Cancer Chemother Pharmacol (1983) 11:51–55

Cancer Chemotherapy and Pharmacology

Distribution, Metabolism, and Irreversible Binding of Hexamethylmelamine in Mice Bearing Ovarian Carcinoma

Enrico Garattini, Tina Colombo, Maria Grazia Donelli, Paolo Catalani, Marina Bianchi, Maurizio D'Incalci, and Claudio Pantarotto

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea, 62, I-20157 Milan, Italy

Summary. *The covalent binding of hexamethylmelamine (HMM) and its metabolites was studied in liver, tumor, blood, kidney, spleen, lung, brain, heart, and small intestine after a single IP injection of 2,4,6-^{14}C-hexamethylmelamine (50 mg/kg) to C57Bl/6J female mice bearing 20-day-old M5076/73A ovarian cancer. Covalent binding to tissue macromolecules was measured 2, 10, and 40 h after injection of the drug. At 2 h liver and small intestine showed the highest levels of irreversibly bound metabolites, the lowest being found in brain and heart. Except in the small intestine, where a decrease was observed between 2 and 10 h, the level of covalent binding was constant up to 40 h.*

HMM metabolism was also studied. Tissue distribution of pentamethylmelamine (PMM), 2,2,4,6-tetramethylmelamine (TMM), and 2,4,6-trimethylmelamine (TriMM) was determined at the three times considered. At 2 h the drug was already extensively metabolized, TriMM being the major metabolite among those determined.

Introduction

HMM or 2,4,6-dimethylamino-*s*-triazine is known as an antineoplastic agent that is especially active in some human malignancies, such as lung and ovarian tumors [10]. Its plasma levels and urinary excretion have already been studied in animals and man [2–5], and its metabolism is known to proceed via a series of oxidative *N*-demethylation steps [7, 12], as shown in Fig. 1.

The mechanism of action of HMM is not yet known, but it can be speculated that the cytotoxic potential may arise from the capacity of HMM metabolites to bind covalently to tissue macromolecules such as DNA, RNA, or proteins. From this point of view the drug does not seem to be a direct alkylating agent like its congener triethylenemelamine, as the molecule appears to need metabolic activation to bind covalently to tissue macromolecules. Recently we reported that in vitro the microsomal fractions from liver and ovarian tumor both had the capacity to metabolize HMM, with the formation of reactive products capable of interacting covalently with microsomal proteins [6]. In the same report we stated that in vivo too, covalent binding of HMM and metabolites to total tissue macromolecules in liver and M5076/73A ovarian tumor in mice was detectable 40 h after injection of radiolabeled HMM.

Reprint requests should be addressed to C. Pantarotto

These preliminary findings prompted us to perform a more systematic study of the covalent binding of anticancer agents to cellular macromolecules [8]. Clarification of this parameter could well assist in explaining the mechanism of action of these drugs and also in the interpretation of their adverse effects.

Materials and Methods

Chemicals. 2,4,6-^{14}C-HMM (specific activity 13.0 mCi/mmol) was used; its radiochemical purity was checked by bidimensional thin-layer chromatography on silicia gel glass plates, which were developed in both dimensions in a solvent system consisting of chloroform : methanol : acetic acid (88 : 10 : 1.5). A second check was made by high-pressure liquid chromatography. Analysis was performed using an isocratic solvent system of 0.01 *M* ammonium formate and acetonitrile (1 : 1) and a μ Bondapak C_{18} column [7]. The labeled drug was found to be more than 99% pure and therefore was utilized as such without being further purified. ^{14}C-Labeled HMM and unlabeled reference standards of PMM, TMM, and TriMM were obtained from the Division of Cancer Treatment, NCI, NIH, Bethesda, Md, USA. All solvents were of the purest grade commercially available.

Treatment of Animals. Nine C57Bl/6J female mice bearing 20-day-old M5076/73A ovarian cancer received IP injections of radiolabeled HMM (25 mCi/kg, corresponding to a dose of 50 mg/kg) dissolved in acetone-corn oil (1 : 9 v/v). The animals were fasted until scheduled death.

Determination of Total Radioactivity (HMM + Metabolites) and Covalent Binding to Macromolecules in Different Tissues and Blood. Three groups of three animals were killed 2, 10, and 40 h after the treatment with radiolabeled HMM. Blood was collected by intracardiac injection, and tissues for analysis were excised. All the samples were kept at −20° C until analysis. Covalent binding to tissue macromolecules and total radioactivity (HMM + metabolites) were determined as already described [6]. Determinations were run in triplicate. The volume of phosphate buffer used for the tissue homogenization ranged from 4 to 10 times the weight of the different tissues. For covalent binding studies, when the amount of acetonitrile-precipitable cellular macromolecules was small a suitable quantity of cold bovine serum albumin (BSA) was added.

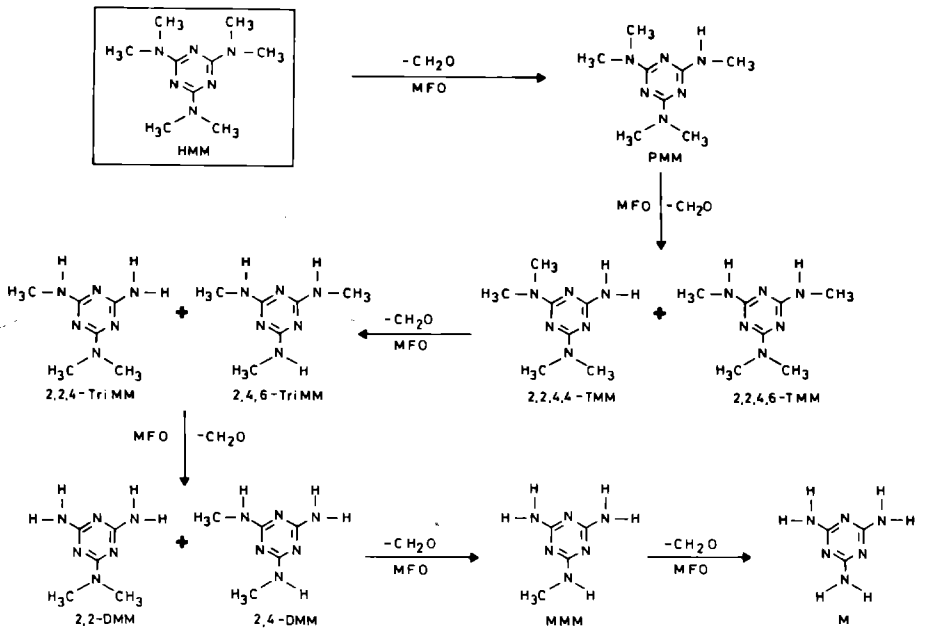

Fig. 1. In vivo oxidative metabolic *N*-demethylations of HMM leading to the melamine moiety. DMM, dimethylmelamine; MMM, monomethylmelamine; M, melamine; MFO, mixed function oxidases

Table 1. Extraction recoveries of HMM, PMM, TMM, and TriMM from blood, tumor, and different tissues of female C57Bl/6J mice

Sample	Extraction recovery (%) ± SE[a]			
	HMM	PMM	TMM	TriMM
Liver	96.4 ± 1.3	98.1 ± 2.6	95.4 ± 1.6	98.0 ± 2.0
Tumor	94.6 ± 1.7	99.0 ± 0.5	101.1 ± 3.7	97.3 ± 1.2
Blood	93.1 ± 3.0	100.7 ± 1.6	98.3 ± 4.4	99.1 ± 2.6
Kidney	98.3 ± 1.3	97.4 ± 2.3	96.1 ± 2.1	96.9 ± 0.8
Spleen	102.0 ± 2.0	97.1 ± 1.6	98.4 ± 3.0	99.5 ± 1.2
Lung	96.4 ± 3.1	98.7 ± 1.0	102.6 ± 1.5	95.4 ± 3.6
Brain	101.2 ± 0.7	100.9 ± 2.4	99.8 ± 2.6	97.1 ± 1.4
Heart	96.0 ± 1.4	103.1 ± 1.1	99.1 ± 0.9	98.9 ± 0.3
Small intestine	97.3 ± 0.9	99.8 ± 2.3	96.4 ± 0.4	102.7 ± 2.0

[a] Each value is the mean ± SE of three different determinations performed with four concentrations

Table 2. Precision of the procedure for quantitation of HMM, PMM, TMM, and TriMM

Sample	% CV[a]			
	HMM	PMM	TMM	TriMM
Liver	4.7	9.2	5.8	7.1
Tumor	6.2	1.7	12.8	4.3
Blood	11.1	5.5	15.5	9.1
Kidney	4.5	8.2	7.5	2.8
Spleen	6.8	5.7	10.5	4.2
Lung	11.1	3.5	5.0	13.1
Brain	2.4	8.2	9.0	5.0
Heart	5.0	3.7	3.1	1.1
Small intestine	3.2	7.9	1.4	6.7

[a] Each value was calculated on four different concentrations run in triplicate

Determination of HMM, PMM, TMM, and TriMM in Blood and Different Tissues. To 50 µl of the blood and tissue homogenates, 50 µl HMM, PMM, TMM, and TriMM solutions in ethanol (1 mg/ml for each compound) and 0.5 ml acetonitrile were added. The samples were mixed on a vortex mixer and centrifuged at 3,000 *g* for 15 min. The supernatant was transferred to conical glass tubes and evaporated under a gentle stream of N_2. The residues were resuspended in 50 µl acetonitrile and spotted in the lower left corner of a 20 × 20 cm silica gel 60 F-254-precoated TLC plate (Merck, Darmstadt, FRG). The tubes were washed with another 50 µl acetonitrile and TLC spotting was repeated. Bidimensional chromatography was then performed using a chloroform : methanol : acetic acid (88 : 10 : 1.5) eluting mixture for both dimensions. The position on the plate of each unlabeled reference standard was determined from the absorption at 254 nm under UV light.

The Rf values in the first dimension were 0.80, 0.52, 0.53, and 0.48, respectively, for HMM, PMM, TMM, and TriMM. The corresponding values were 0.70, 0.54, 0.48, and 0.44 in the second dimension. The spots corresponding to HMM and its metabolites were scraped off and transferred into counting vials containing 10 ml dioxane scintillator (Lumac Systems AG, Basel, Switzerland). The radioactivity was measured in a Nuclear Chicago Isocap 300 liquid scintillation counter. Values were corrected for quenching by the external standardization method.

The sensitivity of the procedure described is very high since it is related to the specific activity of the compound used. By liquid scintillation counting we can easily detect as little as 10 d.p.m.; the minimum detectable amount of each of the compounds analyzed is therefore around 10 pg/sample.

Addition of cold reference standards of HMM, PMM, TMM, and TriMM to drug-free blood and tissues at four different concentrations (10, 25, 50, and 100 µg/ml or g) resulted in the recovery values reported in Table 1. The precision of the procedure utilized for HMM and metabolites quantitation was also determined, and Table 2 reports the calculated percentage coefficients of variation (% CV = standard deviation/mean × 100), which are low enough to indicate that the method is precise. All data in these tables were obtained by processing the samples as described above

Table 3. Levels of ^{14}C-HMM and metabolites and of ^{14}C-HMM and metabolites covalently bound to tissue macromolecules determined at three different times after drug administration

Tissue[a]	^{14}C-HMM and metabolites present in 1 g wet tissue (nmol)			^{14}C-HMM and metabolites irreversible bound to 1 g wet tissue macromolecules (nmol)		
	2 h	10 h	40 h	2 h	10 h	40 h
Liver	79.6 ± 5.5	69.6 ± 8.6	11.0 ± 0.4	4.2 ± 1.9 (5.2)	3.1 ± 1.0 (4.4)	2.5 ± 0.6 (22.7)
Tumor	63.0 ± 2.7	33.1 ± 4.0	9.1 ± 2.8	0.8 ± 0.1 (1.2)	0.7 ± 0.1 (2.2)	0.8 ± 0.1 (8.8)
Blood[b]	43.7 ± 2.0	42.4 ± 4.1	8.9 ± 0.6	0.7 ± 0.1 (1.6)	0.8 ± 0.2 (1.8)	1.0 ± 0.1 (11.2)
Kidney	95.2 ± 6.4	93.2 ± 10.3	13.8 ± 1.6	1.3 ± 0.2 (1.3)	1.6 ± 0.1 (1.7)	1.5 ± 0.3 (10.6)
Spleen	60.3 ± 1.7	63.8 ± 1.7	7.6 ± 1.6	1.4 ± 0.2 (2.3)	1.4 ± 0.1 (2.2)	1.4 ± 0.1 (18.7)
Lung	70.4 ± 4.6	62.2 ± 5.9	6.8 ± 0.7	1.5 ± 0.2 (2.1)	1.7 ± 0.2 (2.8)	1.4 ± 0.1 (20.9)
Brain	32.8 ± 3.8	33.8 ± 4.2	4.2 ± 0.6	0.4 ± 0.1 (1.3)	0.7 ± 0.1 (2.1)	0.7 ± 0.1 (16.7)
Heart	19.3 ± 1.2	23.7 ± 3.3	2.2 ± 0.3	0.5 ± 0.1 (2.5)	0.5 ± 0.1 (2.2)	0.4 ± 0.1 (17.2)
Small intestine	329.8 ± 33.1	127.2 ± 38.6	22.2 ± 8.9	4.6 ± 1.0 (1.4)	0.8 ± 0.1 (0.6)	0.6 ± 0.1 (2.7)

[a] Each value is the mean ± SE of determinations in three animals
[b] Data for the blood are expressed as nmoles of HMM metabolites per milliliter of whole blood. The figures in parentheses show ^{14}C-HMM and metabolites covalently bound as percentages of total ^{14}C-HMM and metabolites

and by quantitatively determining the drug and its metabolites by the gas liquid chromatographic method set up in our laboratory [2, 5].

Results

The left-hand part of Table 3 shows the levels of HMM and metabolites in blood and tissues, expressed as the total radioactivity recovered in the tissues 2, 10, and 40 h after injection of ^{14}C-labeled HMM. A continuous decrease was observed in the levels of radioactivity from 2 to 40 h, the pattern being roughly the same in the different tissues. In our experimental conditions peak levels of radioactivity were found at 2 h in the small intestine, followed by the kidney and the liver; the other tissues showed comparable concentrations although in brain and heart levels were significantly lower.

The right-hand part of Table 3 shows the data on the irreversible binding of HMM and metabolites to tissue and blood macromolecules. The maximum level of binding was already attained after 2 h. This value remained constant at all three times for all tissues, with the exception of the small intestine where a marked decrease was observed between 2 and 10 h. At all times the highest levels of irreversible binding were in the liver, and the lowest were in brain and heart. The figures in parentheses give the amounts of HMM and metabolites bound to tissue macromolecules as percentages of HMM and metabolites present in the same tissues at the same times. This percentage increases with time, reaching 22% of the total radioactivity present in the liver at 40 h. These data also indicate the quantitative importance of the fraction of bound HMM metabolites at 2 h.

The blood and tissue distribution of ^{14}C-HMM is shown in Table 4: the highest levels of the drug were attained at 2 h in all the tissues, decreasing thereafter but still measurable at 40 h. In the small intestine and heart, the highest and lowest levels were attained, respectively, at 2 h and 10 h, and the lowest levels were at 40 h in the spleen and the kidney. Comparison of the concentration of HMM with the total radioactivity present in the different tissues shows that the drug is already extensively metabolized at 2 h. ^{14}C-PMM and ^{14}C-TMM distribution is shown in Tables 5 and 6, respectively; the kinetic pattern was roughly the same as for HMM in all tissues.

As shown in Table 7, TriMM reached higher levels at all times and in all tissues than HMM and the other metabolites determined in this study. TriMM values at 2 h ranged from about 3 nmol/g in the heart to about 9 nmol/g in the kidney, at 10 h from about 1 nmol/g in the heart to 4 nmol/g in the kidney, and at 40 h from about 0.2 nmol/g in the heart to 1.6 nmol/g in the blood.

Table 4. ^{14}C-HMM distribution determined at three different times after drug administration

Tissue[a]	^{14}C-HMM (pmol/g)		
	2 h	10 h	40 h
Liver	1,025 ± 57	305 ± 67	178 ± 76
Tumor	180 ± 67	143 ± 48	63 ± 5
Blood[b]	422 ± 114	262 ± 90	90 ± 7
Kidney	413 ± 5	214 ± 107	46 ± 27
Spleen	1,298 ± 425	188 ± 89	264 ± 132
Lung	1,282 ± 390	201 ± 30	194 ± 92
Brain	230 ± 21	160 ± 18	120 ± 28
Heart	217 ± 72	101 ± 38	94 ± 8
Small intestine	1,953 ± 243	642 ± 126	255 ± 89

[a] Each value is the mean ± SE of determinations in three animals
[b] Data for the blood are expressed as nmoles of HMM metabolites per milliliter of whole blood

Table 5. ^{14}C-PMM distribution determined at three different times after drug administration

Tissue[a]	^{14}C-PMM (pmol/g)		
	2 h	10 h	40 h
Liver	560 ± 11	302 ± 76	132 ± 37
Tumor	138 ± 20	150 ± 64	78 ± 26
Blood[b]	198 ± 47	225 ± 58	310 ± 28
Kidney	350 ± 70	200 ± 48	154 ± 60
Spleen	500 ± 14	313 ± 28	151 ± 59
Lung	1,419 ± 301	436 ± 183	170 ± 21
Brain	180 ± 42	130 ± 57	90 ± 18
Heart	161 ± 50	63 ± 28	74 ± 5
Small intestine	626 ± 28	280 ± 4	160 ± 46

[a] Each value is the mean ± SE of determinations in three animals
[b] Data for the blood are expressed as nmoles of HMM metabolites per milliliter of whole blood

Table 6. ^{14}C-TMM distribution determined at three different times after drug administration

Tissue[a]	^{14}C-TMM (pmol/g)		
	2 h	10 h	40 h
Liver	655 ± 156	382 ± 129	140 ± 18
Tumor	368 ± 119	488 ± 229	90 ± 18
Blood[b]	374 ± 148	245 ± 18	304 ± 146
Kidney	640 ± 126	322 ± 134	192 ± 27
Spleen	1,442 ± 212	1,093 ± 617	168 ± 14
Lung	2,043 ± 860	804 ± 123	242 ± 35
Brain	455 ± 152	245 ± 71	135 ± 4
Heart	645 ± 43	516 ± 264	113 ± 22
Small intestine	865 ± 50	390 ± 67	245 ± 71

[a] Each value is the mean ± SE of determinations in three animals

[b] Data for the blood are expressed as nmoles of HMM metabolites per milliliter of whole blood

Table 7. ^{14}C-TriMM distribution determined at three different times after drug administration

Tissue[a]	^{14}C-TriMM (pmol/g)		
	2 h	10 h	40 h
Liver	7,177 ± 1,001	4,012 ± 2,221	440 ± 50
Tumor	5,598 ± 1,615	3,092 ± 1,626	375 ± 64
Blood[b]	4,098 ± 1,260	2,302 ± 824	1,691 ± 864
Kidney	9,179 ± 2,214	4,095 ± 2,174	567 ± 20
Spleen	6,228 ± 1,125	3,531 ± 2,251	675 ± 39
Lung	9,385 ± 2,101	2,865 ± 1,107	528 ± 30
Brain	5,662 ± 1,214	2,615 ± 1,407	670 ± 180
Heart	3,001 ± 232	1,318 ± 741	162 ± 31
Small intestine	7,518 ± 863	3,700 ± 1,894	625 ± 18

[a] Each value is the mean ± SE of determinations in three animals

[b] Data for the blood are expressed as nmoles of HMM metabolites per milliliter of whole blood

The sum of HMM and all three metabolites determined at 2 h did not account for more than about 20% of the total radioactivity present at that time in the tissues.

Discussion

HMM seems to be a promising tool for investigating the relationship between cytotoxicity and the capacity of an alkylating antitumoral agent for covalent binding of tissue macromolecules. This drug is in fact selective, being relatively active in only a small range of animal and human tumors and having a simple metabolism consisting of a series of *N*-oxidative demethylation steps (see Fig. 1).

In this study the capacity of HMM and its metabolites to covalently bind macromolecules of different tissues including blood was demonstrated in vivo, using C57Bl/6J mice bearing a M5076/73A ovarian cancer. This experimental tumor was chosen because of its sensitivity to HMM at the dosage used. Furthermore, it was decided to inject the labeled drug 20 days after inoculation of the tumor cells, because at this time the tumor is sufficiently developed and still viable with few areas of necrosis.

Covalent binding in all the tissues, with the possible exception of the brain, was already at its maximum after 2 h and remained constant until 40 h. Covalent binding might remain steady longer but it was not possible to make determinations beyond this limit because the maximum survival time of the animals was very close to the time of sacrifice. It therefore appears that in animals at this stage of tumor growth all tissues show a scant capacity for repair. Two hours after drug administration the extent of irreversible binding of HMM and metabolites to blood and tissue macromolecules must be considered high since it ranges from around 1% to 5% of the total radioactivity present. At this time HMM, PMM, and TMM were already extensively metabolized, the only metabolite detectable in noteworthy amounts being TriMM.

These data correlate well with those of a previous report on the distribution of HMM and some of its metabolites in mice bearing the same type of ovarian cancer and treated with a single IP dose of 100 mg/kg [2]. However, on the basis of the total concentrations of HMM and metabolites shown in Table 3, we obviously cannot rule out the presence of appreciable amounts of other isomeric forms of TMM and TriMM, dimethylmelamine isomers, monomethylmelamine, and melamine, which we did not determine because we did not have enough cold reference standards to use as carriers in the thin-layer chromatographic separation method. Although no simple correlations can be extrapolated from covalent binding and the presence of the metabolites determined, the data seem to suggest that the less demethylated metabolites are involved in this interaction (i.e., HMM, PMM, TMM). This hypothesis is supported by at least two pieces of evidence: (1) the only metabolite present in relatively large amounts at 2 h is TriMM, and (2) covalent binding to tissue macromolecules does not increase from 2 h on. If this hypothesis helds true, a correlation can be drawn between covalent binding of HMM and some of its less demethylated metabolites to total tissue macromolecules and cytotoxicity, since it has also been reported that the in vivo cytotoxicity of TMM isomers rapidly decreases down to melamine which is practically non-cytotoxic, whereas the cytotoxicity of HMM, PMM, and TMM isomers is comparable [9]. (Allowance must of course be made for different experimental conditions in our work and the study quoted, especially as regards the different experimental tumors used and treatment schedules.)

The covalent binding data shown in this paper may be relevant from the toxicological point of view, since they demonstrate the presence of HMM and metabolites in brain as well as their capacity to interact irreversibly with brain macromolecules. This interaction in animals might serve as a starting point for a better understanding of the neurological disturbances reported in man after treatment with this drug [1, 11]. However, at present all these considerations remain purely speculative.

The findings offer a good demonstration that HMM is in alkylating agent in vivo, because of its ability to interact covalently with tissue macromolecules. We do not yet know which metabolic intermediate/s are involved in this binding, although considering the chemical structure and the known metabolism of the drug, it can be reasonably assumed that methylol intermediates, arising as shown in Fig. 2 during drug *N*-demethylation and representing reactive species, may be responsible for this interaction [7].

Studies are now needed to check whether covalent binding of total tissue macromolecules correlates with the toxic or

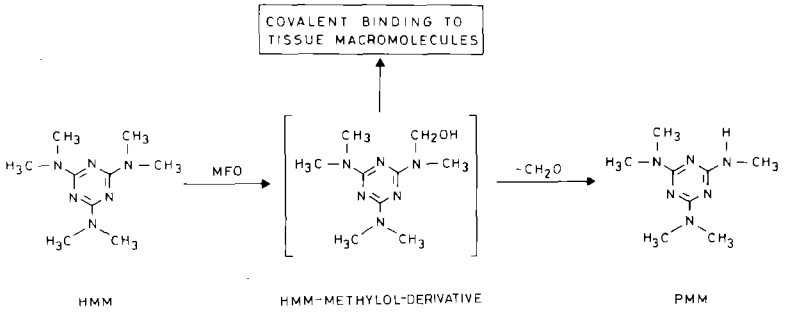

Fig. 2. Rationale for the formation of a methylol derivative of HMM during drug *N*-demethylation to PMM

cytotoxic effects of this anticancer drug. If determination of total covalent binding is too gross a parameter on which to base any of the above correlations, other studies should be designed to define the real target of this interaction (i.e., DNA, RNA, or one or more proteins).

Acknowledgements. We wish to thank Dr R. R. Engle, Chemical Resources Section, Pharmaceutical Resources Branch, Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, USA, for the precious gift of labeled HMM and unlabeled HMM, PMM, TMM, and TriMM. This study was supported by the National Research Council (C.N.R.) within the "Progetto Finalizzato: Controllo della Crescita Neoplastica", Contract No. 80.01539.96, and by PHS grant no. 1-R01 CA29043-01 awarded by the National Cancer Institute, DHHS. The generous contribution of the Italian Association for Cancer Research, Milan, Italy, is also gratefully acknowledged.

References

1. Bonomi PD, Mladineo J, Morrin B, Wilbanks G Jr, Slayton RE (1979) Phase II trial of hexamethylmelamine in ovarian carcinoma resistant to alkylating agents. Cancer Treat Rep 63: 137
2. Broggini M, Rossi C, Colombo T, D'Incalci M (1982) Hexamethylmelamine and pentamethylmelamine tissue distribution in M5076/73A ovarian cancer-bearing mice. Cancer Treat Rep 66: 127
3. Bryan GT, Worzalla JF, Gorske AL, Ramirez G (1968) Plasma levels and urinary excretion of hexamethylmelamine following oral administration to human subjects with cancer. Clin Pharmacol Ther 9: 777
4. D'Incalci M, Bolis C, Mangioni C, Morasca L, Garattini S (1978) Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62: 2117
5. D'Incalci M, Morazzoni P, Pantarotto C (1979) Gas chromatographic determination of hexamethylmelamine in mouse plasma. Anal Biochem 99: 441
6. Garattini E, Donelli MG, Colombo T, Paesani R, Pantarotto C (1981) In vivo and in vitro irreversible binding of hexamethylmelamine to liver and ovarian tumor macromolecules of mice. Biochem Pharmacol 30: 1151
7. Gescher A, D'Incalci M, Fanelli R, Farina P (1980) *N*-Hydroxymethylpentamethylmelamine, a major in vitro metabolite of hexamethylmelamine. Life Sci 26: 147
8. Ghezzi P, Donelli MG, Pantarotto C, Facchinetti T, Garattini S (1981) Evidence for covalent binding of adriamycin to rat liver microsomal proteins. Biochem Pharmacol 30: 175
9. Lake LM, Grunden EE, Johnson BM (1975) Toxicity and antitumor activity of hexamethylmelamine and its *N*-demethylated metabolites in mice with transplantable tumors. Cancer Res 35: 2858
10. Legha SS, Slavik M, Carter SK (1976) Hexamethylmelamine. An evaluation of its role in the therapy of cancer. Cancer 38: 27
11. Wilson WL, Bisel HF, Cole D, Rochlin D, Ramirez G, Madden R (1970) Prolonged low-dosage administration of hexamethylmelamine (NSC 13875) Cancer 25: 568
12. Worzalla JF, Kaiman BD, Johnson BM, Ramirez G, Bryan GT (1974) Metabolism of hexamethylmelamine-ring-^{14}C in rats and man. Cancer Res 34: 2669

Received March 17, 1982/Accepted February 28, 1983