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Hexamethylmelamine-induced Regression of Human Lung Tumors Growing in Immune Deprived Mice[1]

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SUMMARY

Hexamethylmelamine is known to be effective in humans in the treatment of certain malignant tumors, especially bronchial carcinoma. It is, however, quite inactive against a number of animal tumors, making difficult a study of its mechanism of action in experimental systems. In a reexamination of the effects of hexamethylmelamine, two tumors were found to be very sensitive, namely, a mouse plasma cell tumor (PC6) and a human bronchial carcinoma (P246) growing in immune deprived mice. Both tumors undergo a significant and almost complete regression, even when well established, and hence may serve as model systems for the study of the mechanism of action of hexamethylmelamine.

INTRODUCTION

In the course of studies on the antitumor mechanism of action of urethan, Hendry *et al.* (11) examined a series of agents which, like urethan, can condense with amino groups. Included among these was trimethylolmelamine[2] which was at that time used in the textile industry to modify the properties of certain fabrics by covalently linking peptide chains of the textile fiber.

Concurrently, other industrial cross-linking agents were screened for their anticancer activity (10), and one of these, TEM (tretamine) was found to be highly active against Walker carcinoma 256. HMM was also examined as a nonreactive (that is, nonalkylating) analog of TEM.

Both the trimethylol and hexamethyl derivatives of melamine were found to have slight but significant activity against Walker carcinoma 256, and the latter derivatives also have activity against Sarcoma 180 (3). In further, more quantitative tests, however, HMM was found to be without activity against 3 tumors (Leukemia L1210, Carcinoma 755, and Sarcoma 180), then being used by the National Cancer Institute as the basis of their screening program (9).

Despite this marginal activity against animal tumors, HMM was subsequently investigated in humans and was shown to have effects in the treatment of bronchial and ovarian carcinomas, with response rates greater than 20% being obtained (15, 16). Further studies have confirmed the clinical activity of HMM against a number of carcinomas. Of particular interest are the remissions obtained against carcinoma of the lung, which range from 11% for epidermoid carcinomas to 36% for small cell carcinomas (1, 7, 14).

HMM has been mistakenly described as an alkylating agent in a number of publications, and while it can be metabolized *in vivo* to reactive methylols such as trimethylolmelamine, it has no alkylating activity *per se*. Also, although it may resemble alkylating agents in its biological properties and in the types of clinical response obtained, it is quite clear, from its structure-activity relationships (2, 8), that it is considerably different from classical alkylating agents. A study of the mechanism of action of HMM and its analogs might uncover novel pathways by which tumor growth may be selectively inhibited. Unfortunately, such studies are limited because of the lack of an experimental tumor system sensitive to this class of compound.

This paper describes a mouse plasma cell tumor (PC6A), which undergoes complete regression after treatment with dose levels of HMM less than one-tenth of the dose lethal to 50% of the animals tested, and a human carcinoma of the bronchus transplanted to T-cell-deprived mice, which regresses completely after administration of well-tolerated dose levels.

MATERIALS AND METHODS

Animal Tumors. The TLX5 lymphoma was transplanted by s.c. injection of 2×10^5 cells in the inguinal region of CBA/LAC mice. The Walker carcinoma (Wistar rats) and the PC6A plasma cell tumors (BALB/c mice) were obtained by s.c. transplantation of tumor fragments in the flank. Antitumor effectiveness was measured for the PC6A and Walker tumors by comparison of tumor weights of treated and control groups (13) and for the TLX5 tumor by comparison of survival times (6). HMM was administered as a sonically treated suspension in arachis oil by 5 daily i.p. injections. Injections commenced 1 day after transplantation, in the case of the Walker carcinoma 256; 3 days, in the case of the TLX5 lymphoma; but not until 24 days after

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[2] The abbreviations and trivial names used are: trimethylolmelamine, 2,4,6-trihydroxymethylamino-s-triazine; TEM, triethyleneiminomelamine, 2,4,6-triaziridinyl-*s*-triazine; HMM, hexamethylmelamine, 2,4,4-hexamethylamino-*s*-triazine.

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transplantation of the PC6A tumor, when the mean tumor weight was greater than 3 g. Several dose levels of HMM were used, ranging from tumor noneffective to lethal, thus enabling the calculation of a therapeutic index (13).

Human Tumors. As part of a study of xenografted human tumors, pieces of a human adenocarcinoma of the lung (P246), which were obtained at pneumonectomy, were implanted s.c. into the flank of immune deprived mice (4). Growth of the tumor occurred and was visibly established by the 2nd month, when it was harvested and further transplants were made.

The tumor material used for this study was either the 4th or 6th transplant generation.

Immune deprived mice were prepared by a technique of thymectomy, whole-body irradiation, and bone marrow replacement (12), similar to that described by Cobb and Mitchley (5).

Male CBA/LAC mice were thymectomized at 4 weeks of age, and 8 weeks later they were given 900 rads of whole-body irradiation at a rate of 60 rads/min with a 200 kV X-ray machine (half-value layer, 0.4 cu mm; focal distance, 100 cm). Within 2 hr of irradiation, each mouse was given an i.v. injection of 5×10^6 syngeneic femoral bone marrow cells suspended in 0.4 ml of Medium 199 (Wellcome Research Laboratories, Beckenham, England).

The mice were housed in plastic boxes, and bedded on sterilized sawdust and wood-wool. They were fed on a pasteurized diet (Spratts Laboratory Diet No. 1; Lillico and Sons, Wonham Mill, Betchworth, Surrey, England) and given boiled water *ad libitum*.

Tumor fragments, approximately 2 cu mm, were implanted s.c. into the flank 10 to 14 days after irradiation and bone marrow replacement. Treatment was commenced 4 to 5 weeks after transplantation when the tumors were well established. The criterion for an established tumor was one that measured at least 6 mm in 1 diameter.

In the 1st test with HMM, 6 control and 4 solvent control animals were used. HMM was given to 2 groups of 4 mice at 90 and 30 mg/kg i.p. as a suspension in arachis oil. The higher dose level was that which was found in preliminary toxicity studies to be the maximum dose that was well tolerated. Treatment was given daily for 5 days, followed by a rest period of 8 days. This schedule was twice repeated, after which time some mice from each group were sacrificed and their tumors taken for histological examination. The remaining mice in the treatment groups then received 5 more daily injections. The experiment was terminated 8 weeks from the date of the 1st injection.

In a 2nd test, the same procedure was followed, except that HMM was given at dose levels of 90 and 60 mg/kg, and 5 mice were included in each treatment group. Mice in this experiment were killed 7 weeks after the 1st injection, when mice of the control groups had ulcerating tumors.

All mice were observed daily for general health and were weighed and the tumor measured along 2 diameters at right angles to one another, weekly. A tumor volume index was obtained by multiplying the longer diameter by the square of the smaller. The effect of treatment was assessed by plots of tumor volume against time.

RESULTS

The effects of HMM on 3 transplanted rodent tumors are summarized in Table 1. No activity was found against the TLX5 lymphoma, although it is known to be sensitive to nitrosoureas, dimethyltriazenes, and many purines, pyrimidine, and folic acid antagonists. As found in earlier studies, there is a slight but significant effect against Walker carcinoma 256 that is not seen if treatment is delayed until the tumor is established (7 days after transplantation). In contrast, the plasma cell tumor is sensitive to HMM, tumors of larger than 3 g in weight undergoing "complete" regression at these tolerated dose levels. (This tumor is known to be sensitive, even when well-established, to alkylating agents, certain platinum compounds, nitrosoureas, and triazenes, but not to antimetabolites.)

Chart 1 shows the effect of HMM on human bronchial

Table 1

Effect of HMM (5 daily i.p. injections) on 3 experimental tumors

	ID_{90}[a] (mg/kg)	LD_{50} (mg/kg)	Therapeutic index
TLX5 lymphoma	>150	150	<1.0
Walker carcinoma 256	100	133	1.3
PC6 plasma cell tumor	11.0	113	10.3

[a] ID_{90}, dose to cause 90% tumor inhibition; LD_{50}, dose lethal to 50% of animals.

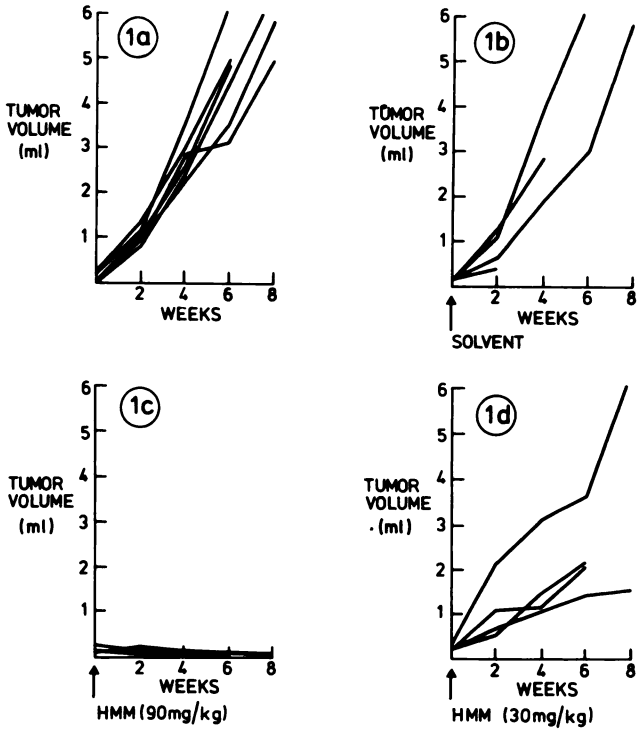

Chart 1. Change in tumor volume, with time, in mice bearing a s.c. transplant of human bronchial carcinoma P246; *a*, 6 untreated control mice; *b*, 4 solvent control mice (2 of these mice died before the end of the experiment); *c* and *d* show the effect of treatment with HMM, 90 and 30 mg/kg, respectively (4 mice in each group). *Arrow*, time at which treatment was started.

carcinoma P246. It can be seen (Chart 1, *a*) that the control tumors grow well. The solvent used may have some slight effect on tumor growth (Chart 1, *b*), and it is now known that because of its very poor absorption from the peritoneal cavity, arachis oil is not a good solvent to use when injections amounting to a large volume of solvent are given over an extended period.

Ninety mg HMM per kg (Chart 1, *c*) are effective in preventing further growth of the tumor immediately after the 1st injection. In the 2nd experiment, the tumors regressed completely at a dose of 90 mg HMM per kg, and their growth was inhibited at 60 mg/kg.

Histological examination of the control material shows a poorly differentiated epidermoid carcinoma with other areas in which tumor cells are arranged in sheets, short cords, or a pseudopapillary pattern. The cells have eosinophilic cytoplasm with pale single ovoid to round nuclei and one or more nucleoli. Pleomorphism is minimal, and between 6 and 8 mitotic figures are present per high-power field (Fig. 1).

After treatment with 90 mg HMM per kg, given as 3 × 5 daily i.p. injections separated by intervals of 8 days, minimal amounts of residual tumor were always seen histologically (Fig. 2). However, most of the cells exhibit marked nuclear and cellular pleomorphism with giant, multinucleate, and bizarre nuclear forms. The nuclei tend to be more vesicular and have prominent punctate chromatin. Mitotic figures are conspicuous by their absence. Eosinophilic hyaline droplets are prominent in the cytoplasm. Some tumor cells show a tendency to spindle forms, and individual cell necrosis is prominent. Around the surviving tumor tissue, fibrosis, hemosiderin-containing macrophages, and occasional round cells are present (Fig. 2).

The tumors treated with lesser amounts of HMM tended to have an intermediate appearance, with some areas similar to the controls and others showing many of the features of the lesions after administration of 90 mg HMM per kg.

DISCUSSION

At least 2 of the tumors described, the PC6 plasma cell tumor and the human lung tumor, are sensitive to HMM, even when well established, and could be used in studies of the mechanism of action of HMM. The plasma cell tumor is sensitive to a range of derivatives that act by alkylation, and its sensitivity to HMM implies that this compound may act *in vivo* after conversion to alkylating methylol derivatives. On the contrary, the lung tumor, although highly sensitive to HMM, has not, in preliminary experiments, shown any sensitivity to cyclophosphamide, and thus HMM may act in a more specific way.

The obvious advantage of studying HMM on human cancer in an *in vivo* system is that any results obtained may be more relevant to the human situation, provided it can be shown that at each transplant generation, the tumors that arise are derived from cells that contain only human chromosomes.

Studies are now in progress to determine whether HMM is active against other lung tumors of human origin and other types of epidermoid carcinoma.

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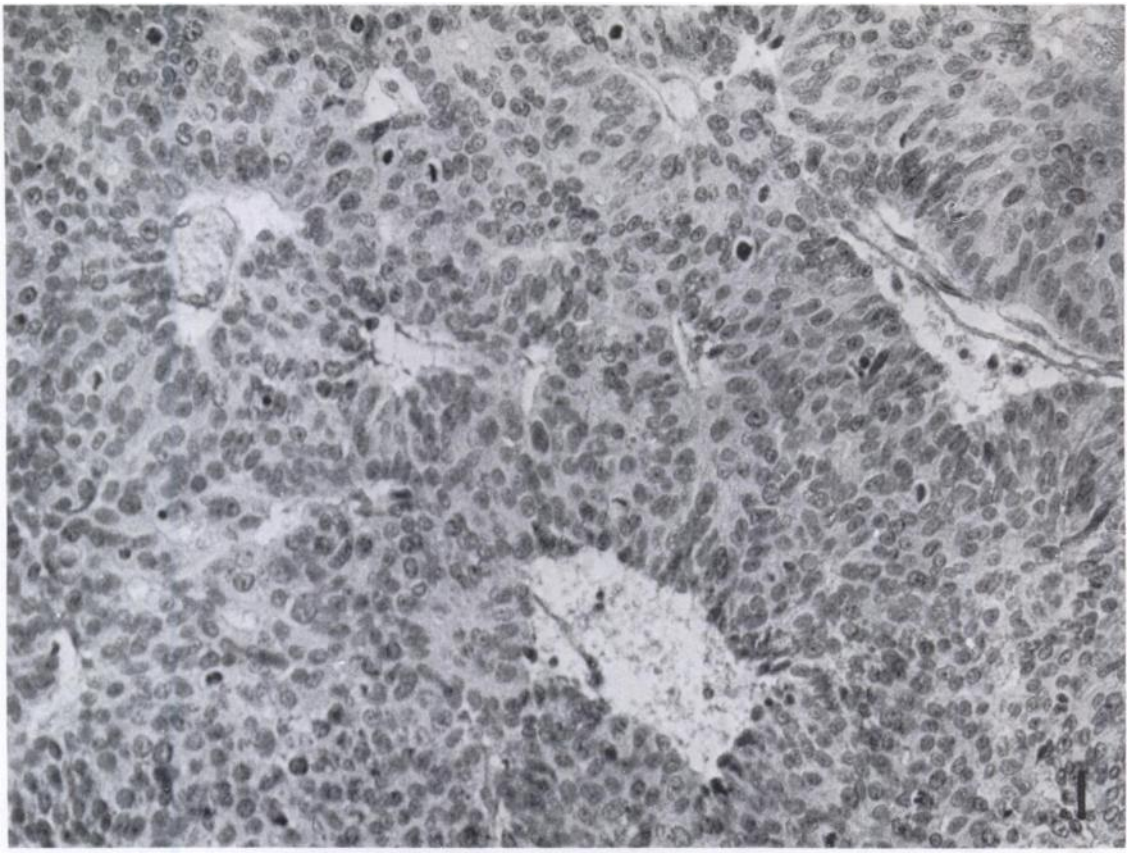

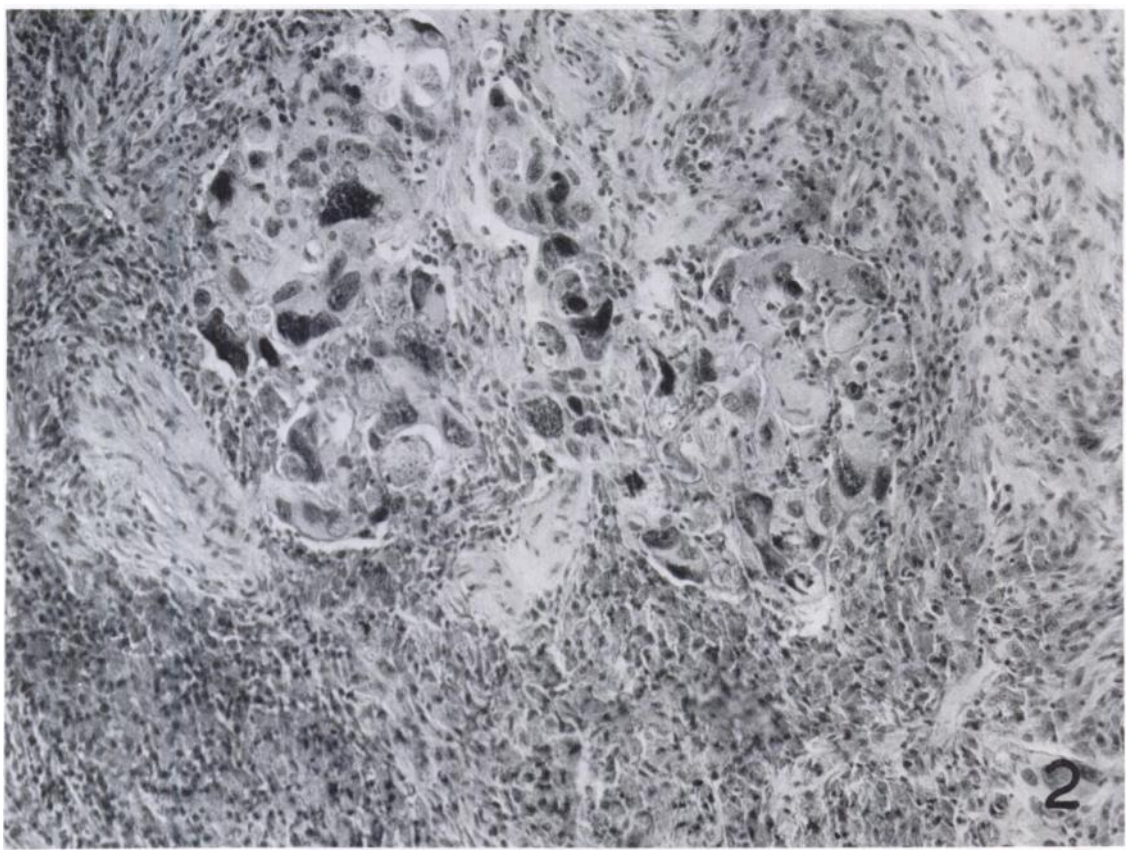

Fig. 1. Transplantable human bronchial carcinoma growing in immune deprived mice, removed 71 days after implantation. The appearances are those of a poorly differentiated epidermoid carcinoma with the tumor cells forming cords and trabeculae. Nuclear pleomorphism is not a prominent feature. Mitotic activity is marked. H & E, × 220.

Fig. 2. Transplantable human bronchial carcinoma growing in immune deprived mice after therapy with 90 mg HMM per kg given as 3 × 5 daily injections separated by intervals of 8 days. Only isolated islands of tumor cells remain. They are large, with eosinophilic cytoplasm, and show considerable nuclear and cellular pleomorphism with giant and bizarre forms. They are surrounded by extensive fibrosis and numerous small round cells. H & L, × 220.