Cancer Chemother Pharmacol (1995) 35: 318–322

ORIGINAL ARTICLE

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Conversion of mitomycin C to 2,7-diaminomitosene and 10-decarbamoyl 2,7-diaminomitosene in tumour tissue in vivo

Received: 9 May 1994 / Accepted: 8 September 1994

Abstract The progress of mitomycin C (MMC) bioreduction was studied in vivo in the rat Sp 107 mammary carcinoma after intra-tumoural injection of either 100 μg or 1 mg. 2,7-Diaminomitosene (2,7-DM) was utilised as a primary bioreductive metabolite and 10-decarbamoyl 2,7-diaminomitosene (DC 2,7-DM) served as a secondary bioreductive metabolite, both of which were measured by high-performance liquid chromatography. 2,7-DM and DC 2,7-DM were produced rapidly, achieving close to their maximal concentrations at the earliest time point studied [5 min]. 2,7-DM was cleared rapidly from the tumour with apparent half-lives of 5 and 35 min after the low and high drug doses, respectively. DC 2,7-DM had a longer apparent half-life of 130 min at the higher dose but, as compared with 2,7-DM, was only a minor metabolite [the area under the curve (AUC) of 2,7-DM was 5.6-fold that of DC 2,7-DM]. At the lower drug dose, DC 2,7-DM was not detectable. Rapid formation and disappearance of bioreductive metabolites of MMC may account for the failure of previous studies to detect these products in vivo.

Key words Mitomycin C · In vivo · Tumour · Metabolism

Introduction

Mitomycin C (MMC) is generally regarded as the prototype anaerobic bioreductive alkylating agent with a proposed mechanism of action involving quinone reduction, resulting in the formation of a number of mono- and bifunctional DNA adducts [2, 7, 8, 13, 18, 21, 22]. Three primary drug metabolites can form as a consequence of MMC monofunctional metabolic activation: 2,7-diaminomitosene (2,7-DM) and the stereoisomers 1,2-cis- and 1,2-trans-1-hydroxy-2,7-diaminomitosene (cis-hydro and trans-hydro) [13, 18, 22]. These products then act as substrates for further enzyme-catalyzed reactions to yield a series of secondary metabolites including 10-decarbamoyl 2,7-diaminomitosene (DC 2,7-DM) [15].

Despite an absolute requirement for bioreduction, to date no clinical pharmacokinetics study (or preclinical animal pharmacology study) has reported the identification of bioreductive metabolites of MMC [5, 6, 10]. Thus, little is known in vivo about the kinetics and preferred metabolic route of the key processes involved in the mechanism of action of this important cancer chemotherapeutic agent. This laboratory has recently reported high-performance liquid chromatographic (HPLC) methods capable of detecting at least five metabolites of MMC, including 2,7-DM, cis- and trans-hydro, in a sub-cutaneously (s.c.) growing rat mammary carcinoma (Sp 107) after intra-tumoural (i.t.) drug administration [1, 3]. In the present work, the progress of MMC bioreduction was studied in vivo in tumour tissue after i.t. drug administration by determining levels of primary (2,7-DM) and secondary (DC 2,7-DM) bioreductive metabolites.

Materials and methods

Drug-analysis techniques

All chromatographic analyses were carried out using a Hewlett-Packard Model 1090 liquid chromatograph equipped with a diode-array detector, and the chromatographic conditions were those previously described elsewhere in detail [1]. Standards of cis-hydro and trans-hydro were synthesised in house by treatment of MMC with 0.1 *M* hydrochloric acid for 25 min at room temperature. Authentic standards of 2,7-DM and DC 2,7-DM were a kind gift from Prof. Maria Tomasz (Department of Chemistry, Hunter College, New York, USA). Extraction of MMC and its metabolites from homogenised tumour tissue was done as previously reported [3].

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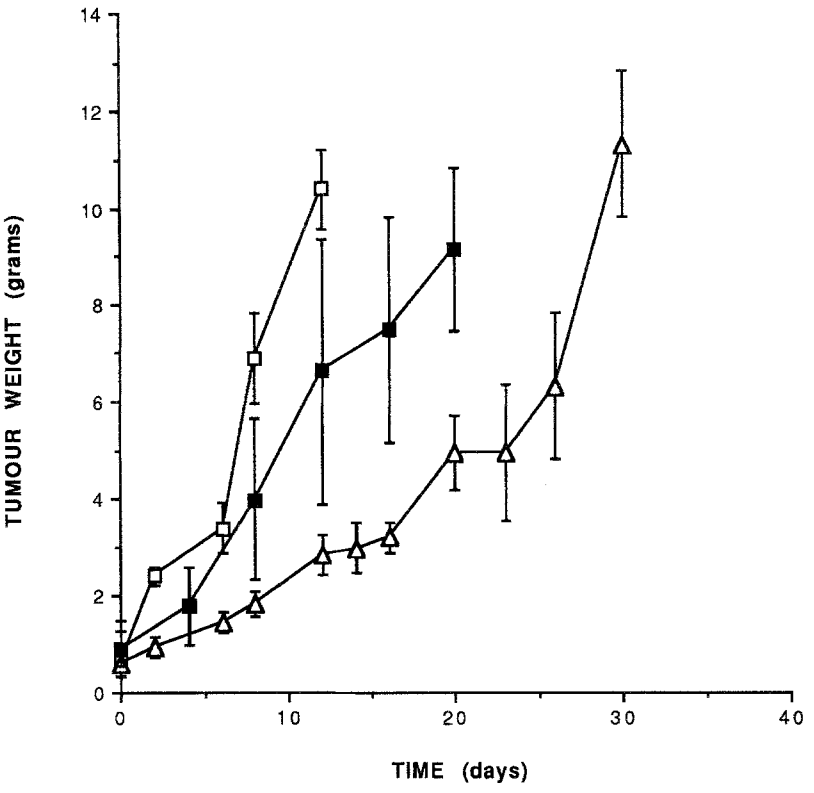


Fig. 1 Antitumour activity of MMC against the rat Sp 107 mammary carcinoma after direct i.t. injection. □ —— □, Control (0.1 ml SDW); ■ —— ■, 100 μg MMC in 0.1 ml SDW; △ —— △, 1 mg MMC in 0.1 ml SDW. Each point represents the mean value ± SD for $n = 6$ animals

Animal model and in vivo drug treatments

The animal model consisted of inbred rats of the WAB/NOT strain and the Sp 107 mammary carcinoma [9]. Animals were kept under standard laboratory conditions and fed on standard laboratory chow. The tumour was maintained by s.c. passage. When tumours became palpable, animals were randomised into three experimental groups containing six animals each for antitumour studies. The first group, which acted as a control, received an i.t. injection of 0.1 ml sterile distilled water (SDW); the second group received 100 μg MMC in 0.1 ml SDW and the third, 1 mg MMC in 0.1 ml SDW. Tumour volumes were determined as described elsewhere [9]. Antitumour activity was assessed as a delay in the number of days required to undergo four tumour doublings. In pharmacokinetic experiments, tumours were allowed to attain a size of 2–2.5 cm in diameter and five animals per time point were killed at the following times after drug administration: 5, 15, 30, 60, 120 and 240 min. Animals were dosed i.t. with 100 μg and 1 mg MMC as described above. Once tumours had been removed they were washed in SDW, then placed in liquid nitrogen and stored at –40 °C prior to drug analysis.

Pharmacokinetic analysis

Peak concentrations and time-to-peak levels for MMC, 2,7-DM and DC 2,7-DM were taken directly from concentration/time profiles. The area under the concentration/time profile (AUC) was calculated by the trapezoidal rule. Tumour parent drug concentration/time profiles were best fitted to a monoexponential decline, and terminal half-lives were calculated from the gradient of the best-fit line. Tumour metabolite concentration/time profiles were not modeled but an apparent half-life was derived from the terminal decline portion of the curve by non-linear regression analysis.

Results

Antitumour studies

Two different i.t. doses were utilised in the present set of experiments: 100 μg and 1 mg. The antitumour activity of MMC at these doses is shown in Fig. 1 and the growth delays produced are contained in Table 1.

Table 1 Time course of MMC metabolic activation in vivo in the rat Sp 107 mammary carcinoma after i.t. administration of drug at two different dose levels. The metabolite 2,7-DM is a product of drug monofunctional metabolic activation and DC 2,7-DM is a product of bifunctional activation (*PC* peak concentration, *t* time to peak concentration, *NE* not evaluable, *ND* not detectable)

	MMC			2,7-DM				DC 2,7-DM				Antitumour
	AUC[a] (μg/g min)	PC (μg/g)	Half-life (min)	AUC (μg/g min)	PC (μg/g)	*t* (min)	Half-life (min)	AUC (μg/g min)	PC (μg/g)	*t* (min)	Half-life (min)	activity[b] (days)
1 mg	708	10.3	36	560	8.4	30	35	97	1.0	30	130	18
100 μg	32	1.2	24	16	0.93	5	NE		ND			8

[a] Area under the tumour concentration/time curve as measured from 0 to 120 min

[b] Antitumour activity was measured as a delay in the number of days required to undergo 4 tumour doublings

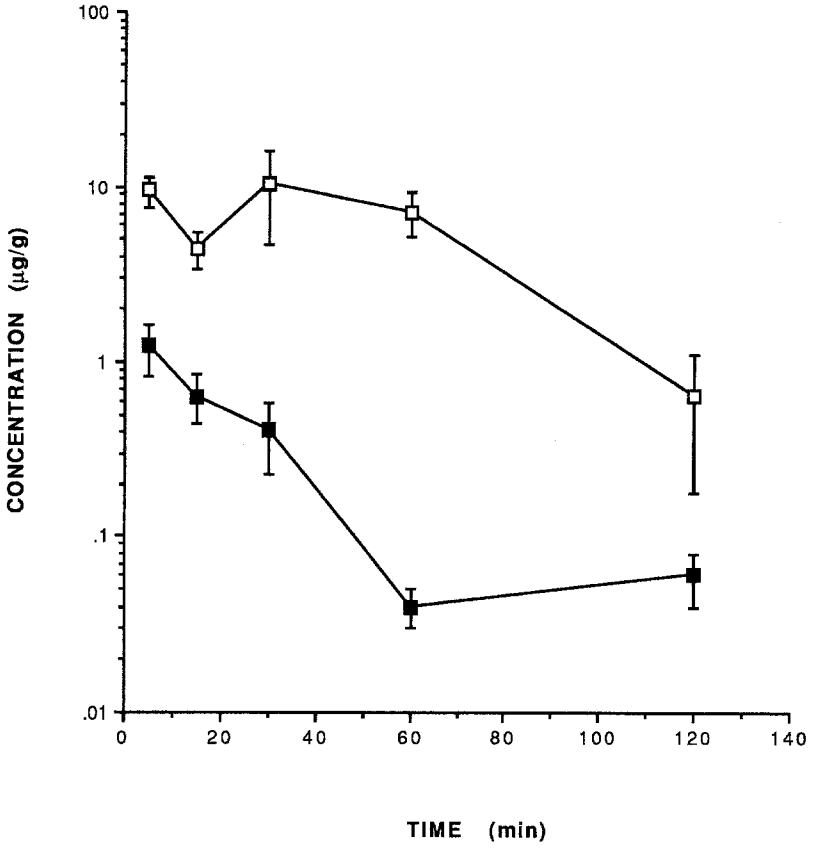


Fig. 2 Concentration/time profiles of MMC in the Sp 107 mammary carcinoma after direct i.t. injection. □ —— □, 1 mg; ■ —— ■, 100 µg. Each point represents the mean value ± SE for $n = 5$ animals

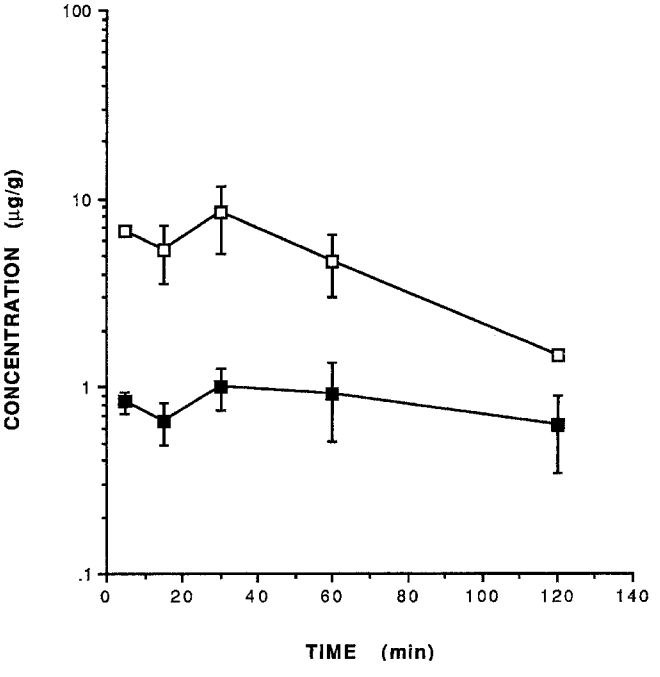
Pharmacokinetics of MMC in the Sp 107 rat mammary carcinoma after i.t. injection

MMC tumour concentration/time profiles obtained after i.t. drug administration of either 100 µg or 1 mg are shown in Fig. 2 and the pharmacokinetic parameters derived from these plots are contained in Table 1. The tumour half-life of MMC was 24 min for the lower dose and 36 min for the higher dose.

Pharmacokinetics of 2,7-DM and DC 2,7-DM in the Sp 107 rat mammary carcinoma after i.t. injection of MMC

Concentration/time profiles for 2,7-DM and DC 2,7-DM are illustrated in Fig. 3 and the pharmacokinetic parameters derived from these plots are contained in Table 1. Only trace levels of cis-hydro and trans-hydro were detected and, consequently, their pharmacokinetics could not be followed. This could indicate that MMC bioreduction in the Sp 107 tumour occurred in a more acidic environment, since that condition favours the formation of 2,7-DM over that of cis- and trans-hydro [15, 20]. 2,7-DM was generated to a much greater level as compared with DC 2,7-DM (5.6-fold larger AUC; 8.4-fold higher peak concentration). However, DC 2,7-DM had a considerably longer half-life of 130 min (see Table 1). Both of these results may reflect that DC 2,7-DM is formed from 2,7-DM through a complex process that is dependent on many kinetic factors [22]. The

Fig. 3 Time course of MMC bioreduction in vivo in the rat Sp 107 mammary carcinoma after i.t. injection of 1 mg MMC. 2,7-DM is the primary bioreductive metabolite and DC 2,7-DM is the secondary bioreductive metabolite. □ —— □, 2,7-DM; ■ —— ■, DC 2,7-DM. Each point represents the mean value ± SE for $n = 5$ animals



main feature of the kinetics of 2,7-DM was its rapid appearance at concentrations close to that of the parent drug at the earliest time point studied (5 min), followed by its rapid disappearance with a half-life of 35 min at the higher dose studied. 2,7-DM has previously been shown to have a half-life of only 13 min in vitro in incubations with either cytochrome P-450 reductase or xanthine oxidase [15]. DC 2,7-DM was also generated quickly at the higher dose but was not detectable at the lower dose.

Discussion

The progress of MMC bioreduction was studied in vivo in a s.c. growing rat mammary carcinoma after i.t. injection of drug. While i.t. drug treatment is not the normal route of MMC administration, although it has occasionally been employed in certain types of human cancer (e.g. pancreatic cancer [14]), it was adopted in an attempt to model processes that to date have proved inaccessible to in vivo study. The observation that the elimination phase of the pharmacokinetics of MMC after tumoural injection followed closely its elimination rate from plasma and tissues after systemic drug treatment may validate the use of this route of administration [5, 12].

The main finding of this work can be summarised as follows: bioreduction of MMC appears to occur very rapidly after instillation of the drug into the tumour but extends only over a relatively short period. At present the significance of 2,7-DM formation in relation to DNA alkylation (and, hence, cytotoxicity) is unclear since these processes may be viewed as competing pathways for a putative quinone methide-reactive intermediate evolved during MMC metabolic activation [13, 18, 22]. Nevertheless, recent studies have shown that 2,7-DM levels do correlate with MMC cytotoxicity in human colon-cancer cell lines [20] and, more significantly, that 2,7-DM levels correlate better than MMC levels to antitumour activity in murine colon cancer [4]. In addition, N-7 monoalkylation of guanine appears to have an absolute requirement for generation of 2,7-DM [19]. Therefore, it seems probable that 2,7-DM formation does reflect to a large degree the level of MMC metabolic activation. From this point of view it is interesting to compare the present in vivo results with published findings on MMC metabolic activation from in vitro studies. Enzymatic reduction of MMC by purified cytochrome P-450 reductase, xanthine oxidase or DT-diaphorase yields almost exclusively (at least 90% of total drug binding to DNA) monofunctionally activated DNA adducts [11, 19, 22]. Secondly, the kinetics of DNA covalent binding after treatment of cells with MMC, or incubation of the drug with purified enzymes, shows that DNA adducts are formed in a rapid burst of activity that occurs during the first 6 min of drug incubation [16, 17]. By 20 min the levels of monofunctional DNA adducts appeared to plateau, after which there was a steady rise in the formation of cross-links for a further 70 min, indicative of a slower bifunctional metabolic process [16].

In summary, the results presented in this report confirm, possibly for the first time in vivo, that MMC bioreduction occurs rapidly and for a short time. The transient nature of the metabolites of MMC evolved in the Sp 107 tumour, if typical for other tissues, may explain why previous clinical pharmacokinetics studies have failed to detect metabolites in patients' plasma [5, 6, 10, 12].

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