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Note

Mitomycin C determination using loop-column extraction: a rapid and sensitive high-performance liquid chromatographic assay for pharmacokinetic studies with Spherex starch particles

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Mitomycin C (MMC, Fig. 1) is a potent antitumour agent, derived from *Streptomyces cyespitosus*, which has been established in chemotherapy for many years. MMC has a high clinical efficacy in a number of neoplastic diseases, but it also shows severe side-effects [1]. Therefore plasma levels of MMC must be controlled in patients with renal or liver impairment to optimize dosage regimens during chemotherapy.

The trend in chemotherapy of tumours is to try to reduce the systemic circulation of the antitumour agent and to concentrate the drug as close as pos-

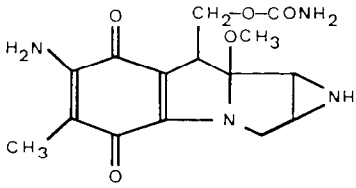

Fig. 1. Structure of mitomycin C.

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sible to the target organ. A very new possibility for achieving this is the embolization of the blood vessel (artery) directly to the tumour or its metastasis (e.g. liver). The use of starch particles (mean size 30 μm) has the great advantage that these particles plug the artery for only 1 h, because they are hydrolysed by endogenous enzymes leading to the formation of natural sugar units as degradation products. The embolization with Spherex particles concentrates MMC close to the tumour and leads to lower plasma levels. Quantitative determination of MMC in plasma under such conditions requires a more sensitive assay than previous clinical studies. In the present paper we report an MMC assay with reduced sample handling by direct injection of plasma samples into a loop-column and a detection limit of ca. 1.0 ng/ml in plasma.

EXPERIMENTAL

Chemicals

MMC hydrochloride (lyophylized powder) was supplied from Ebewe Pharmaceuticals (Unterach, Austria). Deionized and distilled water and methanol (LiChrosolv, Merck, Darmstadt, F.R.G.) were used for the extraction procedure and mobile phase preparation. Disodium hydrogenphosphate (Merck) and phosphoric acid were used for buffer preparation for sample clean-up. Liquemin (Hoffman La Roche, Basle, Switzerland) was used as stabilizer for blood samples.

High-performance liquid chromatographic (HPLC) equipment

The liquid chromatograph consisted of a 420 pump (Kontron, Vienna, Austria), a membrane pulsation-dampener (Scientific Systems, State College, PA, U.S.A.), a LiChrosorb RP-8 guard column (10 μm, 10 mm × 4.2 mm I.D.) and a LiChrosorb RP-8 analytical column (3 μm, 150 mm × 4.2 mm I.D.). Columns were connected by an 'eco-tube' cartridge system (Bischoff, Leonberg, F.R.G.) and thermostatted by a column oven 830 (Kontron). Detection was performed by a Uvikon 430 photometric detector connected with a P-450 integration and controller system (both Kontron). Injection and sample clean-up was performed with a Rheodyne 7125 valve using a loop-column (Spherisorb ODS, 30 μm, 10 mm × 4.2 mm I.D.) instead of a loop. Solvent was degassed by a degasser (Erma ERC-3512, SRD, Vienna, Austria).

Chromatographic conditions

The mobile phase, methanol–water (30:70, v/v), was filtered through a 0.45-μm filter (Reichelt Chemie, Heidelberg, F.R.G.) prior to use and degassed continuously. The HPLC system was operated at 0.8 ml/min (pressure 106 bar) and thermostatted at 35 °C. Detection was performed by measuring the absorbance at 365 nm and a sensitivity of 0.002, with a response time of 2 s.

Patients and sample handling

MMC was administered via rapid bolus injection intra-arterially by a Port-A catheter into the hepatic artery (dosage 0.40 mg/kg body weight) of patients with liver metastases. In the first cycle of treatment MMC was injected without Spherex. In the second cycle (after two weeks) MMC was administered with Spherex particles in 10 ml of sterile and isotonic sodium chloride solution. Blood samples (3 ml) were obtained by venepuncture at 0, 15, 30, 45, 60, 75, 120, 240 and 360 min after administration and stabilized immediately by addition of 50 μl of Liquemin. Plasma was separated by centrifugation of blood samples for 5 min at 2000 *g* and frozen at −70°C until analysis. Frozen storage of plasma samples for a period longer than two weeks was avoided as this can result in a considerable drop in MMC concentration [2].

Extraction procedure

The Rheodyne valve was set to the 'load' position and the loop-column was flushed with 2.0 ml of 0.01 *M* disodium hydrogenphosphate buffer (pH 8.5) followed by injection of 1.0 ml of plasma sample. The matrix components were washed from the loop-column with 2.0 ml of disodium hydrogenphosphate solution and 1.0 ml of distilled water. Finally the valve was switched to the 'inject' position for start of chromatography.

RESULTS AND DISCUSSION

Extraction procedure

Loop-column extraction has been used successfully in clinical and pharmaceutical analysis for determination of drugs from biological matrices [3–6]. Although many high-performance liquid chromatographic methods for the assay of MMC in plasma and urine have been reported, only a single method with loop-column extraction has been described. However, this method, using a Porapak Q loop-column, has a detection limit of 25 ng/ml [7] and was not suitable for our studies.

The reported methods for MMC assay either require a large volume of sample (up to 4 ml of plasma) or have a very time-consuming extraction step or have too limited a sensitivity to quantitate the compound of interest at low nanogram levels [8–10]. Furthermore, extraction of MMC from plasma by solvent-partitioning lowers the precision of the assay. The advantage of the proposed method is based on rapid sample clean-up (within 30 s) and high accuracy. No decomposition and no loss of sample during isolation from the matrix was observed. Moreover, instead of dilution, MMC is preconcentrated on the loop-column by direct injection of samples. The loop-column technique offers high sensitivity by injection of plasma volumes up to 2 ml and can be adapted for fully automated analysis of MMC in routine analysis, if a column-switching module is available. The loop-column has to be renewed after ca. 50

injections of 1.0 ml of plasma owing to increase of back-pressure over 200 bar caused by column clogging. This could be a disadvantage in automated analysis.

Chromatographic isolation

The chromatograms in Fig. 2 show that MMC is well separated from endogenous compounds (retention time 8 min) using a mobile phase of 30% methanol in water. As can be seen, almost no endogenous compound is co-extracted with MMC. Analysis of twelve different normal human and patient plasma samples revealed no interfering bands. Moreover, analysis of methanol standards of co-administered drugs (such as buprenorphine hydrochloride and metoclopramide hydrochloride) suggested no potential interferences.

Photometric detection

Loop-column enrichment of MMC makes possible photometric quantification of the drug down to the low nanogram level. The high detection sensitivity as well as the detection selectivity were obtained by photometric measurement of the eluate at 365 nm. The detection limit was 1 ng/ml by injection of 1 ml of plasma (signal-to-noise ratio 3:1) and could be improved by injection of larger volumes of sample.

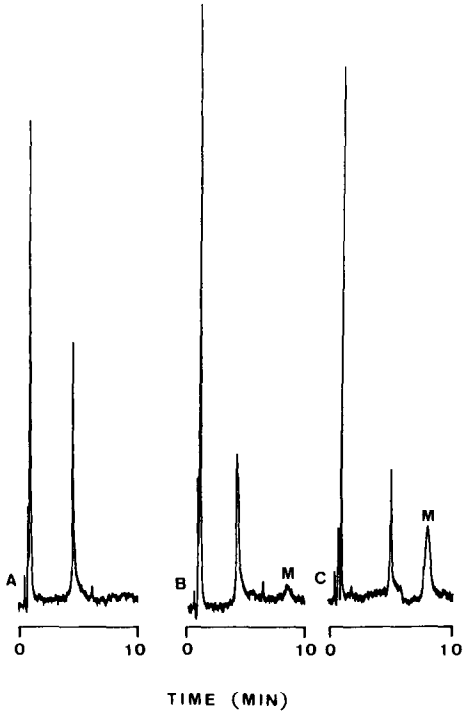

Fig. 2. Chromatograms from (A) blank plasma, (B) a plasma sample containing 4 ng/ml MMC (M) and (C) a plasma sample containing 43 ng/ml MMC. For chromatographic conditions see text.

Quantitation

Quantitation was effected by the external standard method, comparing the peak heights of a calibration graph with the peak heights from sample chromatograms. The use of an internal standard with a suitable chromophore, e.g. mitomycin A, delays the time of analysis to more than 25 min. The calibration curve was linear over the range 1–600 ng/ml [$x=(y-6.89)/1.27$, $r=0.998$, $n=8$] where y is the peak height in mV and x is the drug concentration in ng/ml. Recovery was 100% for standard solutions. Quantitation was performed by running a four-point calibration curve (1, 50, 100 and 250 ng/ml) after each patient (nine plasma samples, twice analysed).

Precision of assay

Analysis of standard curves over a period of six weeks indicated that the day-to-day coefficient of variation (C.V.) was 1.6% and the within-day C.V. was 1.2% for the concentration range 2–350 ng/ml.

Clinical application

Fig. 3 depicts the typical plasma concentration–time curve of MMC in a male patient after bolus injection with and without Spherex starch particles. As can be seen from Fig. 3 the presence of Spherex during injection into the artery leads to significantly lower plasma concentrations, indicating that the amount of the drug that circulates in the systemic blood vessel system is lower. Table I compares the pharmacokinetic parameters for MMC after bolus injection with and without Spherex. This lower concentration profile can be found

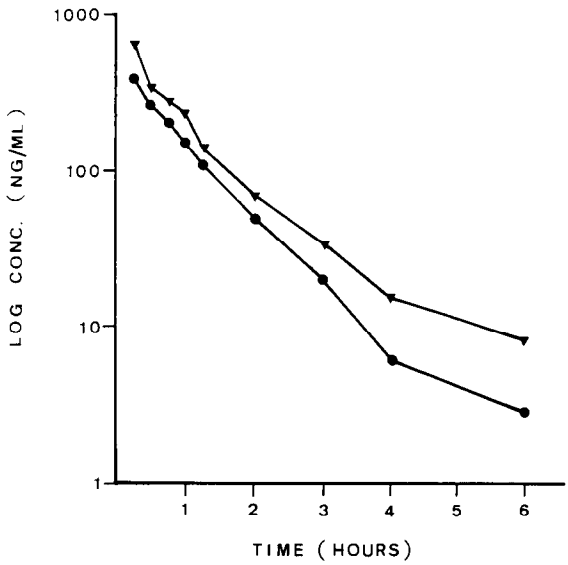

Fig. 3. Plasma concentration–time curve of an MMC-treated patient: first cycle without Spherex (▼); second cycle with Spherex (●).

TABLE I

PHARMACOKINETIC PARAMETERS OF MMC AFTER BOLUS INJECTION WITH AND WITHOUT SPHEREX STARCH PARTICLES

Dose 0.55 mg/kg body weight.

Parameter	With Spherex	Without Spherex
Initial serum concentration at time 0 (c_0) (ng/ml)	743	987
Biological half-life ($t_{1/2}$ biol.) (min)	59	79
Terminal elimination half-life ($t_{1/2}$ terminal) (min)	27	28
Area under the concentration-time curve (AUC) (μg/ml h)	28.6	39.7
Volume of distribution (V_d) (l)	37.7	28.4
Total clearance from the central compartment (Cl_{tot}) (l/min)	0.98	0.71

and verified only by a very sensitive HPLC assay, especially in the terminal elimination phase.

CONCLUSION

The proposed MMC assay provides excellent sensitivity and a rapid sample extraction step from human plasma of MMC-treated patients in special clinical studies. The method was used to monitor plasma levels in eight MMC-treated patients in two cycles of chemotherapy with and without chemobolization by Spherex particles and will be used in further pharmacokinetic and tissue distribution studies of MMC in our laboratories.

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