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Note

High-performance liquid chromatographic assay of mitomycin in biological fluids

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Mitomycin (Mutamycin™) is an anticancer agent isolated from the broths of *Streptomyces caespitosus*, and has been shown to be active against adenocarcinoma of the stomach, breast and lung in adults [1—3]. Mitomycin is a bioreductive alkylating agent and therefore inhibits DNA, RNA and protein synthesis [4—6].

The clinical use of mitomycin has been limited, although it has been under investigation since the late 1950s [7]. Although the pharmacokinetics of mitomycin have been investigated in adults, no data currently exist on the disposition of mitomycin in children with cancer. We have developed a rapid and simple extraction and high-performance liquid chromatographic (HPLC) assay for mitomycin in biological fluids so that we can determine the pharmacokinetics in children.

Fujita [8], using a non-specific microbiological assay, studied the plasma elimination of mitomycin in cancer patients at doses of 2, 10, 20 and 30 mg and reported non-linear pharmacokinetic behavior of mitomycin. However, the bioassay was less sensitive and not specific when compared to HPLC assays developed recently by several investigators [9—14]. Den Hartigh and Van Oort [9] reported a sensitive (detection limit of 1 ng/ml) and specific HPLC method for mitomycin in biological fluids which required extensive sample preparation and liquid—liquid extractions. Eksborg et al. [10], Tjaden et al. [11] and Buice et al. [12] used a solid-phase extraction procedure employing Amberlite XAD-2 resin or C_{18} Sep-Pak cartridges in order to extract the drug from the biological fluid. Mitomycin was eluted from the solid-phase columns by methanol, the methanol was collected, evaporated and finally reconstituted for HPLC injection. Van Hazel and Kovach [13] studied solid-phase extraction of

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mitomycin using a non-ionic exchange resin, Porapak Q, a gas chromatography column resin which has been employed previously for the extraction of anthracycline antibiotics from biological fluids [15]. This report describes an on-line extraction of mitomycin and the internal standard, porfiromycin, from blood and urine using an injector loop-column packed with Porapak Q, and eliminates many of the laborious steps of liquid—liquid extractions. After assay validation, this analytical method was then applied in a phase I-II clinical and pharmacokinetic study of mitomycin in children with refractory solid tumors.

EXPERIMENTAL

Materials and chemicals

Mitomycin and porfiromycin were provided by Bristol Labs. (Syracuse, NY, U.S.A.). Reagents and HPLC-grade solvents were purchased from Sigma (St. Louis, MO, U.S.A.) and Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Porapak Q was obtained from Waters Assoc. (Milford, MA, U.S.A.). Water used for HPLC was deionized, distilled and filtered with Millipore buffer filtration system.

Sample preparation

Of a 25 μg/ml stock solution of porfiromycin 10 μl were added to exactly 1.0 ml of plasma or urine (urine diluted 1:4) in a 1.5-ml Eppendorf centrifuge tube. Spiked samples were centrifuged for 1 min in a high-speed Eppendorf 5414 centrifuge and 100 μl were injected onto the loop-column. A Rheodyne (Berkeley, CA, U.S.A.) Model 7125 injector was modified by placing a 2.3 cm × 3.9 mm I.D. guard column (from Waters Assoc.) packed with Porapak Q in positions 1 and 4 of the Model 7125 injector (the normal sample loop positions). After centrifugation of the spiked plasma and urine samples, 100 μl of centrifuged sample were loaded onto the loop-column which had been washed with 2 ml of HPLC-grade water. Potentially interfering components in the biological samples were removed and mitomycin and porfiromycin concentrated on the solid phase by slowly washing the loop-column (injector still in the LOAD position) twice with 1.0 ml of HPLC-grade water. Mitomycin and porfiromycin were then eluted from the loop-column and onto the reversed-phase analytical column after the injector was switched to the INJECT position. This loop-column procedure reduces sample pretreatment and preparation time to approximately 2 min and has proven to be a very rapid and efficient sample treatment method for many anticancer drugs analyzed in our laboratory.

Chromatographic methods

The HPLC system consisted of an LDC Constametric III pump, the modified Rheodyne Model 7125 injector, a 30 cm × 4.6 mm I.D. μBondapak C_{18} (Waters Assoc.) reversed-phase analytical column (10 μm), a Varian Model UV-5 HPLC detector set at 365 nm (the λ_{max} of mitomycin) and a Spectra Physics SP 4100 computing integrator and recorder. The mobile phase was 0.01 *M* sodium phosphate buffer (pH 6.5)—methanol (70:30) and was pumped isocratically at a flow-rate of 1.5 ml/min.

Assay validation

The extraction efficiency of the loop-column procedure was calculated by dividing the mitomycin peak height and mitomycin/porfiromycin ratio in plasma extracted by the loop-column procedure by the peak height and peak height ratio of an equivalent amount of mitomycin in buffer injected directly onto the HPLC system. In order to assess the precision and accuracy, two calibrators at different concentrations of mitomycin (100 and 500 ng/ml) in plasma and urine were analyzed on five consecutive days to assess the inter-assay precision while replicate assays (n = 10) of the 250 ng/ml calibrator were performed in a single day for the determination of intra-assay precision. Calibrators of the highest and lowest concentrations were analyzed for precision on three separate days. Accuracy was determined by replicate analysis of six operator-blinded plasma samples. The calibration curves (concentration range 25—1000 ng/ml for plasma and 100—5000 ng/ml for urine) were analyzed and calculated by a linear least-squares method. Each urine calibrator was diluted four-fold before the internal standard, porfiromycin, was added.

Drug administration and sampling

Pediatric solid tumor patients were given mitomycin by intravenous infusion over 20 min at a dose of 10 mg/m^2 (n=1 patient) or 12.5 mg/m^2 (n=3). Blood samples were drawn by venipuncture or indwelling central venous catheter 15 min before the dose and at 0, 0.5, 1, 2, 4, 5, 12 and 24 h after the end of the infusion. Samples were collected in 10-ml heparinized Vacutainer® tubes. The plasma was isolated by centrifugation at 1000 g for 5 min, and stored at −70°C until analysis. Urine samples were collected in 6-h fractions for 24 h and stored at −70°C until the time of analysis.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated from the mitomycin plasma concentrations versus time data which were fit to a biexponential equation using the NONLIN least-squares computer program [16]. A weighting factor of $1/y^2$ was used for all data and the initial and terminal rate constants (α and β) were calculated using standard pharmacokinetic equations.

RESULTS AND DISCUSSION

Analytical methods

The extraction efficiency of mitomycin and porfiromycin using the non-ionic resin, Porapak Q and the loop-column ranged from 95.3 to 99.2% at mitomycin concentrations ranging from 25 to 1000 ng/ml. The calibration curves were linear over the concentration range 25—1000 ng/ml in plasma and 100—5000 ng/ml in urine. The lowest, reliable detection limit (peak height three times the baseline and assay coefficient of variation at 10%) was 25 ng/ml, which is four times more sensitive than the microbiological assay of Fujita [8] and in the range of other published HPLC methods (1—50 ng/ml). Results of the precision and accuracy studies showed an intra-assay variability <5% using calibrators of 100 and 500 ng/ml, respectively, and an inter-assay (day-to-day for five consecutive days) variability <10%. Fig. 1 shows the chromato-

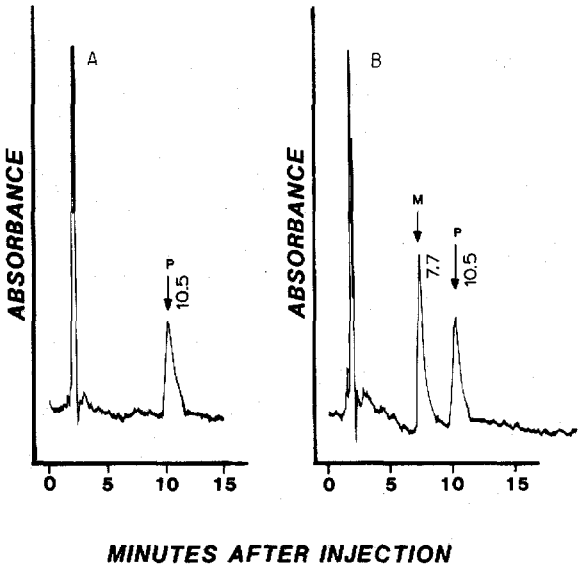

Fig. 1. HPLC profiles of pre (A) and post-dose (B) plasma samples obtained from a patient who received 12.5 mg/m² mitomycin (M) over 20 min. Both samples (1.0 ml of plasma) were spiked with 10 μl of a 25 μg/ml stock solution of porfiromycin (P), the internal standard.

grams obtained by analysis of pre- and post-dose plasma samples from a patient who received 12.5 mg/m² of mitomycin. The retention times and capacity factors (k') were 7.7 min and 4.3 for mitomycin and 10.5 min and 5.8 for porfiromycin, respectively. There were no interfering plasma or urine peaks near the mitomycin and porfiromycin peaks. Using the loop-column extraction procedure, the total time for a single sample analysis from the time the sample is thawed to the end of the chromatographic run is approximately 15 min. We are currently working on a fully automated loop-column extraction procedure using automated switching valves and two pumps which should facilitate unattended operation of the system.

Preliminary pharmacokinetics in children

Fig. 2 shows the plasma concentration versus time profile of mitomycin in four patients administered either 10 or 12.5 mg/m². The initial half-life ($t_{½\alpha}$) ranged from 8.2 to 12.8 min (average of 11.0 min) and the terminal half-life ($t_{½\beta}$) from 57.8 to 115.5 min (average of 88.2 min). The average (± S.D.) systemic clearance of mitomycin was 200.9 ± 55.1 ml/min/m². The amount of unchanged mitomycin eliminated in the patient's urine over 24 h ranged from 4.3 to 27.4% of the total dose administered intravenously.

The primary advantage of this simple, isocratic chromatographic method is the rapid, simple and efficient extraction method for mitomycin and the internal standard, porfiromycin, from plasma proteins and other plasma and urinary components by a loop-column packed with the non-ionic exchange resin, Porapak Q. Both compounds are avidly retained on the packing material during repeated, extensive washing with water and eluted with nearly 100%

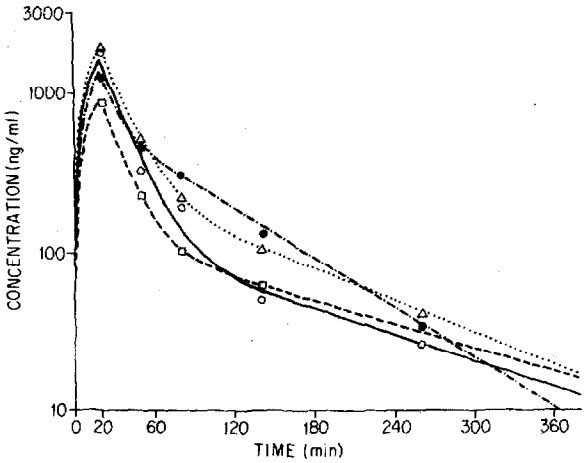

Fig. 2. Least-squares best-fit mitomycin concentration—time curves from four patients with solid tumors following 20-min intravenous infusion of 10.0 mg/m² (one patient) and 12.5 mg/m² (three patients) of mitomycin. □, 10.0 mg/m²; •, ○ and △, 12.5 mg/m².

efficiency by the organic component in the mobile phase. Previous pharmacokinetic studies have shown the concentrations of mitomycin in plasma were usually not detectable (less than 25 ng/ml) after 6 h. However, the plasma concentration versus time data within the first 6 h were adequately fit to a two-compartment pharmacokinetic model using NONLIN. This is in general agreement with other pharmacokinetic results reported in adults receiving similar dosages of mitomycin [13, 17, 18]. Previous publications in adult cancer patients who received similar dosages of mitomycin corroborate our most recent findings regarding the pharmacokinetics of mitomycin in children. The peak plasma levels and volume of distribution in the central compartment were similar as well as the elimination rate constant (K_{el}) and mean terminal half-life ($t_{½\beta}$) of 50—60 min. Systemic clearance, when normalized per body surface area (ml/min/m²) was also strikingly similar [13]. The relatively small amount of mitomycin eliminated in the patient's urine suggests that non-renal elimination (metabolism and/or biliary secretion) may be the primary route of elimination for mitomycin. We continue to monitor the plasma disappearance of mitomycin in order to evaluate the effects of altered liver function on drug disappearance using this rapid and simple HPLC procedure.

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