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Note

High-performance liquid chromatographic analysis of pentamethylmelamine and its metabolites in biological fluids

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Pentamethylmelamine (PMM) (Fig. 1), an antitumor agent currently in clinical trial, is an aqueous soluble demethylated metabolite of hexamethylmelamine (HMM). HMM is effective in the treatment of a number of solid tumors, particularly ovarian carcinoma and lung cancer [1]; however, because of its water insolubility HMM is administered orally. PMM has activity similar to that of HMM against a number of animal tumors [2, 3], and both undergo extensive demethylation in vivo [4, 5]. These demethylated metabolites also have antitumor activity [2, 3].

Previous methods for determination of HMM and PMM include gas chromatography [5—7], gas chromatography—mass spectrometry [4], high-performance liquid chromatography (HPLC) [8], and thin-layer chromatography [9]. We now report a rapid, specific HPLC assay that will allow for the determination of the pharmacokinetics of not only PMM but also its demethylated metabolites.

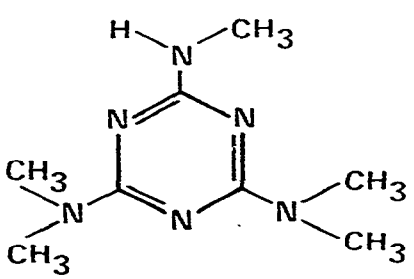

Fig. 1. Structure of pentamethylmelamine.

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MATERIALS AND METHODS

Chemicals

Pentamethylmelamine (PMM) and its metabolites N^2,N^2-dimethylmelamine (2,2-DMM); N^2,N^4,N^6-trimethylmelamine (2,4,6-TrMM); N^2,N^2,N^4-trimethylmelamine (2,2,4-TrMM); N^2,N^2,N^4,N^4-tetramethylmelamine (2,2,4,4-TeMM); N^2,N^2,N^4,N^6-tetramethylmelamine (2,2,4,6-TeMM), and monomethylmelamine (MMM) were kindly supplied by Leonard H. Kedda (Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute). Glass-distilled methanol was obtained from Burdick and Jackson Labs. (Saginaw, MI, U.S.A.). All other chemicals were obtained from regular commercial suppliers.

Sample preparation

Biological samples were obtained from patients receiving 80—1500 mg/m^2 of PMM. Blood was drawn into tubes containing heparin and centrifuged to obtain plasma. Urine was collected as voided, and cerebrospinal fluid (CSF) was obtained by lumbar puncture. To 3-ml Clin-Elut tubes (Analytichem International, Lawndale, CA, U.S.A.) containing inert cellulose were added 2 ml of the biological fluid. The tubes were eluted twice with 3 ml of ethyl acetate, with a 5-min delay between elutions. The eluents were collected, combined, and evaporated with a stream of nitrogen. The residues were reconstituted with 100 μl methanol. Aliquots of 10 μl were analyzed by HPLC. Control plasma extracted by the same procedure showed no interfering peaks.

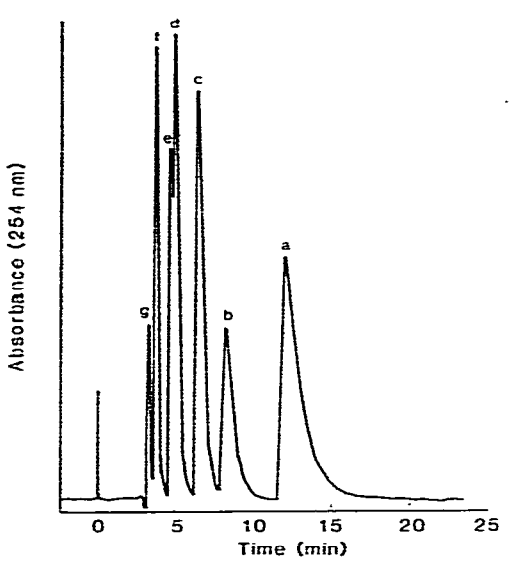
HPLC analysis

Analyses were performed on a Waters Assoc. liquid chromatograph (Milford, MA, U.S.A.) equipped with a M6000 pump, U6K injector, and Model 440 UV detector operating at 254 nm. Peak areas and retention times were determined by a Shimadzu (Kyoto, Japan) Chromatopec-EIA electronic integrator. Separations were achieved on a μ Bondapak C_{18} (10-μm particle size) column using 0.01 *M* ammonium formate (pH 3.5)—methanol (60:40) as eluent at a flow-rate of 1 ml/min.

RESULTS AND DISCUSSION

Fig. 2 shows a chromatogram of an aqueous solution of PMM and its demethylated metabolites. Except for the trimethylmelamines, the PMM metabolites are all well separated, with MMM eluting near the void volume. The capacity factors (k') for the compounds are: PMM, 2.54; 2,2,4,6-TeMM, 1.42; 2,2,4,4-TeMM, 0.91; 2,2,4-TrMM, 0.52; 2,4,6-TrMM, 0.42; DMM, 0.15; MMM, 0.01. The coefficients of variation for three determinations of the extracts of a 1 μg/ml plasma standard were: PMM, 1.9; 2,2,4,6-TeMM, 1.7; 2,2,4,4-TeMM, 1.2; 2,4,6-TrMM, 1.1; 2,2,4-TrMM, 0.64; DMM, 0.98; MMM, 2.4.

Fig. 3 shows the elution profile of plasma from a patient 8 h after the intravenous administration of 640 mg/m^2 of PMM. In this patient, as in most patients studied, no 2,2,4-TrMM was detected; however, all other metabolites were present. The metabolite 2,2,4-TrMM was detected in the plasma of only



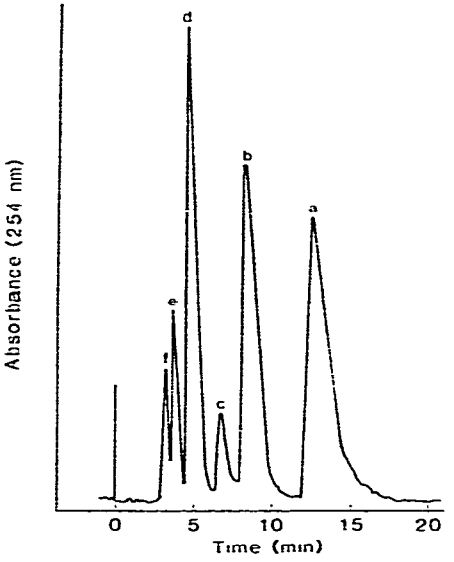


Fig. 2. Elution profile of an aqueous standard of PMM and its demethylated metabolites. Peaks: a = PMM, b = 2,2,4,6-TeMM, c = 2,2,4,4-TeMM, d = 2,2,4-TrMM, e = 2,4,6-TrMM, f = DMM, g = MMM.

Fig. 3. Chromatogram of patient's plasma 8 h after intravenous administration of 640 mg/m² of PMM. Peaks: a = PMM, b = 2,2,4,6-TeMM, c = 2,2,4,4-TeMM, d = 2,4,6-TrMM, e = DMM, f = MMM.

three of 21 patients, whereas 2,2,4,4-TeMM was observed in the plasma of two patients.

The plasma clearance of PMM and metabolites in a patient after 1 g/m² of PMM was administered is shown in Fig. 4. PMM and 2,2,4,6-TeMM are

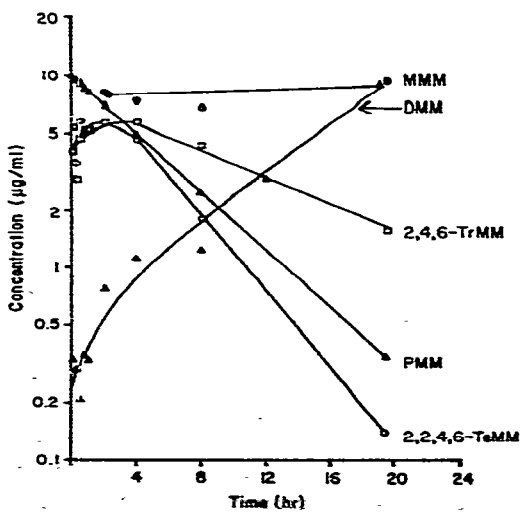


Fig. 4. Plasma clearance of PMM and metabolites after administration of 1 g/m² of PMM.

rapidly cleared from plasma, but 2,4,6-TrMM is cleared more slowly. DMM and MMM concentrations continue to rise during this time.

The urinary excretion of PMM and metabolites was low. Fig. 5 shows the elution profile of a patient's urine during the infusion of 1.5 g/m² of PMM. As shown, the major urinary excretion products are 2,4,6-TrMM and DMM. The chromatographic profile of a patient's CSF 24 h after administration of 2.0 g/m² of PMM is shown in Fig. 6. Again, the major constituents observed are the TrMM and DMM.

Thus, the HPLC assay described is an efficient and effective method for the determination of PMM and its metabolites in biological fluids. This assay is currently being used to study the clinical pharmacology of PMM.

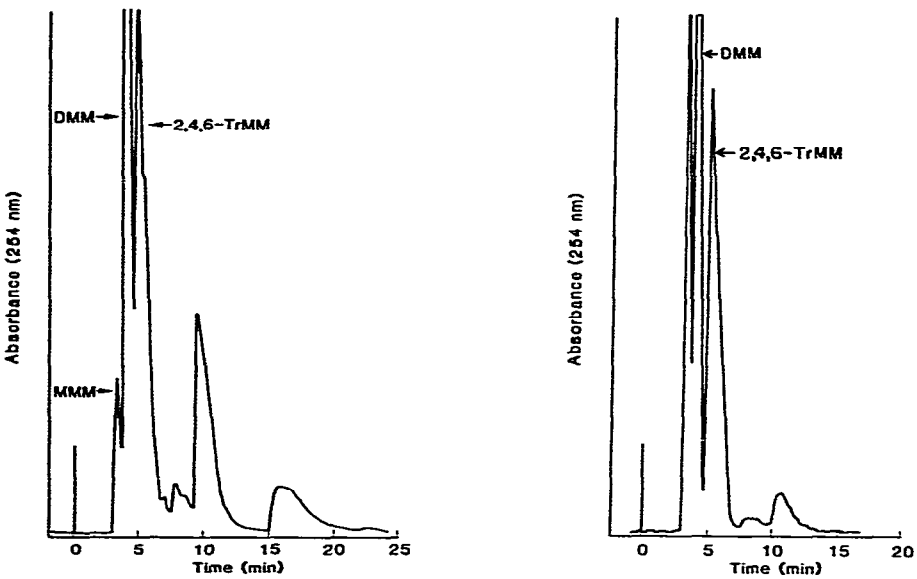


Fig. 5. Chromatogram of patient's urine during infusion of 1.5 g/m² of PMM.

Fig. 6. Elution profile of patient's CSF 24 h after administration of 2 g/m² of PMM.

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