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Influence of Ascites on the Pharmacokinetics of Hexamethylmelamine and *N*-Demethylated Metabolites in Ovarian Cancer Patients*

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Abstract—*Levels of hexamethylmelamine (HMM) and its metabolites pentamethylmelamine (PMM), $N_2N_2N_4N_6$ tetramethylmelamine (TMM) and $N_2N_4N_6$ trimethylmelamine (TriMM) were determined in plasma of* 31 *patients with advanced ovarian cancer receiving HMM. In* 14 *of them with ascites the drug metabolites PMM, TMM and TriMM were assayed in this fluid. Both in plasma and in ascites a GLC method with nitrogen detection was applied after extraction with* n-*hexane and ethyl acetate at pH* 11.5. *Plasmatic HMM* $T/2\alpha$ *and* $T/2\beta$ *were respectively* 47 ± 6 *and* 403 ± 37 min. *Mean* $AUC_{o \to 12\ hr}$ *values* ±*S.E.M., expressed in* μg/ml × min, *were* 312 ± 95 *for HMM,* 117 ± 32 *for PMM,* 260 ± 50 *for TMM and* 311 ± 82 *for TriMM. AUCs of HMM, PMM, TMM and TriMM in ascites calculated up to* 6 *hr were* 78 ± 13, 31 ± 7, 60 ± 12 *and* 129 ± 38 μg/ml × min *respectively. The presence of ascites, the patient's age, the concomitant administration of adriamycin and cyclophosphamide and fatness did not appear to influence the kinetic behaviour of HMM and metabolites. HMM was not detectable in urine and PMM, TMM and TriMM accounted for less than* 1% *of the HMM dose.*

INTRODUCTION

ONLY limited information is available on the influence of neoplastic effusions on the pharmacokinetics of anticancer agents. As about 50% of advanced ovarian cancer patients present ascites, it could be relevant to know whether the disposition of drugs used in the therapy of this malignancy is modified by the presence of this peritoneal fluid compartment. Further, the concentrations of drugs in ascites may have a direct cytotoxic effect on cancer cells floating in the effusion or implanted as micrometastases in the peritoneum.

In this study we compared the pharmacokinetics of hexamethylmelamine (HMM)—an established drug for ovarian cancer [1]—and of some *N*-demethylated metabolites in two populations of ovarian cancer patients, similar except for the presence of ascites. We also determined the concentrations of these compounds in ascites. Other factors possibly influencing the pharmacokinetics, such as age, fat constitution or concomitant treatment with other anticancer agents, were also analysed.

MATERIALS AND METHODS

Patients

Thirty-one patients were studied, all with epithelial ovarian cancer, stage III or IV (FIGO), and normal liver and renal function. Fourteen patients presented ascites; they were aged between 16 and 77 years (Group A); 17 patients without ascites were aged between 28 and 70 years (Group B). The patients were classified as being of fat, normal or thin constitution on the basis of average weight tables for women of different weight and age [2].

The oral doses of HMM ranged from 115 to 200 mg/m^2 and differences in doses were well balanced in the two groups. Five patients in group A and 7 in group B received adriamycin (50 mg/m^2 i.v.) and cyclophosphamide (70 mg/m^2 i.m.) in combination with HMM.

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Sample collection

Before HMM administration and at intervals up to 12 hr blood samples of 3 ml were taken from an arm vein; 6 ml of ascites were taken by paracentesis through a sylastic drain at the same intervals, but only in 3 particularly cooperative patients was it possible to continue for 12 hr, the others lasting only 6 hr.

Urine samples were collected during 24 hr after HMM treatment in 10 patients (5 in group A and 5 in group B) and 8 ml were stored at −20°C until analyzed.

Analytical assay

Half a milliliter of plasma, ascites or urine was made alkaline with NaOH (pH 11.5) and extracted once with *n*-hexane and once with ethyl acetate. Then a GLC method with nitrogen detector, previously described [3], was applied. HMM, pentamethylmelamine (PMM), $N_2N_2N_4N_6$ tetramethylmelamine (TMM) and $N_2N_4N_6$ trimethylmelamine (TriMM) used as standards were kindly supplied by NCI, NIH, Bethesda, MD, U.S.A.

Pharmacokinetic analysis

We applied the peeling method to the decreasing plasma concentrations after the peak and calculated the pharmacokinetic parameters on a Hewlett Packard 9810 computer. In 12 cases there were not enough points to calculate the first disappearance phase after the plasma peak and only the concentrations measured from 4 or 5 to 12 hr were considered in calculating the elimination half-life. The areas under the curve of concentration vs time (AUC) were calculated by the trapezoidal rule.

RESULTS

Figure 1 depicts mean levels of HMM in plasma and ascites from patients in group A. Figure 2 shows the plasma concentrations in patients without ascites (group B). Both populations showed considerable variability in HMM plasma levels and no appreciable differences were found between the two groups.

Table 1 shows the pharmacokinetics parameters of HMM and metabolites PMM, TMM and TriMM with and without ascites. The high HMM peak level found in one patient (20.8 μg/ml) raises the mean in group B, but if this patient is omitted the mean peak falls to 1.3 ± 0.4 μg/ml, which is close to the 1.6 ± 0.3 found in group A. The distribution half-life ($T/2\alpha$) in groups A and B was 42 ± 9 and 53 ± 9 min respectively, the elimination half-life ($T/2\beta$), 402 ± 81 and 412 ± 37 min. Mean plasma AUC values of the parent compound and metabolites appeared to be slightly higher, though not significantly different, in the group of patients without ascites. The overall (groups A + B) mean $AUC_{0\to 12\,hr}$ values ± S.E.M., expressed in μg/ml × min, were 312 ± 95 for HMM, 117 ± 32 for PMM, 260 ± 50 for TMM and 311 ± 82 for TriMM. AUCs of HMM and metabolites PMM, TMM and TriMM in ascites calculated up to 6 hr were 78 ± 13, 31 ± 7, 60 ± 12 and 129 ± 38 μg/ml × min respectively, lower than the corresponding plasma values.

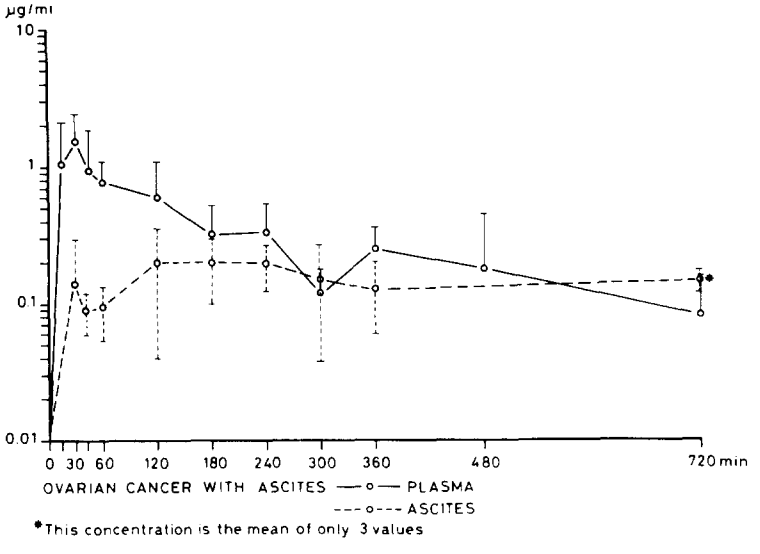

Fig. 1. Mean plasma levels (±S.E.M.) of HMM ——— and mean ascites levels (±S.E.M.) of HMM -------- in ovarian cancer patients with ascites (group A). At 720 min the mean was calculated only on 3 values.

Table 1. Pharmacokinetic parameters of HMM and of the metabolites PMM, TMM and TriMM in patients with or without ascites

	HMM						PMM		TMM		TriMM	
	Peak level μg/ml	Time min	Half life α min	Half life β min	$AUC_{\rightarrow 12\,hr}$ plasma μm/ml × min	$AUC_{\rightarrow 6\,hr}$ ascites μg/ml × min	$AUC_{\rightarrow 12\,hr}$ plasma μg/ml × min	$AUC_{\rightarrow 6\,hr}$ ascites μg/ml × min	$AUC_{\rightarrow 12\,hr}$ plasma μg/ml × min	$AUC_{\rightarrow 6\,hr}$ ascites μg/ml × min	$AUC_{\rightarrow 12\,hr}$ plasma μg/ml × min	$AUC_{\rightarrow 6\,hr}$ ascites μg/ml × min
With ascites $\bar{m} \pm$ S.E.M.	1.5 ± 0.3	107 ± 21	42 ± 9	402 ± 81	216 ± 52	78 ± 13	69 ± 17	31 ± 7	201 ± 62	60 ± 12	289 ± 132	129 ± 38
Range	0.25–4.28	30–240	15–95	140–821	7–786	12–174	12–185	4–72	24–497	9–135	50–643	17–312
Without ascites $\bar{m} \pm$ S.E.M.	2.5 ± 1.2	85 ± 11	53 ± 9	412 ± 37	397 ± 174		163 ± 60		301 ± 74		339 ± 106	
Range	0.12–20.8	30–180	30–81	294–602	34–2915		4–747		15–786		136–494	
Total $m \mp$ S.E.M.	2.01 ± 0.64	95 ± 11	47 ± 6	403 ± 37	312 ± 95	78 ± 13	117 ± 32	31 ± 7	260 ± 50	60 ± 12	311 ± 82	129 ± 38
Range	0.12–20.8	30–240	15–95	140–602	7–2915	12–174	4–747	4–72	15–786	9–135	50–643	17–312

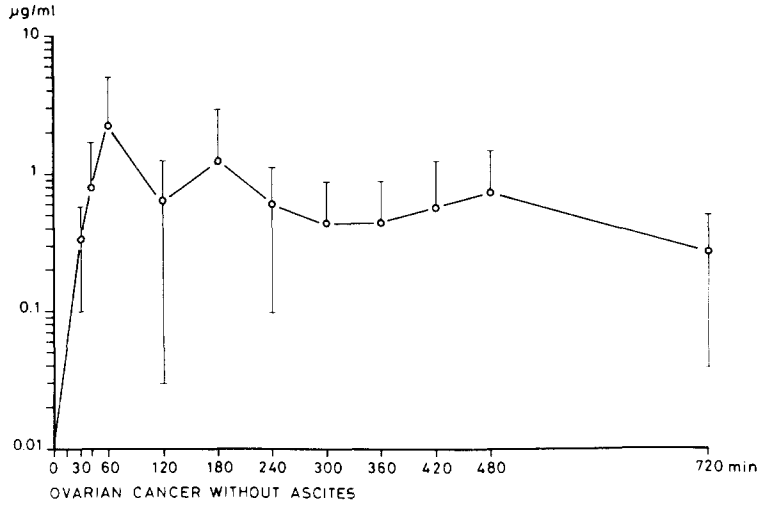

Fig. 2. Mean plasma levels (±S.E.M.) of HMM in ovarian cancer patients without ascites (group B).

Plasma AUC values were also analyzed according to factors other than ascites, such as age, other drugs combined or not and the patient's fat, normal or thin constitution. As can be seen in Fig. 3, none of these factors appeared to influence drug disposition.

HMM as unchanged drug was not detectable in the urine of 10 patients investigated. PMM, TMM and TriMM were excreted in small amounts. In the urine 24 hr after HMM administration 68 ± 25, 1517 ± 332 and 1000 ± 231 μg respectively were found, accounting altogether for approximately 1% of the HMM dose.

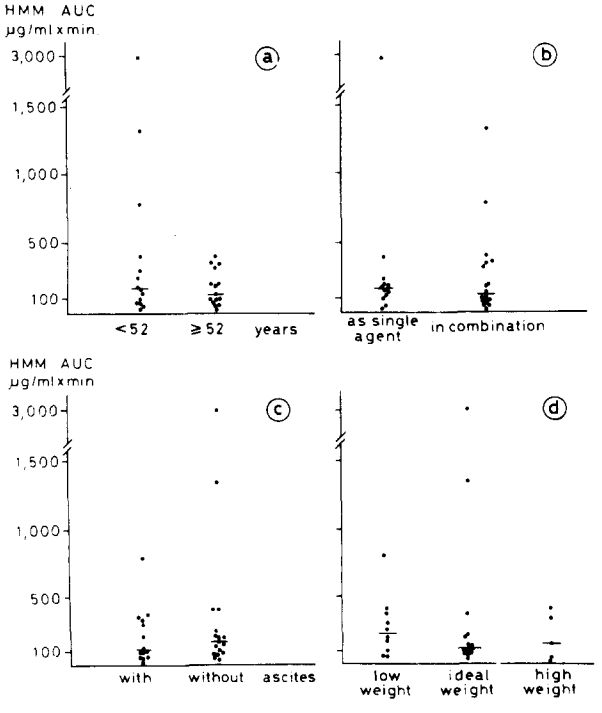

Fig. 3. AUC values of HMM in ovarian cancer patients according to age (a); other drug therapy (b); presence of ascites (c); and body weight (d).

DISCUSSION

As already reported [4], plasma levels of HMM vary widely among patients receiving the drug. A similar high variability was found in the present study for the concentrations of the *N*-demethylated metabolites PMM, TMM and TriMM. Such variability in the disposition of HMM and metabolites may be one of the reasons for the variable response shown by patients treated with similar doses of HMM. No differences were found between ovarian cancer patients with or without ascites. Although any such differences would be difficult to demonstrate because of the wide variability in both groups, the finding that concentrations of the drug and metabolites were lower in the effusion than in plasma provides evidence of the lack of influence of a 'third space' on the pharmacokinetics of HMM. As it has been reported that the higher the polarity of a compound the greater its tendency to concentrate in the peritoneal compartment [5], it might be expected that the more polar *N*-demethylated metabolites would reach higher concentrations in ascites than the parent compound. However, even though PMM and TMM are more water-soluble than HMM, their polarity is relatively low too, as indicated by their *n*-octanol/water partition coefficients, respectively 67 and 16 [6]. The fact that ascites appears not to influence the pharmacokinetic behaviour of HMM suggests there is no need to modify the drug dosage in patients presenting neoplastic effusions.

Though the mode of action of HMM has not yet been elucidated, and it is still not clear whether metabolites such as hydroxymethylmelamines, formed as intermediates of *N*-demethylation [7], are important for HMM

activity, there is an indication that 0.1 μg/ml of HMM can inhibit nucleic acid synthesis in a human ovarian cancer cell line *in vitro* [8] without necessarily requiring metabolic activation. The drug concentrations in ascites, particularly in the first hours after HMM ingestion, are lower than in plasma—approximately 0.1 μg/ml for at least 12 hr, so they could just be cytotoxic against malignant cells present in ascites or against peritoneal micrometastatic foci.

Age, other associated drugs or constitution appeared not to influence the kinetic behaviour of this compound, which probably depends more on individual variability in absorption and metabolism. Both factors are under detailed investigation at the moment in our laboratory.

The finding that renal excretion of HMM, PMM, TMM and TriMM is negligible in man is in line with previous reports, indicating N_2N_4 dimethylmelamine as the major urinary metabolite of HMM [9, 10].

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