, the rabbits were killed at 0.5-, 1-, 2-, 3-hour intervals after drug administration. Then, 100 to 200 μL of aqueous humour were drained via a 26-gauge needle, and excised a section of conjunctiva measuring 15 x 15mm and a section of sclera 15 x 10mm; each section weighed 150 to 250 mg.

Extraction of MMC from Ocular Tissues

For the conjunctiva and sclera, 5mL of cold acetonitrile was added to the 200mg specimen and homogenized. For the aqueous humour, 4 mL of ethyl

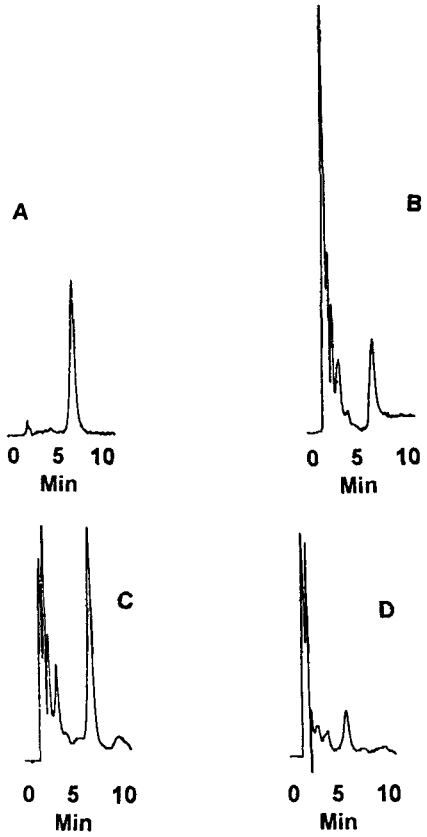

Figure 1. Typical chromatograms (A) 10 μL of MMC standard at a concentration of 0.2 μg/mL; (B) conjunctiva extract, (C) sclera extract and (D) aqueous humour extract after administration.

acetate was added to 0.2 mL of the aqueous humour sample and stirred. Then the samples were ultrasonicated for 30min. After centrifugation at 3000rpm for 10 min at 4 °C, the organic phase was separated and evaporated completely at 40 °C in the vaccum desiccator. After dissolving the residue in 0.4 mL mobile phase, sonication was performed. The filtration was carried out before injecting the sample into the chromatograph. In control extraction, ocular tissues were collected from non-drug administration rabbits and MMC (0.1-400 μg/g) was added to 200 mg conjunctiva and sclera or 0.2 mL aqueous humour. The late extraction was as described above.

Table 1

Recovery of Mitomycin C from Rabbit Ocular Tissues (Mean±SD)

Amount/g Tissue (μg)	Extraction Efficiency (%) Conjunctiva	Sclera	Aq. Humour
400	68 ± 4	59 ± 9	49 ± 12
200	72 ± 8	75 ± 6	68 ± 9
100	74 ± 7	71 ± 13	64 ± 11
50	83 ± 6	81 ± 4	80 ± 7
10	86 ± 9	83 ± 5	81 ± 6
1	92 ± 7	91 ± 9	89 ± 8
0.5	81 ± 10	79 ± 12	85 ± 3
0.1	65 ± 11	64 ± 2	54 ± 5

n = 5 for each concentration.

Table 2

Effect of Evaporation Temperature on the Stability of MMC Extract from Rabbit Ocular Tissue

Temperature (°C)	Recovery, % (Mean ± SD) Conjunctiva	Sclera	Aq. Humour
30	92 ± 11	91 ± 13	88 ± 12
40	92 ± 7	91 ± 9	89 ± 12
50	87 ± 10	80 ± 6	78 ± 9
60	81 ± 8	74 ± 8	62 ± 7
70	76 ± 12	70 ± 5	49 ± 10
80	58 ± 9	52 ± 10	51 ± 13

1.0 μg MMC was added to each tissue per gram and extracted as described in the METHODS section. n = 4.

Stability of MMC

A solution of 0.2 μg/mL of MMC was prepared with 20 mM sodium phosphate of various pH values, and kept it in the dark for various durations. The amounts of chromatography detection were compared with each other and an optimial pH value of solution was determined.

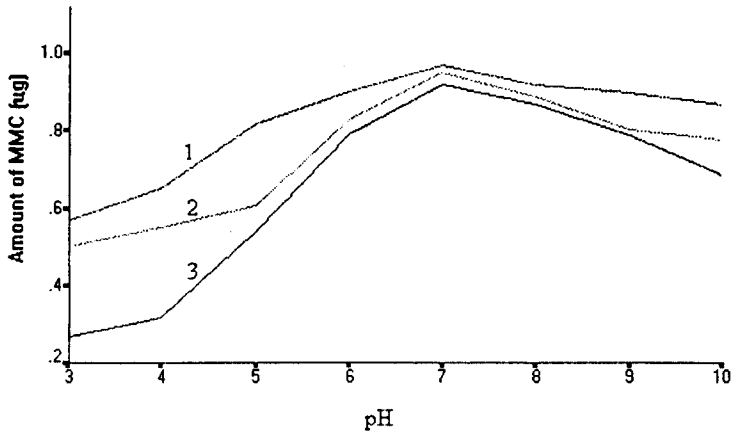

Figure 2. Degradation curve of MMC. 1 μg of MMC was dissolved in 5.0 mL 20 mmol sodium phosphate buffer, at various pH values, and kept at 20 °C in the dark for (1) 24 hrs; (2) 72 hrs; (3) 168 hrs.

RESULTS

The chromtograms of MMC standard and extracts of ocular tissues are shown in Figure 1. The retention time of MMC in this condition was 5.6 min. The detection limit of MMC for this method is 0.02 μg/g tissue or 0.02 μg/mL aqueous humour. The efficiency of extraction in each tissue is listed in Table 1.

The effect of different pH values on Mitomycin C stability is shown in Figure 2. When the pH value is below 6 or above 8, MMC is unstable. The effect of evaporation temperature on stability of MMC extract from rabbit ocular tissues is shown in Table 2. The best appropriate evaporating temperature was at 40 °C, at which there was a better recovery and a shorter evaporation time. The mean concentration of MMC in ocular tissues at different time intervals after cellulose sponge administration is shown in Table 3. The halflives of Mitomycin C were 0.58 hours in conjunctiva, 0.45 hours in sclera, respectively. The peak concentration of MMC in aqueous humor was reached at 1 hour.

DISCUSSION

Andrews[12] obtained different hydrolytic products from MMC with hydrochoric acid. Pan[13] reported that MMC and its metabolites reduction was catalyzed by NADPH-cytochrome P-450 reductase. Neither author, however, investigated systematically, the effect of pH value on the stability of MMC.

Table 3

Mitomycin C Concentration Changes of Ocular Tissue after Administration (Mean ± SD)

Hours	n	Conjunctiva (μg/g)	n	Sclera (μg/g)	n	Aq. Humour (μg/mL)
0	6	1.3859 ± 0.6208	4	2.4686 ± 0.8375	4	0.0330 ± 0.0050
0.5	6	0.7614 ± 0.1743	5	1.1518 ± 0.3604	4	0.0943 ± 0.0312
1	7	0.3691 ± 0.2635	6	0.5082 ± 0.3287	6	0.1214 ± 0.0413
2	4	0.1429 ± 0.0933	4	0.1632 ± 0.1136	5	0.0867 ± 0.0256
3	4	0.0895 ± 0.0796	4	0.1074 ± 0.0871	4	0.0743 ± 0.0154

This experiment studied the degradation of MMC in solution at various pH values, ranging from 3.0-10.0, at different time intervals (Figure2). When the pH was at 7.0, the amounts of MMC loss were 3%, 5% and 8% in 24, 72 and 168 hours, respectively; but when the pH value was at 3.0, they were 43%, 50% and 73%, and 13%, 22% and 31% at a pH value of 10.0. The results indicated that MMC is unstable in acidic and basic conditions, especially in acid.

Cummings et al.[9] chose the mobile phase at pH 5.8, improving the resolution between MMC and its metabolites. Our study showed that MMC was degraded, obviously, at pH 5.8. This fact points out that MMC was also degraded during analysis in Jeffrey's method. Thus, we chose sodium phosphate buffer, pH 7.0-methanol (70:30) as mobile phase to reduce degradation of MMC during the analysis.

The evaporation temperature of organic extract can also influence the stability of MMC. When the temperature is above 50 °C, the degradation becomes remarkably obvious in our experiments (Table 2). Thus, we determined the evaporation temperature at 40 °C in which the drying time was shorter and degradation of MMC was minimized.

The nature of the extraction solvents were compared between acetonitrile and chlorform/2-propanal/ethyl actate. Our studies showed that the former was more advantageous for extracting MMC from conjunctiva and sclera. For aqueous humour, ethyl acetate was used for extraction solvent. The efficiency of liquid-liquid extraction is shown in Table 1. Across a wide range of concentrations (0.1-400 μg/g), the extraction efficiency of MMC remained high. The average recovery was 77.6 ± 9.4%, 75.4 ± 10.4% and 71.4 ± 14.8%, for conjunctiva, sclera and aqueous humour, respectively. These data gave a satisfactoy extraction efficiency of MMC from ocular tissues and was superior to Cummings' report.[9] The present study determined the pharmacokinetics of

conjunctiva, sclera and aqueous humour MMC after postoperative topical administration in rabbit eyes. The half-lives were calculated to be 0.58 hours for the conjunctiva and 0.45 hours for the sclera. The peak concentration of MMC in aqueous humor was achieved 1 hour after cellulose sponge administration.

The initial concentration of sclera MMC is higher than conjunctiva, but the half-life of MMC in conjunctiva is longer than sclera. The clearance times of MMC are 3 and 6 hours for sclera and conjunctiva, respectively. These results indicate that the absorption of MMC of sclera is more rapid than conjunctiva and the clearance is also rapid.

REFERENCES

1. T. Yamamoto, J. Varani, H. K. Soong, P. R. Lichter, "Effects of 5-Fluorouracil and Mitomycin C on Cultured Rabbit Subconjunctival Fibroblasts," Ophthalmology, **97**, 1204-10 (1990).

2. G. L. Skuta, R. K. H. Parrish, "Wound Healing in Glaucoma Filtering Surgery," Surv. Ophthalmol., **32**, 149-70 (1987).

3. M. M. Tahery, D. A. Lee, "Review: Pharmacologic Control of Wound Healing in Glaucoma Filtration Surgery," J. Ocul. Pharmacol., **5**, 155-79 (1989).

4. Y. Kitazawa, K. Kawase, H. Matsushita, M. Minobe, "Trabeculectomy with Mitomycin. A Comparative Study with Fluorouracil," Arch. Ophthalmol., **109**, 1693-8 (1991).

5. J. H. Rabowsky, J. M. Ruderman, "Low-Dose 5-Fluorouracil and Glaucoma Filtration Surgery," Ophthalmic. Surg., **20**, 347-9 (1989).

6. G. L. Skuta, C. C. Beeson, E. J. Higginbotham, "Intraoperative Mitomycin versus Postoperative 5-Fluorouracil in High Risk Glaucoma Filtering Surgery," Ophthalmology, **99**, 438-44 (1992).

7. S. C. Srivastava, U. Hornemann, "High-Pressure Liquid Chromatography of the Antibiotics Mitomycin A, B and C and of polar Mitomycin C Conversion Products," J. Chromatogr., **161**, 393-395 (1978).

8. K. Kawase, H. Matsusluta, T. Yamamota, Y. Kitazawa, "Mitomycin Concentration in Rabbit and Human Ocular Tissues after Topical Administration," Ophthalmology, **99**, 203-7 (1992).

9. J. Cummings, L. Chirrey, N. Willmott, G. W. Halbert, J. F. Smyth, "Determination of Mitomycin C, 2,7-Diaminomitose, 1,2-cis-and 1,2-trans-1-hydroxy-2,7-Diaminomitosene in Tumour Tissue by High-Performance Liquid Chromatography," J. Chromatogr., **612**, 105-13 (1993)

10. D. Seigel, N. W. Gibson, P. C. Preusch, D. Ross, "Metabolism of Mitomycin C by DC-Diaphorase: Role in Mitomycin C-induced DNA Damage and Cytotoxicity in Human Colon Carcinoma Cells," Cancer Res., **50**, 7483 (1990).

11. P. R. Hoban, M. I. Walta, C. N. Robson, J. Godden, I. J. Stratford, P. Workman, A. L. Harris, I. D. Hickson, "Decreased NADPH:Cytochome P-450 Reductase Activity and Impaired Drug Activation in a Mammalian Cell Line Resistant to Mitomycin C under Aerobic but not Hypoxic Condition," Cancer Res., **50**, 4692 (1990).

12. P. A. Andrews, Su- Shu Pan, N. R. Bachur, "Liquid Chromatographic and Mass Spectral Analysis of Mitosane and Mitosene Derivatives of Mitomycin C," J. Chromatogr., **262**, 231-247 (1983).

13. Su-Shu Pan, P. A. Andrews, C. J. Glover, N. R. Bachur, "Reductive Activation of Mitomycin C and Mitomycin C Metabolites Catalyzed by NADPH-Cytochrome P-450 Reductase and Xanthine Oxidase," J. Biol. Chem., **259**, 959 (1984).

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