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Pharmacokinetics of Mitomycin C in Rabbit and Human

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Summary. *A sensitive and specific high-pressure liquid chromatographic assay was developed to characterize the plasma elimination and urinary excretion of mitomycin C in humans. Extraction of mitomycin C and an internal standard, porfiromycin, from plasma by chromatography over a non-ionic resin, Porapak Q, yields high recovery of both compounds and facilitates measurement of as little as 5 ng mitomycin C by reversed-phase high-pressure liquid chromatography. The assay was used to characterize the plasma elimination of mitomycin C in rabbits and was shown to be applicable to the characterization of the pharmacokinetics of mitomycin C in humans receiving as little as 8 mg/m^2.*

Introduction

It is over two decades since several workers in Japan reported anticancer activity of mitomycin C in humans [4]. Because subsequent studies of mitomycin C in the United States were not as encouraging, little effort was made to characterize the pharmacokinetic behavior of mitomycin C in humans. In 1971 Fujita [3], using a microbiological assay, studied the plasma elimination of mitomycin C in cancer patients given total doses of 2, 10, 20, and 30 mg. He reported that plasma elimination of the drug depended upon the amount of drug administered and concluded that intermittent administration of a large dose of mitomycin C results in a higher total amount of drug in the blood than does frequent administration of small doses. Some years prior to Fujita's observations, Hata et al. had noted that a single high dose of mitomycin C appeared to be superior to repeated smaller doses in the Ehrlich ascites murine model [7], and Kenis reported 'massive' doses to be more effective than smaller doses in human patients [8]. As Baker and Vaitkevicius point out [1], whether because of the results of Fujita or because of the above studies, several workers re-explored the clinical anticancer activity of mitomycin C given according to acute intermittent schedules and achieved an improved therapeutic index [2, 5,17]. With reports of the success of various combinations of mitomycin C against gastric [14], pulmonary [13], and breast carcinomas [9], the scheduling of mitomycin C in these programs has become the subject of considerable clinical interest. Unfortunately, most clinical studies of mitomycin C over the past decade have not been accompanied by studies of its pharmacokinetic behavior. Indeed, the observation of Fujita that mitomycin shows marked schedule dependence has never been confirmed.

Lack of pharmacologic data probably stems from problems inherent in setting up microbiologic assays and from the impossibility, without considerable effort to identify active components in crude plasma samples, of being certain that only the compound of interest is being detected. Certainly characterization of the pharmacokinetic behavior of mitomycin C in the presence of other active cytostatic agents with a microbiological assay would be difficult if not impossible. In 1979, Kono et al. [10] reported a high-pressure liquid chromatography (HPLC) assay for mitomycin C following extraction of the drug from plasma by ethyl acetate. At approximately the same time, Srivastava et al. [16], in studies of various chemically formed adducts of mitomycin C, demonstrated that mitomycin A, B, and C can be separated by reversed-phase HPLC. After studying the ability of several organic solvents to extract mitomycin C from plasma, we found that separation of mitomycin C and porfiromycin from plasma proteins and other plasma components can be accomplished with high recovery of both compounds by passage over a non-ionic

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exchange resin, Porapak Q. This procedure facilitates accurate measurement by HPLC of mitomycin C in the range of concentrations expected in humans receiving mitomycin C in combination therapy programs.

Materials and Methods

Mitomycin C was purchased from Bristol Laboratories (Syracuse, NY). Male New Zealand white rabbits were placed in a rabbit restrainer (Plas Labs, Lansing MI). Control venous blood was taken from the marginal vein of one ear. Mitomycin C was injected rapidly into the opposite ear. Blood was sampled at intervals into heparinized (143 U) venoject tubes and centrifuged at 3,000 rpm for 5 min. Red blood cells and plasma were separated and frozen at −70° C until analysis. Mitomycin C was stable in plasma stored at −70° C for at least 4 weeks.

Patients receiving mitomycin C by rapid IV injection had venous blood samples taken prior to administration of mitomycin C and then at intervals over 3 h from an indwelling catheter kept open with 5% dextrose in water. These samples were processed and frozen as described for samples from the rabbit.

At the time of analysis, known volumes (0.2–2.5 ml) of thawed red blood cells and plasma were spiked with known amounts of porfiromycin (0.1–0.2 μg/sample) as internal standard. Samples were diluted with five volumes of distilled water. Red blood cell ghosts were sedimented by centrifugation at 8,000 *g* for 90 min. Mitomycin C and porfiromycin were partially purified by column chromatography over 1 ml Porapak Q (Waters Associates, Milford, MA, USA) in 0.8 × 4 cm polypropylene columns (Econo-Columns, Bio-Rad Laboratories, Richmond, CA). Porapak resin was prepared by washing with 20 volumes of methanol and then 40 volumes of water prior to use. The samples were pipetted onto the columns and washed with 20 ml water. The mitomycin C and porfiromycin were eluted with 6 ml methanol. The entire methanol eluate was collected in conical tubes and evaporated to dryness under a stream of N_2 at 37° C. The residue was dissolved in 0.2 ml water and 0.1 ml was injected onto a Hewlett-Packard C8 10 μ reversed-phase column. Mitomycin C and porfiromycin were eluted against a gradient from 5% acetonitrile to 41% acetonitrile in water over 8 min at a flow rate of 2 ml/min in a Hewlett-Packard Model 1084B high-pressure liquid chromatograph. Both compounds were detected by UV absorption at 360 nm. After each chromatographic run, the column was washed with 100% acetonitrile for 5 min and then with 5% acetonitrile for 5 min. Gradient elution of mitomycin C and porfiromycin and re-equilibration of the column required approximately 20 min. Standard curves were calculated at the time of each analysis from pooled human plasma or rabbit plasma containing known amounts of mitomycin C and porfiromycin. Urine samples were diluted 100-fold in rabbits and 10-fold in humans, spiked with porfiromycin, and injected without purification onto the column. Mitomycin C concentrations versus time were fitted to a multi-exponential equation with the aid of a nonlinear regression computer program, NONLIN, with a weighting factor equal to the reciprocal of the concentration of mitomycin C [11].

Results

Recovery of mitomycin was 85% from 0.2 ml plasma and 65% from 2.5 ml plasma. Mitomycin C was completely separated from porfiromycin (Fig. 1). The retention times of mitomycin C and porfiromycin were 6.2 and 6.7 min. The assay was linear from 5 to 1,000 ng injected on the column (Fig. 2). By injecting 0.1 ml of the methanol fraction of our plasma samples reconstituted to the original volume of the plasma, we measured 50 ng/ml accurately.

Four rabbits were given 10 mg mitomycin C by rapid IV injection. The plasma pharmacokinetic parameters are shown in Table 1. The mean $t_{1/2}\alpha \pm$ SEM (standard error of the mean) was 2.1 ± 1.1 min (range 1.0–3.4 min); the mean $t_{1/2}\beta$ was 9.3 ± 1.1 min (range 7.1–12.1 min), and the mean clearance, 34.0 ± 7.4 ml/min/kg (range 27.5–44.3). Peak concentrations of mitomycin C in red blood cells and the disappearance of drug from red blood cells were virtually identical to those found for plasma (data not shown). Urinary excretion was measured for 4 h in one rabbit. Of the total dose, 11% was recovered over the first 30 min, 2.4% over the next 30 min, and 0.4% over the final 180 min.

Two patients, one with advanced lung cancer and one with advanced colon cancer, received mitomycin C alone at 15 mg/m^2 and one patient with advanced lung cancer received mitomycin C, 8 mg/m^2, in combination with doxorubicin, 40 mg/m^2, and *cis*-platinum, 60 mg/m^2. All three patients had normal serum creatinine values and normal hepatic function except the lung cancer patient treated at 15 mg/m^2, who had an SGOT level five times normal

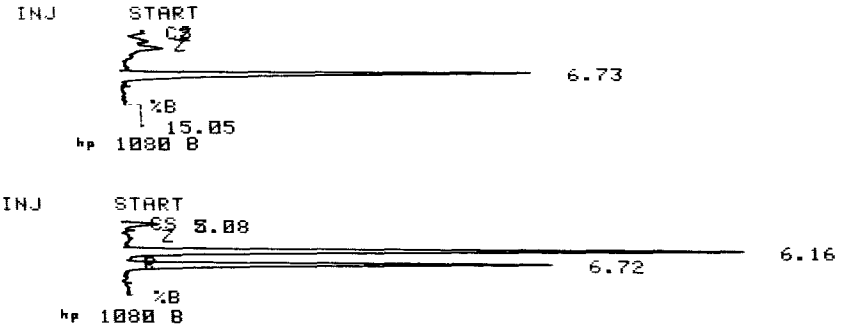

Fig. 1. Chromatograms of plasma samples obtained from a patient prior to *(above)* and 15 min following *(below)* administration of mitomycin C, 15 mg/m^2, by IV injection. Porfiromycin, 50 ng, was added to both samples

Table 1. Pharmacokinetic parameters in rabbits following rapid intravenous administration of mitomycin C (10 mg)

	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	V_1 (ml/kg)	V_2 (ml/kg)	Total body clearance (ml/min/kg)	Peak plasma level measured	
						µg/ml	Time
1.	1.4	7.1	123.9	343.2	29.82	10.3	3 min
2.	1.0	8.5	133.8	775.1	44.29	5.8	3 min
3.	2.5	9.6	174.1	992.1	34.26	10.0	3 min
4.	3.4	12.1	216.0	698.6	27.49	8.1	5 min
Mean (± SEM)	2.1 (± 0.6)	9.3 (± 1.1)	161.9 (± 21.0)	702.3 (± 134.9)	34.0 (± 7.4)	8.6 (± 1.0)	

Table 2. Pharmacokinetic parameters in humans following rapid intravenous administration of mitomycin C

	Mitomycin dose (mg/m^2)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	V_1 (ml/kg)	V_2 (ml/kg)	Clearance (ml/min/kg)	Peak plasma level (µg/ml)
1.	15.0	10.3	44.3	209.4	525.2	7.27	1.22
2.	15.0	13.2	62.0	257.6	329.2	5.16	1.25
3.	8.0	7.4	35.7	208.7	62.0	4.94	0.78

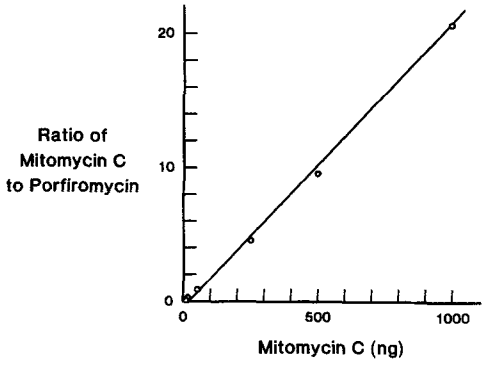

Fig. 2. A standard curve of the ratios of several concentrations of mitomycin C to a constant concentration of porfiromycin (0.5 µg/ml) in human plasma versus mitomycin C concentration

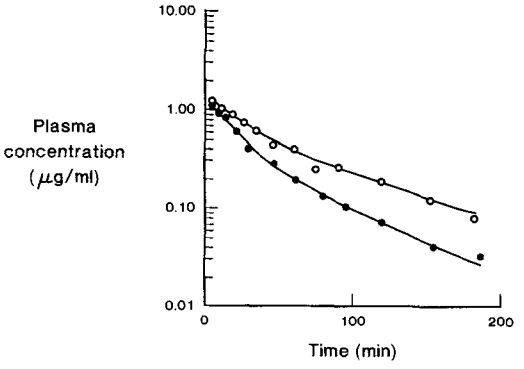

Fig. 3. Plasma concentrations of mitomycin C after rapid IV injection of 15 mg/m^2 in a patient with normal liver function (●) and in a patient with abnormal liver function (○)

and an alkaline phosphatase level twice normal. As shown in Table 2, there was rapid distribution and relatively rapid elimination of mitomycin C from plasma in the three patients. The longest secondary half-life of plasma elimination was in the patient with abnormal liver function (Fig. 3). The patient treated with the combination of drugs had received concomitant manitol diuresis (25 g mannitol in 1,000 ml D5/½ N.S. over 2 h and eliminated 8% of the total dose of mitomycin C in the urine collected 2 h after treatment.

Discussion

The primary advantage of the assay we have developed is the ease with which mitomycin C and a convenient internal standard, porfiromycin, can be separated from plasma proteins and other plasma components by chromatography over the non-ionic exchange resin, Porapak Q. Provided sufficient resin is used, virtually 100% of small (nanogram) quantities of mitomycin C present in relatively large volumes (2.5 ml) of human or rabbit plasma is bound to the resin and is retained during extensive washing with water. Both drugs are eluted completely with methanol facilitating subsequent concentration of samples.

We were surprised at the rapid secondary half-life of elimination of mitomycin C from the plasma in rabbits (range 7.1–12.1 min), compared with the secondary half-lives of elimination of mitomycin C in

three humans (range 35–62 min). The basis for the difference in plasma half-life of mitomycin C between these two species is not certain but is likely to be related to differences in rates of metabolism (inactivation) of the drug. In humans and rabbits urinary excretion does not appear to be a major route of elimination, since the majority of drug disappeared from plasma at a time when less than 10% of the drug was present in the urine.

Our values for the secondary half-life of plasma elimination of mitomycin C in humans are consistent with the data of Fujita [3]. Although Fujita reported very short half-lives based on linear plots of plasma concentration versus time, when his data were recalculated by Reich [12] from semilogarithmic plots the half-lives were 30, 33, and 49 min after total doses of mitomycin C of 10, 20, and 30 mg, respectively.

As has been demonstrated in the rat [15], we believe variations in the plasma half-life of mitomycin C in humans are likely to depend upon hepatic function. Gutierrez et al. [6] noted slow elimination of mitomycin C in one child with hepatic metastases compared with elimination of the drug in two children without liver disease. In our study, mitomycin C was eliminated most slowly in the one patient with hepatic dysfunction. We are continuing studies of plasma disappearance of mitomycin C, to look for effects of altered liver function on drug disappearance and to assess the suggestion of Fujita that mitomycin C exhibits dose-dependent pharmacokinetics.

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