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The Pharmacokinetics of Mitomycin C in the Mitomycin C, Ifosfamide and Cisplatin (MIC) Regimen

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Abstract: The purpose of this study was to investigate the pharmacokinetics of mitomycin C when administered with ifosfamide and cisplatin as part of the mitomycin C, ifosfamide and cisplatin (MIC) regimen. Eleven patients with advanced non-small cell lung cancer, aged 49-73 years, were treated with mitomycin C (6 mg m^{-2}), ifosfamide and cisplatin. Mitomycin C concentrations in plasma and erythrocytes were determined using HPLC with UV absorbance detection at 360 nm. The plasma β half life of mitomycin C was 44 min and the clearance 16.38 L h^{-1} m^{-2}. A mean erythrocyte/plasma ratio of 0.87 (SD±0.35) was obtained. At low plasma concentrations, mitomycin C was undetectable in the erythrocyte. The mean plasma mitomycin C concentration when this occurred was 50 ng mL^{-1} (SD±35). The plasma pharmacokinetics of mitomycin C in the MIC regimen are consistent with the pharmacokinetic data obtained as a single agent, or as a component of other chemotherapy regimens. Mitomycin C is taken up into erythrocytes and is detectable within these cells for two hs following injection. The erythrocyte may act as a transporter of this compound in the circulation.

Key words: Mitomycin C, pharmacokinetics, erythrocytes, non-small cell lung cancer

INTRODUCTION

Mitomycin C is an anti-tumour antibiotic with activity in gastrointestinal malignancies and non-small cell lung cancer (Cullen *et al*., 1988). In non-small cell lung cancer, it is commonly used with ifosfamide (I) and cisplatin (C) in the MIC regimen. Mitomycin C is a bioreductive alkylating agent and is metabolised under reducing conditions to form a bifunctional compound, which forms cross links between strands of DNA (Fracasso *et al*., 1986). This cross linking is enhanced as the pH of the environment is lowered. Activation of mitomycin C can also occur under aerobic conditions, resulting in the generation of oxygen radicals.

Initial pharmacokinetic studies of mitomycin C were restricted by a detection limit of 100 ng mL^{-1} with undetectable levels two hs after administration (Schwartz and Philips, 1961). Early data of longer serum half-lives with higher doses suggested saturable metabolic pathways (Reich, 1979), but subsequent studies have not confirmed dose dependent pharmacokinetics (Sarna *et al*., 1982; Den Hartigh *et al*., 1983; Buice *et al*., 1984; Schilcher *et al*., 1984) and plasma concentrations decay rapidly in a biphasic manner (Van Hazel *et al*., 1983; Buice *et al*., 1984; Lankelma *et al*., 1988; Dennis *et al*., 1993).

Differences in the clearance and β-half-life of mitomycin C have been observed depending on whether mitomycin C is given as a single agent or in combination with other cytotoxics, although pharmacokinetic studies of mitomycin C administered in the MIC regimen have not been described. As a possible explanation, variations in binding to blood constituents have been proposed (Den Hartigh *et al*., 1983a; Verweij *et al*., 1986). The erythrocyte is a site of potential importance as the metabolism of mitomycin C is influenced by conditions of pH and oxygen tension. NADH: cytochrome b_5 reductase, a pH dependent enzyme capable of activating mitomycin C, is present in human erythrocytes (Fisher and Olsen, 1982). There is no *in vivo* information available regarding the uptake of mitomycin C into erythrocytes, only limited in vitro data, but recent reports have

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highlighted this compartment in the clinical pharmacokinetics of cytotoxic agents, including ifosfamide (Schrijvers *et al*., 1999). We describe a pharmacokinetic study of mitomycin C, analysing both plasma and erythrocyte fractions.

MATERIALS AND METHODS

Study design and subjects: Eleven patients receiving 6 mg m^{-2} mitomycin C, as part of the MIC regimen for advanced non-small cell lung cancer, were studied during the first course of treatment. In the first four patients (patients 1 to 4), an intravenous bolus of 800 mg m^{-2} mesna was given over two min, to prevent ifosfamide associated urotoxicity, followed by a 2 min bolus injection of mitomycin C. An infusion of 3 g m^{-2} ifosfamide and 2 g m^{-2} mesna was then administered over 6 h. Pre-hydration for 2.5 hs was followed by a 3 h infusion of 50 mg m^{-2} cisplatin and post-hydration over 8 h. In the remaining seven patients (patients 5 to 11), ifosfamide and mesna was administered first, with an infusion duration of 2 h, followed by the mitomycin C as a 2 min bolus injection and then the cisplatin with hydration. Intravenous dexamethasone and ondansetron were used as antiemetics in all patients. The study protocol was approved by the Guy's and St Thomas's ethics committee, Guy's Hospital, London, UK. Written informed consent was obtained from all patients.

Blood sampling: Blood samples (5 mL) were collected in lithium heparin tubes at approximately 5, 15, 30, 60, 120, 180 and 240 min after the administration of mitomycin C. The plasma and erythrocyte fractions were processed immediately using the MESED (measurement of sediment) instrument (Driessen *et al*., 1994). This enabled the separation of the fractions without disturbing the equilibrium existing between erythrocytes and plasma; 100 mg (110 μL) of pure erythrocyte sediment was obtained and confirmed by weighing. Samples were stored at -20 °C until analysis.

Bioanalyses: The plasma and erythrocytes were analysed using HPLC with UV absorbance detection at 360 nm, providing a detection limit of 1 ng mL^{-1} (Tjaden *et al*., 1982). Blank plasma samples of 500 μL, spiked with mitomycin C, were used for the construction of calibration curves. Extracts from 500 μL aliquots of plasma were obtained using XAD-2 resin and injected onto the column. The erythrocyte sediment was totally haemolysed by adding 0.9 mL of HPLC quality water, vortexing and leaving the mixture at room temperature for 30 min. The erythrocyte lysate was extracted in the same way as the plasma samples using XAD-2 resin. The concentration in the erythrocyte compartment was determined using the spiked plasma calibration curve (Momerency *et al*., 1996), after normalisation of the concentrations measured by multiplying by the factor:-

$$\frac{500}{\text{Volume of erythrocytes (}\mu\text{L)}}$$

Plasma pharmacokinetic analysis was performed using non-compartmental methods (Holford, 1990).

RESULTS

The patient group comprised eight males and three females, with a median age of 63 years (range 49 to 73 years). The erythrocyte profile of mitomycin C closely paralleled and was slightly below that of plasma, until 126 min after injection, when mitomycin C was no longer detected in erythrocytes, despite appreciable quantities in plasma (Fig.1). A similar pattern was seen in the other patients.

The mean erythrocyte concentration/plasma concentration (E/P) ratio, when mitomycin C was detected in the erythrocyte fraction, was 0.87 (SD = 0.35; n = 28). The concentration of mitomycin C fell to undetectable levels in the erythrocyte, whilst still present in the plasma. The mean plasma concentration at which this occurred was 50 ng mL^{-1} (SD = 35) (Table 1). Mitomycin C was not detected in the erythrocyte beyond 2 h. When the haematocrit was taken into account, the mean percentage of whole blood mitomycin C associated with the erythrocyte compartment was 34% (SD = 9; n = 25).

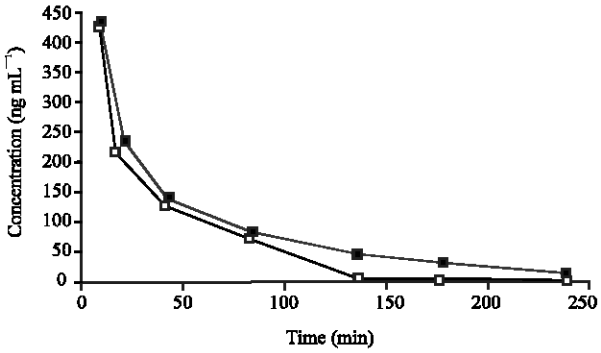

Fig. 1: Plasma (■) and erythrocyte (□) mitomycin C profiles in patient 6

Table 1: Plasma pharmacokinetic values and erythrocyte distribution of mitomycin C

	Plasma							
Patient	Clearance (L h^{-1} m^{-2})	Vss (L m^{-2})	β-half-life (min)	AUC[a] (μg min mL^{-1})	C[b] (ng mL^{-1})	Mean E/P ratio	HCT[c]	Percentage in Erythrocyte
1	15.35	10.06	33	23	77	0.66	0.419	32
2	11.81	13.41	52	30	53	0.67	0.310	23
3	13.54	13.06	47	27	31	1.01	0.302	30
4	16.88	10.55	33	21	10	0.60	0.364	25
5	15.76	10.94	31	23	32	0.86	0.302	27
6	16.36	20.88	54	22	50	0.92	0.422	44
7	21.27	36.47	76	17	88	0.66	0.434	33
8	23.60	18.50	34	15	128	0.76	0.445	38
9	14.63	11.93	44	25	18	1.01	0.341	34
10	17.17	12.13	39	21	35	1.07	0.392	40
11	13.95	9.38	36	26	26	0.59	-	-
Mean	16.39	15.21	44	23	50			
SD	3.41	7.90	14	4	35			

[a]AUC = area under the time-concentration curve. [b]C = Highest plasma concentration at which mitomycin C was undetectable in the erythrocyte. [c]HCT = haematocrit. Percentage in erythrocyte = E*HCT/(E*HCT)+(P[1-HCT]), where E and P are the concentrations of mitomycin C in erythrocytes and plasma respectively. SD = standard deviation

DISCUSSION

The plasma β half-life of mitomycin C was 44 min and the clearance 16.39 L h^{-1} m^{-2}. The plasma pharmacokinetic data are in agreement with previously published results. A plasma β half-life of 40 to 55 min, apparent volume of distribution at the steady state (Vss) values of 15 to 32 L m^{-2} and total clearances of 11 to 56 L h^{-1} m^{-2}, have been described (Den Hartigh *et al.*, 1983a; Van Hazel *et al.*, 1983; Buice *et al.*, 1984; Schilcher *et al.*, 1984; Verweij *et al.*, 1986; Lankelma *et al.*, 1988; Dennis *et al.*, 1993).

Differences in plasma mitomycin C pharmacokinetics, depending on whether mitomycin C is given alone or in combination, have been reported. Lankelma *et al.* (1988) studied repeated intravenous bolus injections of 5 to 15 mg m^{-2} mitomycin C, either alone or in combination with doxorubicin, vincristine, vindesine, bleomycin, cisplatin and 5-fluorouracil. Combination treatment was given to nine patients and in the one who received cisplatin, mitomycin C was the last drug given. The total body clearance of mitomycin C, following single agent administration, was higher after a second injection, whereas when mitomycin C was given with the other cytotoxics no such difference was observed.

A total mitomycin C clearance of 18 L h^{-1} m^{-2} when given as a single agent and one of 28 L h^{-1} m^{-2} when administered as part of combination chemotherapy, was reported by Den Hartigh *et al.* (1983a). The β half-life of mitomycin C was shorter when given with other cytotoxics. In a further study, a lower AUC and a higher total clearance of mitomycin C was observed in combination with 5-fluorouracil and doxorubicin, but the β half-life and volume of distribution were unchanged (Verweij *et al.*, 1986). However, the rates of elimination of mitomycin C given in different multiple regimens in another study were virtually identical (Van Hazel *et al.*, 1983). In six patients receiving mitomycin C, vinblastine and cisplatin, mitomycin C serum concentration-time curves approached linearity within 45 min of administration, with most concentrations below the assay sensitivity of 10 ng mL^{-1} after 180 min (Buice *et al.*, 1984). The binding of mitomycin C to erythrocytes and plasma proteins has been investigated *in vitro* (Den Hartigh *et al.*, 1983b). Incubation of an erythrocyte suspension at 37 °C resulted in an uptake of mitomycin C into erythrocytes of 57±2%. Equilibrium was reached within 10 min and the equilibrium constant was unaffected by mitomycin C concentrations of up to 6 μg mL^{-1}. The binding of mitomycin C to erythrocytes was only partially reversible and the extent of mitomycin C uptake was not influenced by doxorubicin, epirubicin, 5-fluorouracil, or cisplatin. Plasma protein binding of mitomycin C was 24±4%; the degree of binding was similar at mitomycin C concentrations up to 3 μg mL^{-1} and not affected by doxorubicin, epirubicin, or 5-fluorouracil. However the presence of high concentrations of cisplatin (20 μg mL^{-1}) led to a 30% reduction in mitomycin C binding.

Studies of the erythrocyte compartment *in vivo* have previously been limited to animals. In an early report of intravenous mitomycin C administration to rats, unwashed haemolysed erythrocytes contained scarce quantities of mitomycin C one h after injection, whilst the plasma level at this time was 600 ng mL^{-1} (Schwartz and Philips, 1961). In a later study in rabbits, the mean plasma β half-life was

9.3 min, considerably shorter than in humans (Van Hazel *et al*., 1982). Peak concentrations of mitomycin C in erythrocytes and its disappearance from this compartment, were virtually identical to those found for plasma.

It is not possible to identify the location of mitomycin C within the erythrocyte. However the effect of mitomycin C on the physical properties of the human erythrocyte membrane *in vitro*, determined by electron spin resonance, does not support membrane localisation (Korkmaz and Korkmaz, 1997). In the range 0-10 μmol (0 -3343 ng mL^{-1}) mitomycin C, there are no changes in the dynamic parameters of spin-labels inserted in the erythrocyte cell membrane, implying that mitomycin C is unable to incorporate into this structure.

The uptake of 87±35% of mitomycin C into erythrocytes during MIC chemotherapy is consistent with the reported *in vitro* study (Den Hartigh *et al*., 1983b). Mitomycin C was not detected in erythrocytes at the time of cisplatin administration and therefore it was not possible to identify any effect of cisplatin on the distribution of mitomycin C in erythrocytes.

The plasma pharmacokinetics of mitomycin C in the MIC regimen are consistent with the pharmacokinetic data obtained as a single agent, or as a component of other chemotherapy regimens. In the first 2 h following a bolus injection, it enters the erythrocyte compartment with an E/P ratio of 0.87 and 34% of whole blood mitomycin C is located in the erythrocyte compartment at plasma concentrations of greater than 50 ng mL^{-1}. This distribution may be important in the pharmacokinetics of mitomycin C, particularly regarding the differences between single agent and various combination regimens. The absence of mitomycin C in erythrocytes at low plasma concentrations requires further investigation.

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