

Superior efficacy of trimelamol to hexamethylmelamine in human ovarian cancer xenografts

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Summary. A series of eight human ovarian cancer lines grown in nude mice were used to compare the activity of hexamethylmelamine (HMM) and *N*²,*N*⁴,*N*⁶-trihydroxymethyl-*N*²,*N*⁴,*N*⁶-trimethylmelamine (trimelamol). The tumor lines differed in histological subtype and growth rate. The drugs were administered i.p. at the maximum tolerated dose at alternate days. Differences in volume of treated and control tumors were endpoints of the study. The tumor lines varied widely in sensitivity to HMM and in four lines a T/C% below 25% was achieved. Trimelamol appeared to be more active than HMM and achieved a T/C below 25% in seven tumor lines. Thus far, the drug has demonstrated significant activity in a phase I trial in ovarian cancer patients. Comparative clinical studies of HMM vs trimelamol have not yet been performed.

Introduction

Hexamethylmelamine [HMM, Fig. 1], a synthetic *S*-triazine compound, has demonstrated activity against several experimental tumor systems, such as the Crocker mouse sarcoma and the Walker 256 rat carcinosarcoma [5, 15]. The drug was introduced into clinical practice in 1964, and responses were reported in a variety of human tumor types, including cancer of the lung, ovary, cervix, breast, and lymphomas [15]. In particular, its activity in alkylating agent-refractory ovarian cancer [2, 12] led to the use of HMM in combination chemotherapy in ovarian cancer patients [17, 20].

Dose-limiting side effects of HMM manifest as gastrointestinal toxicity, which seems to be dose-related and occurs in 50%–70% of treated patients [15]. Less frequent side effects are hematological toxicity and peripheral neuropathy. Because of its poor water solubility, HMM needs to be administered p.o.

In a search for water-soluble HMM analogues with antitumor activity, two derivatives were selected as potential candidates for clinical trials using the i.v. route of administration [8]. The first HMM analogue to be studied clinically was the monodemethylated derivative pentamethylmelamine (PMM, Fig. 1). Phase I trials with PMM revealed its severe gastrointestinal toxicity and frequent central nervous system disturbances, because of which the

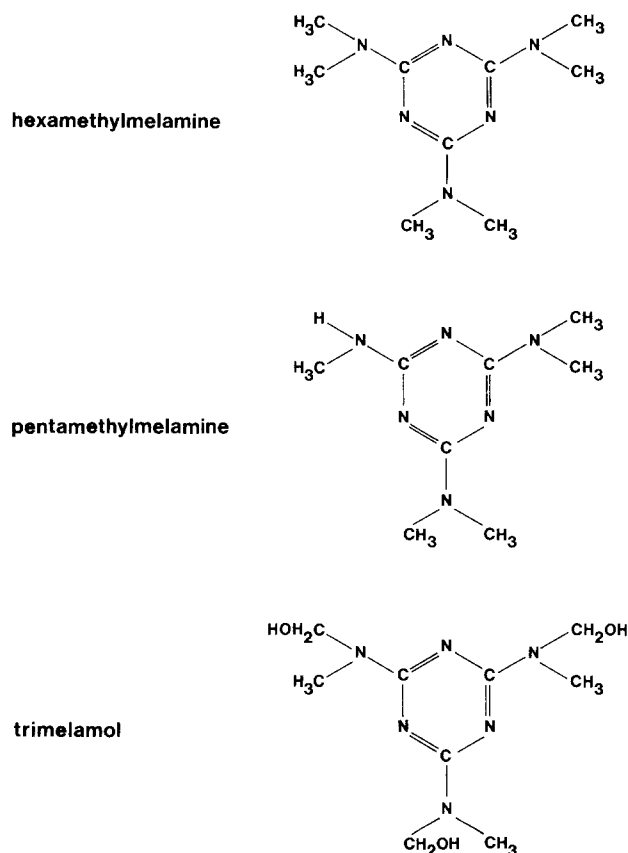


Fig. 1. Chemical structures of hexamethylmelamine, its monodemethylated derivative pentamethylmelamine and *N*²,*N*⁴,*N*⁶-trihydroxymethyl-*N*²,*N*⁴,*N*⁶-trimethylmelamine (trimelamol)

compound was excluded from further clinical investigation [6, 11, 16, 19].

In the present study, we report on the activity of the second water-soluble, but less stable HMM analogue, *N*²,*N*⁴,*N*⁶-trihydroxymethyl-*N*²,*N*⁴,*N*⁶-trimethylmelamine (trimelamol, Fig. 1) in a series of human ovarian cancer xenografts. The efficacy of trimelamol at its maximum tolerated dose (MTD) was compared with that of HMM. Trimelamol was demonstrated to have marked activity in the human lung cancer line P246 in an earlier study [7]. As an advantage over HMM and PMM, trimelamol dose not require metabolic activation to exert its cytotoxic action [18].

The compound is currently under clinical investigation in a phase I trial [13]. Thus far, trimelamol has shown antitumor activity in ovarian cancer patients, while it appears less toxic than PMM.

Materials and methods

Animals and tumor lines. Female B10 LP/Cpb nude (nu/nu) mice (TNO, Zeist, NL) were maintained in cages with paper filter covers. Cages, covers, bedding, food and water were changed and sterilized weekly. Animal handling was done in a laminar down-flow hood.

The following human ovarian cancer lines were used: Ov.Pe and Ov.He, both moderately differentiated mucinous adenocarcinomas; MRI-H-207 and A 2780, both undifferentiated adenocarcinomas; Ov.G1, a poorly differentiated and FKo, a moderately differentiated serous adenocarcinoma; FMa, a poorly differentiated mucinous carcinoma (cervical origin?), and Ov.Me, a carcinosarcoma of the ovary. MRI-H-207 was kindly provided by Dr A. E. Bogden, Mason Research Institute, Worcester, Mass, USA; FKo and FMa were kindly provided by Dr W. Kleine, Albert-Ludwigs University, Freiburg, FRG; A 2780 has been described before as an ovarian cancer cell line [10]. Tumor lines were maintained by serial s.c. transplantation of tumor fragments 2–3 mm in diameter in both flanks of 8- to 10-week-old animals.

Treatment and evaluation. HMM was used as HMM $\text{HCl} \cdot 2\text{H}_2\text{O}$ in aqueous solution 5 mg/ml at pH 2.9 (kindly provided by Dr A. Hulshoff, University of Utrecht, NL), as described earlier [14]. Trimelamol (kindly provided by Dr K. R. Harrap, Institute of Cancer Research, Sutton, Surrey, GB) was dissolved in 95% glucose 5%/5% DMSO, 2.86 mg/ml at pH 8.0 just before each experiment and kept at 4 °C until use. Single agents were injected i.p. at the MTD on alternate days. At this MTD the mice were allowed to lose 10%–15% of their initial weight within 1 week after the first injection [4].

In each experiment, groups of 12–18 tumor-bearing mice were randomized to give at least 5 animals for the treatment group and 5 animals for the control group. At the start of treatment tumors had a mean volume between 50 and 150 mm³. Tumors were measured weekly in three dimensions by the same observer with a slide caliper. The volume was calculated by the equation length \times width \times thickness \times 0.5, and expressed in cubic millimeters. Because of the variation in size at the initiation of treatment, volumes were calculated in relation to the initial tumor volume. The relative tumor volume was expressed by the formula V_T/V_0 , where V_T is the volume at any given day and V_0 the volume at the start of treatment. The ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100 (T/C%) was calculated at each evaluation. For each experiment the lowest value within 5 weeks after the last treatment day was considered the optimal ratio. Deaths within 2 weeks after the final injection were considered as toxic deaths and these animals were excluded from the study. Complete remission (CR) was defined as the total disappearance of the tumors without regrowth within the following month.

Statistics. Antitumor activity of HMM and trimelamol was evaluated with Student's *t*-test.

Results

In the present study we used eight human tumor lines originating from various histological subtypes of ovarian cancer. From Fig. 2 it is clear that their growth rates vary, MRI-H-207 and A 2780 having the shortest tumor-doubling time. For drug administration an alternate day schedule was chosen, in an attempt to approach the clinical schedule of 2 weeks' exposure to HMM [17, 20]. Various doses and varying numbers of injections were administered, to find the optimal schedules suitable with our criteria of MTD. For HMM the MTD appeared to be 150 mg/kg i.p. on days 0, 2, 4, and 6, while for trimelamol the MTD was 125 mg/kg i.p. on days 0, 2, 4, 6, and 8.

As can be expected from the clinical situation, the pattern of efficacy of HMM in the tumor lines varied from highly sensitive to insensitive (Table 1). With this compound it was possible to achieve a T/C% below 50% in all but two tumor lines and below 25% in four tumor lines. In MRI-H-207 a CR was reached. Trimelamol at its MTD was even more active than HMM and a T/C% below 25% was achieved in all but one tumor line. In each of MRI-H-207, FMa, and Ov.Me a CR was reached. Except for one Ov.Me tumor, none of these tumors had reappeared after 1 month. It is of interest that lower doses of HMM administered in Ov.Me, or of trimelamol administered in Ov.Pe, FMa, and Ov.Me were less effective than the MTD of these compounds.

Comparison of the antitumor activity results of HMM and trimelamol showed superior efficacy for the latter drug in Ov.Pe, MRI-H-207, Ov.G1, FKo, FMa, and Ov.Me ($P < 0.01$). The efficacy of both drugs was similar in A 2780, while Ov.He was the only tumor line with better sensitivity to HMM ($P < 0.05$).

Discussion

Using a series of human ovarian cancer lines with differences in histology and growth rate, we were able to demonstrate a superior efficacy of the HMM analogue trimelamol compared with that of the parent compound. As previous studies in our human ovarian cancer model showed its predictive potential and its reliability, our results may well indicate a superior antitumor potential of trimelamol to HMM in the clinical situation.

Theoretically, there are several advantages in substituting trimelamol for HMM in the clinic. First, HMM and also PMM require metabolic activation by a liver microsomal fraction to elicit their antitumor effects [1]. Oxidative *N*-demethylation occurs in vivo and *N*-methylolmelamine intermediates are thought to be responsible for cytotoxic activity [18]. Therefore, the direct administration of an *N*-methylolmelamine such as trimelamol, would obviously facilitate drug action. Second, in patients with gastrointestinal toxicity the oral administration of HMM is unreliable. Moreover, the absorption of HMM results in variable plasma concentrations [9]. With the improved water solubility of trimelamol as compared to HMM, i.v. administration became possible. This should optimize serum levels of the active agent required for an antitumor effect. Third, in our human ovarian cancer lines trimelamol proved more effective than HMM if administered at MTD. Our findings in combination with the acceptable toxicity and proven antitumor activity of trimelamol thus far observed in a

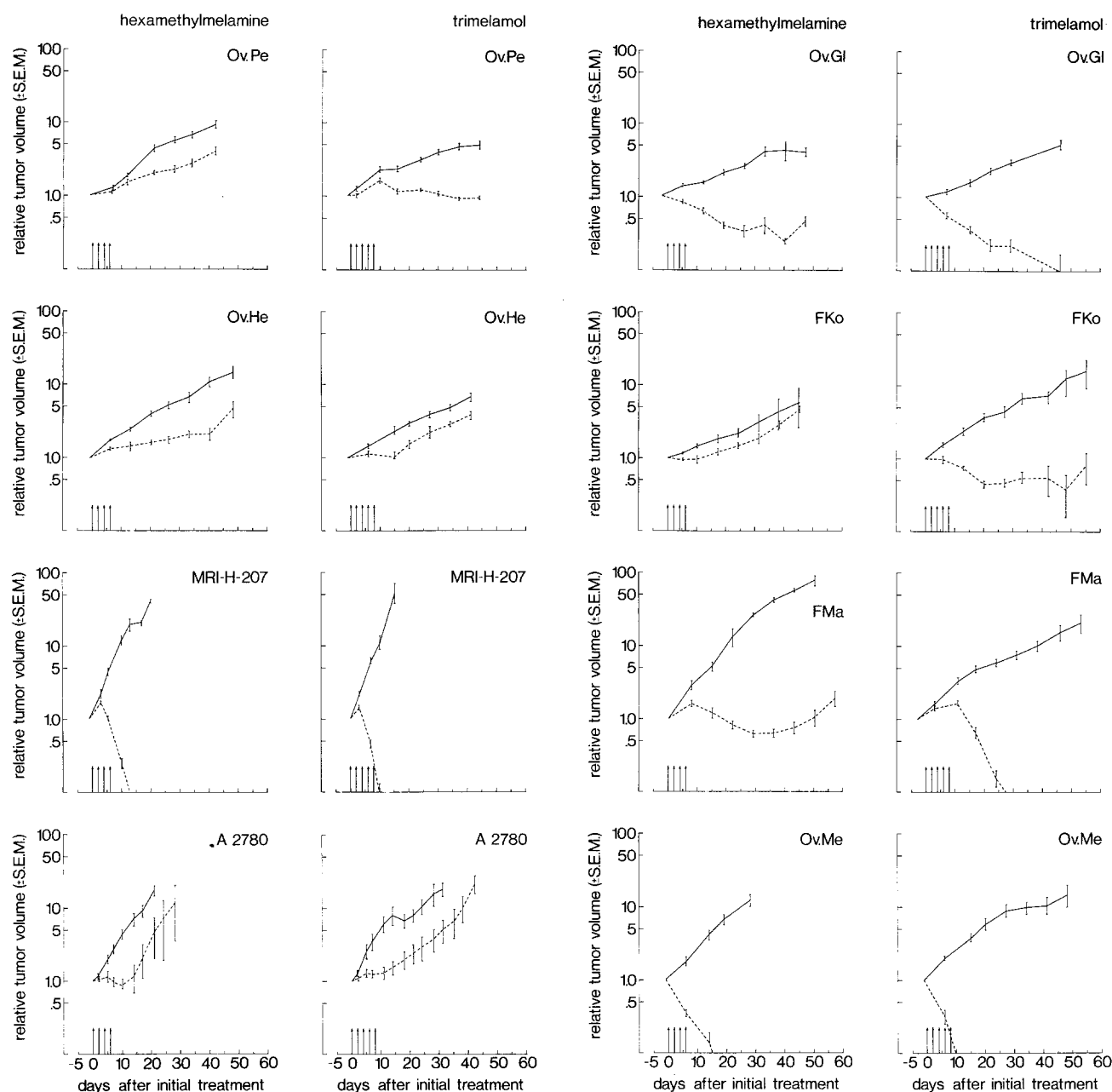


Fig. 2. Treatment results obtained with hexamethylmelamine 150 mg/kg i.p. days 0, 2, 4, and 6 (MRI-H-207: 100 mg/kg) and trimelamol 125 mg/kg i.p. days 0, 2, 4, 6, and 8, compared with controls. The relative tumor volume is the tumor volume at any given day V_T / the volume at the start of treatment V_0 . -----, mean of relative volumes of treated tumors; ———, mean of relative volumes of control tumors

phase I clinical trial [13] may indicate an increased therapeutic index for this HMM analogue.

The activity of HMM in our human ovarian cancer model is higher than that observed in patients. The different routes of administration resulting in different bioavailabilities of the drug may not be the only factor to explain this discrepancy. A species difference in the metabolism of HMM may also exist with murine plasma concentrations of active intermediates being higher than those achievable in man. Ruddy et al. [18] compared the pharmacokinetic behavior of PMM in man and mouse. They found a rapid metabolism of PMM with extensive formation of *N*-methylolmelamines in the mouse, whereas in man PMM metabolism was slower, with nondetectable levels of *N*-methylol-

melamines. Comparison of the species difference in *N*-methylolmelamine levels, pharmacokinetic behavior, and antitumor activity of trimelamol may eventually provide a better insight into the mechanism of action and the cytotoxicity of this compound.

Recently, we reported on the activity of a variety of cisplatin analogues in our human ovarian cancer lines [3, 4]. Our findings correlated well with clinical results obtained with platinum compounds in ovarian cancer. Our model appeared highly reliable, because in a number of tumor lines differing in sensitivity to cisplatin a platinum compound with lower activity than cisplatin consistently had reduced efficacy. The tumor lines presently used also vary in sensitivity to HMM. With the exception of one tu-

Table 1. Activity of hexamethylmelamine and trimelamol in a series of human ovarian cancer lines

Tumor line	Hexamethylmelamine				Trimelamol			
	Dose (mg/kg)	Treatment schedule	Optimum T/C% (day) ^a	Toxic deaths ^b	Dose (mg/kg)	Treatment schedule	Optimum T/C% (day) ^a	Toxic deaths ^b
Ov.Pe	150	q2dx4	40 ^c (28)	1/7	100	q2dx5	60 ^c (35)	0/6
					125	q2dx5	19 ^c (37)	1/7
Ov.He	150	q2dx4	27 ^c (33)	1/8	125	q2dx5	58 ^c (27)	1/6
MRI-H-207	150	q2dx4	CR ^c (28)	1/6	100	q2dx5	CR ^c (18)	0/7
A 2780	150	q2dx4	20 ^c (10)	0/6	125	q2dx5	21 ^c (14)	1/8
Ov.GI	150	q2dx4	13 ^c (26)	1/7	125	q2dx5	7 ^c (29)	1/6
FKo	150	q2dx4	65 ^c (31)	2/8	125	q2dx5	8 ^c (33)	1/8
FMa	150	q2dx4	1.5 ^c (36)	1/5	100	q2dx5	39 ^c (35)	1/9
					125	q2dx5	CR ^c (38)	0/7
Ov.Me	100	q2dx7	2.1 ^c (28)	1/6	100	q2dx5	16 ^c (27)	0/6
	150	q2dx4	0.2 ^c (28)	1/7	125	q2dx5	CR ^c (27)	1/7

^a T/C%, optimal value of the mean of relative tumor volume in treated animals/mean of relative volume in control animals $\times 100$, calculated within 5 weeks after completion of chemotherapy

^b Toxic deaths include deaths occurring within 2 weeks after completion of chemotherapy and are excluded from the study

^c Significant difference ($P < 0.05$) between treated and control tumors evaluated with Student's *t*-test; CR, complete remission

mor line, we again consistently observed superior antitumor activity with trimelamol.

In conclusion, comparative activity of HMM and trimelamol in our human ovarian cancer model demonstrates superiority for the latter compound. Unlike HMM, trimelamol can be administered i.v. and does not require metabolic activation. These data may well indicate an increased therapeutic index for trimelamol in the clinical situation. Preliminary results of a phase I trial with trimelamol are encouraging and appear to open new perspectives for improving treatment results in ovarian cancer patients.

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