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LIQUID CHROMATOGRAPHIC DETERMINATION OF MITOMYCIN C IN HUMAN PLASMA AND URINE

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SUMMARY

A method is given for the determination of the antineoplastic drug mitomycin C in plasma and urine samples. Mitomycin is isolated from the biological matrix with the aid of a Sep-Pak C_{18} extraction column and eluted with methanol. The methanol is evaporated and the residue is redissolved in the chromatographic mobile phase (methanolic phosphate buffer). Mitomycin C is separated from coextracted compounds by reversed-phase liquid chromatography on a LiChrosorb RP-8 column. A high detection sensitivity and selectivity was obtained by photometric measurements at 365 nm. The precision of the determinations was better than 6% relative standard deviation for plasma samples within the range 2—1000 ng/ml, and for urine samples within the range 0.5—4.4 μg/ml. The pH-dependent stability of mitomycin in buffer solutions has been studied.

INTRODUCTION

Mitomycin C (Fig. 1), an antitumour antibiotic, was discovered in the late 1950's. Early clinical trials, however, revealed severe toxicity and the impor-

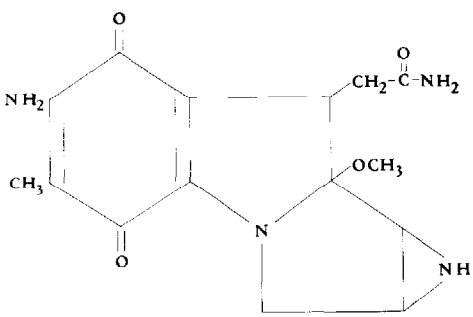

Fig. 1. Structural formula of mitomycin C.

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tance of proper dosage schedules to reduce toxicity has been emphasized. The mechanism of action of mitomycin C has been extensively studied in model systems [1—3]. Mitomycin C behaves as a bifunctional "alkylating" agent upon chemical or enzymatic reduction [2]. Only limited data of the clinical pharmacokinetics of mitomycin C are available, due to the lack of suitable assay methods. The microbiologic assay methods [4, 5] suffer from low selectivity and sensitivity. High-performance liquid chromatography (HPLC) has been used for separation of mitomycin from impurities [6] as well as for studies of the stability of mitomycin in aqueous solutions [7]. Recently, HPLC methods have been used for quantitation of mitomycin in serum [8—10].

Physicochemical investigations of porfiromycin, which differs from mitomycin C by having a methyl substituent on the aziridine nitrogen, have indicated that the chemical degradation of this compound is complex and strongly pH-dependent, as studied by photometric technique [11, 12]. These findings were recently confirmed also to be valid for mitomycin C [7].

The present paper gives a liquid chromatographic method for the analysis of mitomycin C in plasma and urine samples including a simplified extraction method.

The stability of mitomycin C was investigated in the present study with the aim of finding conditions under which the degradation of mitomycin C was negligible for a proper handling of biological samples as well as for the use of a suitable liquid chromatographic isolation procedure.

EXPERIMENTAL

Apparatus

Photometric measurements were performed with a Zeiss PMQ III Spectral photometer. An Orion Research Model 701/digital pH meter equipped with an Ingold combined electrode type 401 was used for pH measurements.

Chromatographic system

The pump was of the LDC 711 Solvent Delivery System type, and the columns were of stainless steel (length 150 mm, I.D. 4 mm, O.D. 6.35 mm). The LiChrosorb RP-8 support (E. Merck, Darmstadt, G.F.R.) had a mean diameter of 5 μm, and the chromatographic system was operated at ambient temperature (25 ± 2°C). A Rheodyne (Model 7125) injection valve with a sample loop of 150 μl was used. The chromatographic detectors used were an Altex 253 photometric detector measuring at 253.7 nm or an LDC Spectromonitor III, measuring at 365 nm. Both detectors had cells with a 10-mm path length and a volume of 8 μl.

Chemicals

All chemicals used were of analytical grade and used without further purification. Mitomycin C was kindly supplied by Bristol Laboratorier AB (Solna, Sweden). Desipramine chloride was obtained from AB Hässle, (Mölndal, Sweden). The mobile phases were prepared from acetonitrile (Uvasol, E.

Merck), methanol (p.a., E. Merck), phosphate buffer (pH 7.0, μ = 0.1) or citrate buffer (pH 5.1, μ = 0.1), and distilled water.

The buffers used in studies of the stability of mitomycin C were prepared from phosphoric acid, citric acid and sodium hydroxide to give pH values within the range 2.0—8.0. Unless otherwise stated the buffer solutions had an ionic strength of 0.1. Sep-Pak C_{18}® extraction columns were obtained from Waters Assoc. (Milford, MA, U.S.A.).

Chromatographic technique

The chromatographic columns were packed by slurry packing [13] using glycerol—methanol (1:3) as the suspension medium. The slurry was forced into the column at a flow-rate of 9 ml/min or a pressure of 34 bar, whichever was the limiting factor. The mobile phases were passed through the chromatographic system until constant retention was obtained. Usually less than 50 ml were required. The interstitial volume, V_m, of the columns was determined by the injection of 0.1 *M* phosphoric acid (10 μl).

The lifetime of the separation column was considerably increased by the use of a 7-cm pre-column, packed with the same support as the separation column, inserted between the pump and the injector.

All capacity factors (k') given are averages from at least three determinations. The mobile phase flow-rate was 0.7 ml/min throughout this study.

Drug administration

A solution containing 0.5 mg/ml mitomycin C in sterile water was used. A dose of 0.15—0.35 mg/kg body weight was given intravenously as a bolus injection over a period of 3 min. Intrahepatically 0.35 mg/kg body weight was injected into the arteria hepatica, the injection time being 3—5 min.

Plasma samples

Blood samples (5—7 ml) were taken by venipuncture from mitomycin-treated cancer patients at various time intervals. The samples were collected in 10 ml test tubes (Vacutainer®) containing 250 IU of heparin (freeze-dried). The plasma fractions were isolated by centrifugation at 325 *g* for 10 min. The separated plasma fractions were stored at —70°C until analysis.

Urine samples

Aliquots of urine samples from mitomycin-treated patients were collected in glass test tubes and stored at —70°C until analysis.

Spiking of urine and plasma samples

Appropriate amounts of mitomycin C were dissolved in methanol. To each millilitre of blank urine or plasma sample were added 100 μl of the methanolic solution.

Degradation of mitomycin C

(1) A stock solution of mitomycin C in distilled water (1.56 mg/ml) was diluted five times with citrate or phosphate buffer (final ionic strength 0.08).

The degradation of mitomycin C was followed by photometric measurement at 355 nm.

(2) A stock solution of mitomycin C in distilled water (36.8 μg/ml) was diluted five times with citrate or phosphate buffer (final ionic strength 0.08). The degradation of mitomycin C was followed by reversed-phase liquid chromatography with photometric detection at 253.7 nm. The mobile phase used was a mixture of 10% acetonitrile in citrate buffer pH 5.1.

Unless otherwise stated the degradation experiments were performed at 25.0 ± 0.1°C.

Analytical method

Extraction procedure

The Sep-Pak columns were pretreated, according to instructions from the manufacturer, by passage of 2 ml of methanol followed by 5 ml of distilled water with the aid of a glass syringe. A 1-ml volume of plasma or urine sample was mixed with 100 μl of desipramine chloride solution (100 μg/ml) and forced through the Sep-Pak cartridge. The cartridge was washed with 3 ml of water (discarded) and mitomycin was eluted with 4 ml of methanol. The methanol was evaporated to dryness under nitrogen flow. The residue was dissolved in 200 μl of mobile phase with the aid of a vortex-type mixer. After centrifugation, 100 μl of the clear liquid was injected into the liquid chromatograph.

Liquid chromatography

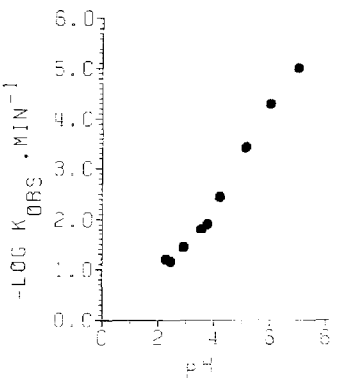
Mitomycin was separated from coextracted compounds on a LiChrosorb RP-8, 5 μm, column, eluted with an aqueous mobile phase containing 10% of phosphate buffer (pH 7.0 μ = 0.1) and methanol, 20 and 25% for urine and plasma samples, respectively. The absorbance of the eluate was measured at 365 nm. Quantitation was based on peak area measurements and the molar absorptivity of mitomycin. All plasma and urine levels of mitomycin were corrected for recoveries.

RESULTS AND DISCUSSION

Stability of mitomycin C

The chemical degradation of mitomycin C was studied by the photometric technique previously used by Garrett et al. [11, 12] for degradation studies of porfiromycin, and also by reversed-phase liquid chromatography [7]. The results (Fig. 2) show that the stability of mitomycin C increases with increasing pH within the pH range 2—7, the half-lives being about 10 min and 50 days at pH 2.3 and 7.0, respectively. The constants determined by the photometric technique were about 30% lower than the constants determined from chromatographic data, which may be the result of difficulties in determination of the absorbance values at infinite time for the first degradation step [11]. The data for the degradation of mitomycin C presented in Fig. 2 are very close to those found by Garrett et al. for porfiromycin [11, 12].

The stability of mitomycin C was strongly temperature-dependent. The



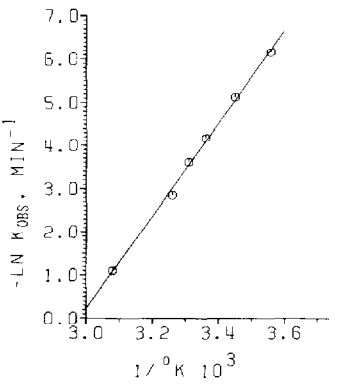


Fig. 2. Degradation of mitomycin C — influence of pH.

Fig. 3. Temperature dependence of rate constants. pH 3.51; chromatographic data.

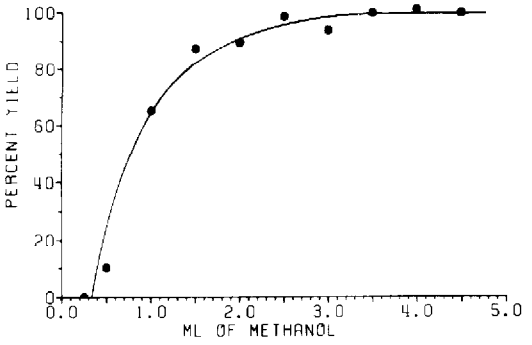
Arrhenius activation energy, calculated from the slope of the plot in Fig. 3, was 98 kJ/mol in citrate buffer pH 3.51 (liquid chromatographic data). A value of 81.1 kJ/mol has been found in unbuffered medium [7]. The rate constants for the degradation of mitomycin C in 10^{-2} *M* phosphoric acid were almost unaffected by the addition of NaCl (10^{-2} to 1 *M*), i.e. the rate constants were essentially unaffected by the ionic strength.

Extraction procedure

In the previously published HPLC analytical methods for mitomycin C [8, 9] the drug was isolated from the biological matrix by liquid—liquid extraction. Since mitomycin C has a low partition coefficient into the organic extractants used, a large ratio of organic phase to aqueous phase was necessary to obtain quantitative transfer of the drug from the plasma. Since large amounts of organic extractants with high boiling points were used and an evaporation temperature not exceeding 40°C is recommended to avoid decomposition of mitomycin C [9], the extraction procedure seemed too time-consuming for routine analysis. Isolation of mitomycin C from plasma with the aid of a non-ionic resin, Porapak Q, gave greatly varying recoveries (65—85%) [10].

In the analytical procedure for mitomycin C proposed in the present paper the drug was isolated from the biological matrix with the aid of a Sep-Pak C_{18} cartridge. The polar constituents of the biological matrix were removed by a wash with distilled water. Mitomycin C was eluted from the extraction column with methanol. A quantitative recovery of mitomycin C was obtained when the amount of methanol used for the elution exceeded 3.5 ml (Fig. 4).

To avoid decomposition of mitomycin C the evaporation of the methanol extract was performed under a nitrogen stream at a temperature not exceeding 40°C [9]. Hartigh et al. [9] found that the dissolution of mitomycin C from evaporated plasma extracts using a mobile phase containing 25% of methanol was neither complete nor reproducible, which necessitated the use of pure methanol for the dissolution of the drug. However, injection of samples dissolved in a liquid of a higher solvent strength than the mobile phase used often gives rise to disturbances of the chromatographic behaviour [14, 15] and should



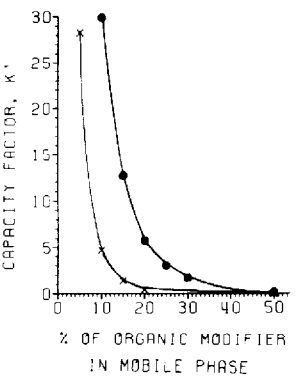


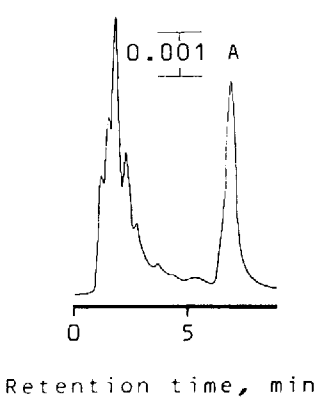
Fig. 4. Elution of mitomycin C from Sep-Pak C_{18} extraction columns.

Fig. 5. Capacity factors and concentration of organic modifier in mobile phase. Mobile phase: organic modifier in phosphate buffer pH 7.0 with a final ionic strength of 0.01. Organic modifier: X = acetonitrile; • = methanol.

accordingly be avoided. In the present paper complete dissolution of mitomycin C in the chromatographic mobile phase was obtained after the addition of desipramine, previously shown to prevent adsorption on glass surfaces effectively [14—17].

Chromatographic isolation

To avoid decomposition of mitomycin C during the liquid chromatographic procedure too low a pH of the mobile phase should be avoided; cf. Fig. 2. The retention of mitomycin C was strongly dependent upon the concentration of organic modifiers in the mobile phase (Fig. 5). Acetonitrile was the most suitable modifier for the separation of mitomycin and its degradation products. However, in the proposed analytical method, aimed for routine analysis of mitomycin in biological samples, methanol was preferred as modifier due to



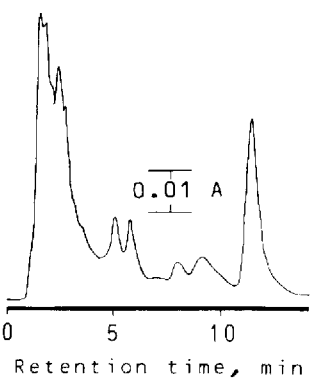


Fig. 6. Chromatogram of plasma sample from a mitomycin-treated cancer patient. The chromatographic peak corresponds to 62 ng of mitomycin per ml of plasma.

Fig. 7. Chromatogram of a urine sample from a mitomycin-treated cancer patient. The chromatographic peak corresponds to 7 μg of mitomycin per ml of urine.

its lower toxicity. Mitomycin in plasma extracts was completely isolated from endogenous compounds using a mobile phase containing 25% of methanol (Fig. 6). For the analysis of mitomycin in urine samples a decrease of the methanol concentration in the mobile phase to 20% was necessary to avoid interference (Fig. 7).

Photometric detection

The absorption spectra of mitomycin in the mobile phase used in the analytical procedures (containing 20 and 25% of methanol, respectively) were identical and showed that the commonly used fixed-wavelength ultraviolet detectors measuring at 254 nm are unsuitable for the analysis of mitomycin in the low concentration range. The high detection sensitivity as well as the high detection selectivity in the present method was obtained by photometric measurement of the eluate at 365 nm; cf. ref. 18. The signal-to-noise ratio was better than 10 for an injected amount of 1 ng of mitomycin C.

Quantitative determination

Quantitation was based on peak area measurement and the molar absorptivity of mitomycin C in the mobile phase ($\epsilon = 1.65 \cdot 10^4$) according to the principles given in ref. 19.

The amount of sample (in mmol), M, can be calculated from the equation

$$M = Y \times u \times b \times \epsilon^{-1}$$

where Y = peak area in mm^2, u = ml/mm chart paper, b is absorbance/mm chart paper. From the equation it follows that the peak area is not dependent on chromatographic parameters such as column efficiency and capacity factors of the solutes, as well as length and diameter of the chromatographic column. Hence, once the molar absorptivity has been determined no calibration graph need be constructed.

Recovery and precision

The recovery and precision of the method for the determination of mitomycin in plasma and urine samples are presented in Table I. The precision of the determinations was better than 6% relative standard deviation for plasma

TABLE I

RECOVERY AND PRECISION FROM SPIKED PLASMA AND URINE SAMPLES

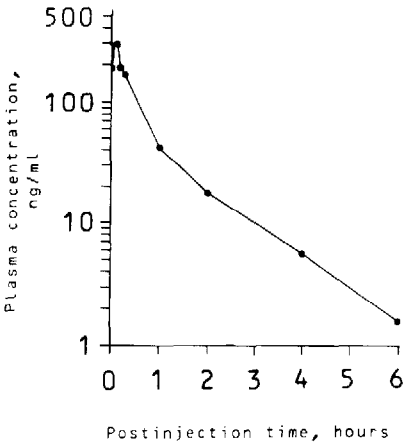
Biological sample	Drug concentration (ng/ml)	Recovery* (%)
Plasma	2.13	71.5 ± 5.0
Plasma	4.94	94.6 ± 4.3
Plasma	12.6	97.2 ± 1.5
Plasma	105	101.9 ± 0.7
Plasma	1050	102.9 ± 2.5
Urine	450	87.0 ± 1.5
Urine	930	89.8 ± 0.4
Urine	2120	82.2 ± 5.9
Urine	4370	84.6 ± 4.1

*Recovery ± relative standard deviation (n = 6).

samples within the range 2—1000 ng/ml and for urine samples within the range 0.5—4.4 μg/ml.

Clinical applications

Plasma levels of mitomycin after an intrahepatic dose of 0.35 mg/kg are given in Fig. 8, the terminal half-life being 1.1 h. About 12% of mitomycin was excreted unchanged in the urine after an intravenous dose of 0.15 mg/kg (Fig. 9).



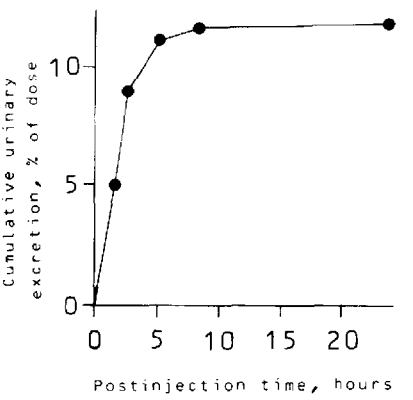


Fig. 8. Plasma concentration—time curve after intrahepatic administration of mitomycin C. Mitomycin, 0.35 mg/kg body weight, was given as a bolus injection in the arteria hepatica.

Fig. 9. Urinary excretion of mitomycin C. Mitomycin, 0.15 mg/kg body weight, was given as an intravenous bolus injection.

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