Cancer Chemother Pharmacol (1986) 17: 149–152

Cancer Chemotherapy and Pharmacology

The clinical pharmacology of mitozantrone

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Summary. The pharmacological disposition of the anthracenedione mitozantrone has been measured in 11 patients with six different tumour types. Administered at 14 mg/m^2 as a 30-min infusion, the drug was assayed by a high-pressure liquid chromatographic technique sensitive to 1 ng mitozantrone/ml plasma. The mean half-lives for mitozantrone in plasma were as follows: α, 9.4 min; β, 1.6 h; γ, 23 h. The mean volume of distribution (V_d) was 1565 l. For two patients with impaired liver function the T ½ γ and V_d were 63.1 h and 4853 l, respectively. Less than 5% of the administered drug was excreted in urine, but two urinary metabolites were identified. These were not influenced by pre incubation of urine samples with β-glucuronidase or sulphatase, suggesting that neither metabolite is a glucuronide or a sulphate conjugate of mitozantrone. Hepatic metabolism is the major route of elimination of mitozantrone, and caution should be exercised when using this drug for patients with hepatic dysfunction.

Introduction

Mitozantrone, 1,4-dihydroxy-5,8-bis[(2-hydroxyethyl)amino]ethyl[amino]9,10-anthracenedione dihydrochloride, is the first of a new generation of anticancer drugs now under investigation for the treatment of breast carcinoma and haematological malignancies. Developed from a screening programme investigating compounds with structures related to the anthracyclines, mitozantrone was the most active of a series of bis-substituted (amino alkyl amino) anthraquinones when tested against a series of transplantable animal tumours including the P388 and L1210 leukaemias and the B16 melanomas [6, 11]. Since the amino sugar of the anthracyclines was lacking it was speculated that mitozantrone might not cause cardiac damage, and in preclinical tests of toxicology mitozantrone was markedly less cardiotoxic than doxorubicin in the beagle dog and cynomolgus monkey [5, 7]. Phase I clinical studies demonstrated that myelosuppression is the acute dose-limiting toxicity [1]. In phase II studies mitozantrone has been shown to have activity in breast cancer [3], non-Hodgkin's lymphoma [4] and acute leukaemia [9]. In all these studies mitozantrone has been remarkable for its patient acceptance, with a very low incidence of nausea, vomiting or alopecia. There is, however, accumulating evidence to suggest that with chronic administration mitozantrone can cause cardiomyopathy in man [3, 8, 10].

To optimise the clinical prescription of mitozantrone we have developed a highly sensitive assay for the drug in biological fluids. We report here a study evaluating the distribution and metabolism of mitozantrone in patients with a variety of solid tumours.

Patients, materials and methods

Mitozantrone was supplied by Lederle Laboratories of Cyanamid GB Ltd as a sterile solution at 2 mg free base/ml of solution.

Organic solvents methanol and acetonitrile (HPLC grade) and formic acid (HPLC grade) were obtained from Rathburn Chemicals Ltd, Peebles, Scotland. Ammonia solution 33% (Analar grade) was obtained from May & Baker. Organic solvents were vacuum-filtered through a Millipore 0.6 μM filter and aqueous solvents, vacuum-filtered through a 0.45 μM Whatman cellulose nitrate membrane filter.

Eleven patients with six different tumour types were studied. In no case had there been any prior exposure to mitozantrone. Disease sites ranged from single to multiple, five patients having ascites or pleural effusion, and two having hepatic metastases, one with grossly abnormal liver function tests, including bilirubin more than twice the upper limit of normal. No patient had impaired renal function as judged by blood urea and creatinine. Mitozantrone at 14 mg/m^2 was infused over 30 min in 100 ml 5% dextrose. Blood samples were collected via an indwelling cannula prior to infusion and at 10-min intervals for the first hour, thereafter at 1-h intervals and finally at 4-h intervals up to 48 h. Blood samples were transferred to 10-ml lithium heparin tubes and immediately centrifuged at 2500 rpm for 10 min. The separated plasma was frozen and stored at −20 °C until ready for analysis. Urine was collected at intervals up to 48 h. Volumes were measured and an aliquot of each stored in glass at −20 °C for later analysis. The pretreatment sample was also analysed.

Determination of mitozantrone

The plasma constituents were absorbed using C_{18} mini-chromatographic columns (Waters Associates) and eluted

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under vacuum. Prior to sample application, the C_{18} cartridges were washed with 10–15 ml methanol and 10–15 ml water. After application, the samples were washed with 10–15 ml 10 m*M* hydrochloric acid followed by 300 µl methanol to concentrate the mitozantrone band. The drug was then further eluted with 750 µl methanol and evaporated to dryness under nitrogen. The residue was redissolved in 300 µl mobile phase, vortexed, and centrifuged for 5 min at 3200 rpm; aliquots of 100–150 µL were then analysed using HPLC. A reverse-phase µBondapack C_{18} (3.9 mm ID × 30 cm; particle size 10 µm) Waters Associates column preceded by a guard column packed with Bondapack C_{18} corasil was used. The mobile phase, 0.55 *M* ammonium formate (pH 4.3)/acetonitrile 73:27, was delivered isocratically by a model 6000A pump (0.5 ml/min). Prior to use the mobile phase was degassed by sonication for 30 min. Sampling from limited volume inserts was by a WISP 710B autoinjector, and detection of the column eluant was monitored at 658 nm. Quantitation was by the external standard method and standard solutions of mitozantrone were prepared in the mobile phase. Peaks were automatically integrated and results calculated with a Waters 730 Data Module. Human plasma and urine at room temperature were spiked with a range of concentrations of mitozantrone and the samples processed immediately for drug quantitation by HPLC. Recovery of mitozantrone was quantitated by calibration against known concentrations of standards in the mobile phase.

Enzyme assays

The presence of possible glucuronide or sulphate conjugates of mitozantrone in patients' urine was determined. Urine samples obtained 4–8 h after treatment were incubated in 0.2 *M* Na acetate, pH 5.0, at 37 °C for 1 h with β-glucuronidase or sulphatase (low in β-glucuronidase activity). Samples were subsequently analysed by HPLC and compared with control samples at pH 5.0 without enzyme.

Results

Recovery of mitozantrone

The immediate recovery of mitozantrone from plasma samples spiked with concentrations ranging between 1 ng and 1 µg/ml at room temperature was 94.9% ± 5.01% (mean ± SD). However, in samples left at room temperature for 24 h the average recovery was 56%. For urine samples analysed immediately, recoveries of 103.5% ± 2.07%, 97.4% ± 1.53% and 95.3% ± 0.85%, respectively, were obtained for mitozantrone concentrations of 2.0 µg/ml, 500 ng/ml and 100 ng/ml, with an average mean recovery of 92.1% for samples left at room temperature for 24 h.

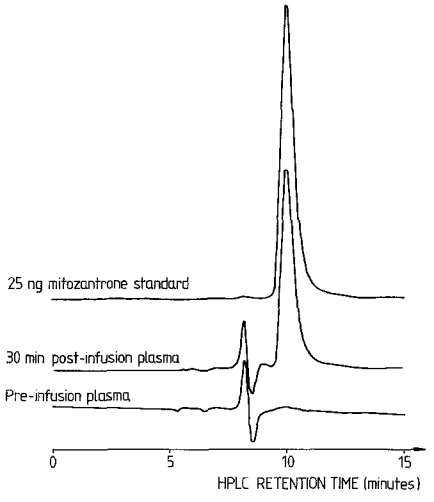

Fig. 1. Chromatographic profiles of mitozantrone in plasma

Monitoring at 658 nm produced a maximum absorption band of mitozantrone eliminating any interference from plasma constituents. This is demonstrated in Fig. 1, which illustrates the chromatographic profile of a preinfusion plasma sample and typical profiles of standard mitozantrone and mitozantrone extracted from a postinfusion plasma sample. The assay, being sensitive to 1 ng mitozantrone/ml in plasma, allows for the quantitation of drug beyond 24–48 h of treatment.

Results on Clinical Samples

Patient data are summarised in Table 1 and the corresponding pharmacokinetic data, in Table 2. The maximum plasma concentrations were achieved at the end of the infusion, with a mean value of 550 ng mitozantrone/ml plasma. For all patients there was a rapid initial decline in the plasma levels following the end of infusion. Within 1 h the plasma levels were less than 10% of the maximum plasma concentration (see Fig. 2). The initial half-life for all patients, including the one patient who was jaundiced (MD), was short, with a mean level of 9.45 min, followed by a longer second half-life β-phase of 1.61 h. The terminal elimination phase was long, strikingly so for the patients who exhibited liver involvement, whether there was elevation of the bilirubin or not (patients IB and MD). Excluding these two patients the half-life is calculated at 22.96 h and the volume of distribution calculated at 1565 l. For the two patients with liver involvement the results are 63.1 h and 4853 l.

The cumulative urinary excretion of mitozantrone as unchanged drug was low (Table 3), with less than 5% of the dose excreted in 48 h, 65% of which was accounted for in the first 4 h after treatment. In addition to unchanged drug, two other peaks were detected in urine. These presumptive metabolites were most abundant 4–8 h following infusion (Fig. 3). Both peaks were still detectable at 48 h, but at much lower levels. Table 4 summarises the cumulative urinary excretion of both metabolites expressed as total peak area units.

Following incubation of the urine samples with β-glucuronidase or sulphatase, HPLC analysis did not demonstrate any difference between the control and the test samples, suggesting that neither of the metabolites is a glucuronide or sulphate conjugate of mitozantrone.

Patient MV had malignant ascites, and 6 days after treatment an ascites sample was assayed for mitozantrone; however, no drug was detected.

Determination of mitozantrone in red blood cells during and after infusion showed a slow intracellular accumulation of drug to give a 3:1 ratio at 5 h (see Table 5).

Discussion

Using the assay described in this paper mitozantrone can be reproducibly detected in biological fluids to concentrations as low as 1 ng/ml. Excluding patients with deranged

Table 1. Clinical details

Patient	Age	Sex	Primary diagnosis of cancer	Previous chemotherapy	Sites of disease	Biochemistry	Urea/ creatinine
MV	48	F	Ovary	Yes	Ascites, iliac fossa mass	Raised LDH	Normal
MS	77	F	Breast	No	Breast, axillary nodes	Normal	Normal
RS	57	F	Ovary	Yes[a]	Ascites, nodes, pelvic mass	Raised LDH	Normal
IB	43	F	Rectum (carcinoma)	No	Liver	Normal	Normal
DC	64	M	Kidney (adenocarcinoma)	Yes	Pleural effusion, nodes, epigastric mass	Raised LDH	Normal
HS	65	F	Skin (melanoma)	Yes	Skin nodes	Raised LDH	Normal
GL	72	F	Ovary	Yes	Pleural effusion, pelvic mass	Normal	Normal
MG	51	F	Breast	Yes[a]	Bone, soft tissue	Raised alk. phos.	Normal
JD	60	F	Skin (melanoma)	Yes	Liver, lung, node, skin, pleural effusion	Raised LDH and alk. phos.	Normal
PT	56	F	Breast	Yes[a]	Bone	Normal	Normal
MD	68	F	Breast	No	Liver, bone, skin, breast	Raised bilirubin, raised γ-GT, raised alk. phos.	Normal

[a] Including doxorubicin

Table 2. Pharmacokinetics

Patient	Half-lives			Volume of distribution (l)	Plasma clearance (l/h)
	α (min)	β (h)	γ (h)		
MV	8	2.05	20	1383	55.3
MS	8	2.10	16	1271	61.6
RS	12	1.53	20.5	1637	55.4
IB	10	1.82	64.2	4545	49.5
DC	8	1.29	10.8	903	57.7
HS	10	2.07	20	1348	50.0
GL	10	1.00	11	681	46.3
MG	9	1.15	20	1230	52.8
JD	8	1.00	41	3323	51.9
PT	10	1.50	47.4	2307	34.0
MD	11	2.30	62	5161	64.0

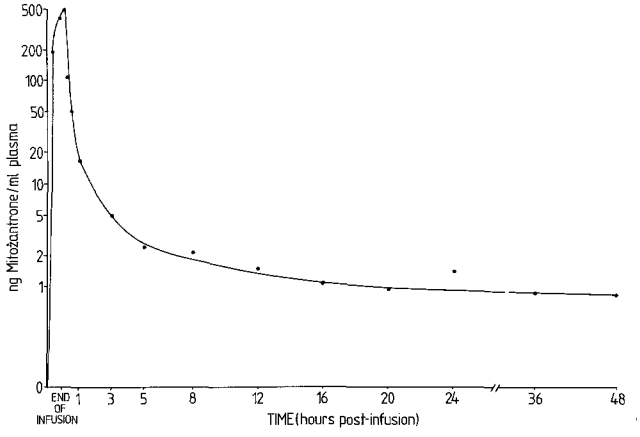

Fig. 2. Example of plasma clearance of mitozantrone (patient 1B). All patients received 14 mg/m^2 mitozantrone in 100 ml 5% dextrose infused over 30 min

Table 3. Cumulative urinary excretion of unchanged mitozantrone expressed as a percentage of dose administered

Time after infusion	$\bar{x} \pm SD$
1 h	1.31% ± 0.67%
2 h	1.81% ± 0.61%
4 h	2.06% ± 0.51%
12 h	2.52% ± 0.66%
24 h	2.76% ± 0.81%
36 h	3.09% ± 0.23%
48 h	3.26% ± 0.36%

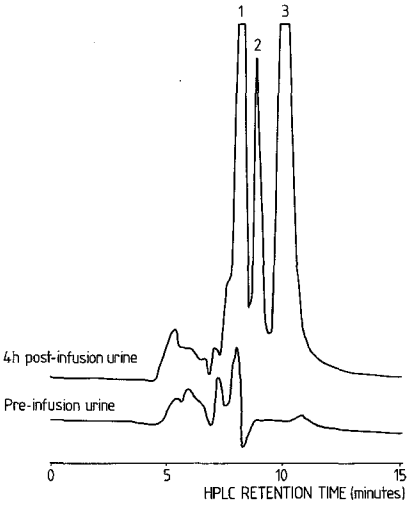

Fig. 3. Chromatographic profiles of urine samples. *1*, metabolite with retention time 8.16 min; *2*, metabolite with retention time 8.80 min; *3*, unchanged drug with retention time 9.97 min

Table 4. Cumulative urinary excretion of "metabolites" M1 and M2, expressed as peak area units

Time after infusion	M1 $\bar{x} \pm$ SD	M2 $\bar{x} \pm$ SD
1 h	1.8	0.71 ± 0.64
2 h	3.28 ± 2.52	1.99 ± 1.61
4 h	7.62 ± 7.0	4.0 ± 2.15
12 h	22.86 ± 14.0	8.59 ± 2.36
24 h	25.8 ± 18.2	8.95 ± 3.94
36 h	44.7 ± 24.0	14.9 ± 2.02
48 h	48.0 ± 26.39	15.8 ± 1.82

Table 5. Accumulation of mitozantrone in red blood cells of two patients during and after 30 min drug infusion

Time	Plasma	Red cell pellet
End of infusion	600 ng/ml	62.4 ng/ml
1 h after infusion	19.35 ng/ml	13.2 ng/ml
5 h after infusion	1.49 ng/ml	4.42 ng/ml
10 min during infusion	83.8 ng/ml	14.8 ng/ml
End of infusion	600 ng/ml	110.0 ng/ml
15 min after infusion	214 ng/ml	44.8 ng/ml
1 h after infusion	16.6 ng/ml	8.0 ng/ml

liver function the results show a β half-life of 1.6 h and a terminal phase of 23 h. This prolonged terminal phase together with the very large volume of distribution (V_d) indicates that mitozantrone distributes to a deep tissue compartment from which it is slowly released. Our data are in close agreement with those of Alberts et al. [2], who reported a V_d of 1875 ± 670 l/m^2 in five patients. The mean V_d in our eleven patients was 1565 l but in two patients V_d was markedly smaller (903 and 681) while in two others it was very large, at 4545 and 5161 l. These values correlated with short and particularly long terminal half-lives – 10.8 and 11 h, and 64.2 and 62 h, respectively.

In tissue culture experiments using the human breast cancer cell line MCF7 grown in soft agar (Courtenay assay) we have demonstrated that mitozantrone at concentrations of 1×10^{-8} *M*, 5×10^{-9} *M* and 1×10^{-9} *M* reduces cell viability to 0.6%, 7% and 43% of control, respectively. In the patient study the maximum plasma concentration achieved in patients was 10^{-6} *M* mitozantrone, falling to 5×10^{-9} *M* at 8 h, thus confirming that cytotoxic plasma levels were sustained for at least this period of time when mitozantrone was infused in this way. Our data do not indicate that fluid accumulations such as a pleural or ascitic effusion influence the elimination of mitozantrone from the peripheral compartment.

The low urinary excretion of parent drug and its metabolites suggests that hepatic metabolism is the most likely route of elimination of mitozantrone. This is supported by the prolonged terminal half-life seen in our patients whose liver function was deranged. No unexpected clinical toxicity resulted from this, but we advise caution in the use of this drug in patients with marked hepatic dysfunction, particularly if marrow reserve is compromised for other reasons.

The two urinary metabolites that were detected do not appear to represent the products of direct glucuronidation of sulphation of the parent drug, and further studies are necessary to elucidate this aspect of mitozantrone metabolism.

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Received September 30, 1985/Accepted January 7, 1986