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Note

High-performance liquid chromatographic analysis of the anticancer agent methylglyoxal bis(guanylhydrazone) (MGBG, NSC-32946) in biological fluids

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In 1958, Freedlander and French demonstrated that methylglyoxal bis-(guanylhydrazone) (MGBG, Fig. 1) was an effective agent against murine L1210 and P388 leukemias [1]. In the ensuing phase I and phase II clinical trials, MGBG was shown to have significant antileukemic activity [2—5]. In one study, 72% of patients with acute myeloblastic leukemia whose blast cells had granules or were Auer body positive obtained a remission [3]. However, efficacious therapy was often accompanied by toxicities that were not significantly attenuated by dose scheduling. As a result, further therapeutic trials with MGBG were discontinued.

Recently, Knight et al. [6] reported that the dose-limiting toxicity of MGBG may be largely diminished utilizing a weekly i.v. dose schedule. As a result, MGBG is being intensively re-evaluated for remission induction in patients with oligoleukemia.

To facilitate the renewed clinical trial of MGBG, it is advantageous to have knowledge of certain pharmacokinetic properties of this agent. Accordingly, a fast, sensitive, specific, and reproducible assay for MGBG is desirable. This paper describes a high-performance liquid chromatographic (HPLC) method to quantify MGBG in biological fluids. It is readily applicable to the monitoring of MGBG in cancer patients during therapy so that toxicity may be avoided.

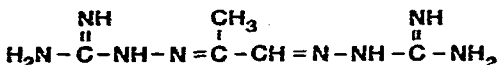

Fig. 1. Structure of MGBG.

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EXPERIMENTAL

Chromatography

All analyses were performed with a Waters Assoc. (Milford, MA, U.S.A.) Model 204 liquid chromatograph equipped with a Model M 6000A pump, a variable-wavelength UV detector (Varian Vari Chrom) set at 283 nm and a Varian recorder (Model 9176). Peak areas were determined by electronic integration (Varian Model CDS-111). An analytical reversed-phase μBondapak C_{18} column (Waters, 4 mm × 30 cm, 10 μm particle size) equipped with a reversed-phase μBondapak C_{18} guard column (Waters 1 mm × 6 cm, 10 μm particle size) was used for separation. Only reagents of analytical grade were used and all solvents were filtered and vacuum-degassed before use. The mobile phase was 0.03 *M* sodium acetate adjusted to pH 4.3 with glacial acetic acid and contained 5% methanol (Burdick and Jackson Labs., Muskegon, MI, U.S.A.). The flow-rate was 2 ml/min.

Preparation of biological fluids

Plasma. Aliquots (1 ml) of plasma containing MGBG (supplied by the Drug Development Branch, National Cancer Institute) were placed in 10-ml plastic centrifuge tubes. Plasma proteins were precipitated with 100 μl of cold perchloric acid (PCA). The tubes were centrifuged at 8,000 *g* (Sorvall centrifuge model RC2B) for 20 min and the supernatants were decanted into centrifuge tubes containing 100 μl of cold 10 *N* potassium hydroxide. The potassium perchlorate precipitate was removed by centrifugation at 5,000 *g* for 10 min and the supernatants were chromatographed as described above.

Urine. Urine samples of 1 ml containing various amounts of MGBG were acidified to pH 1 with 12 *N* hydrochloric acid and added to centrifuge tubes in which was placed 100 mg of XAD-2 resin (Supelco, Bellefonte, PA, U.S.A.) previously equilibrated in 1 *N* hydrochloric acid. The samples were vigorously stirred for 5 min and the supernatants were decanted, neutralized with 10 *N* potassium hydroxide and chromatographed as described previously.

Cerebrospinal fluid. Cerebrospinal fluid (CSF) was assayed for MGBG by the precipitation of contaminating proteins with PCA. Excess PCA was removed by addition of 10 *N* potassium hydroxide.

Leukocytes. A sample of MGBG was added to one ml of phosphate-buffered saline (PBS) containing $1 \cdot 10^8$ leukocytes. The cells were disrupted by sonication (Fisher Sonic Dismenbrator) and the proteins were precipitated with PCA. The PCA soluble fractions were neutralized and chromatographed.

RESULTS AND DISCUSSION

Its intense absorbance in the ultraviolet region (λ_{max} = 28 nm, ϵ = 33,800 at pH 4), permits MGBG to be readily assayed in leukocytes and biological fluids by means of HPLC. Under the conditions described in Experimental, MGBG was eluted from the reversed-phase column 6 min after injection (Fig. 2). In complex mixtures such as urine (Fig. 2E), the separation of MGBG from other components may have been slightly enhanced by a 40% reduction in the methanol content of the mobile phase. However, this slight advantage was offset by exaggerated tailing of the MGBG peak.

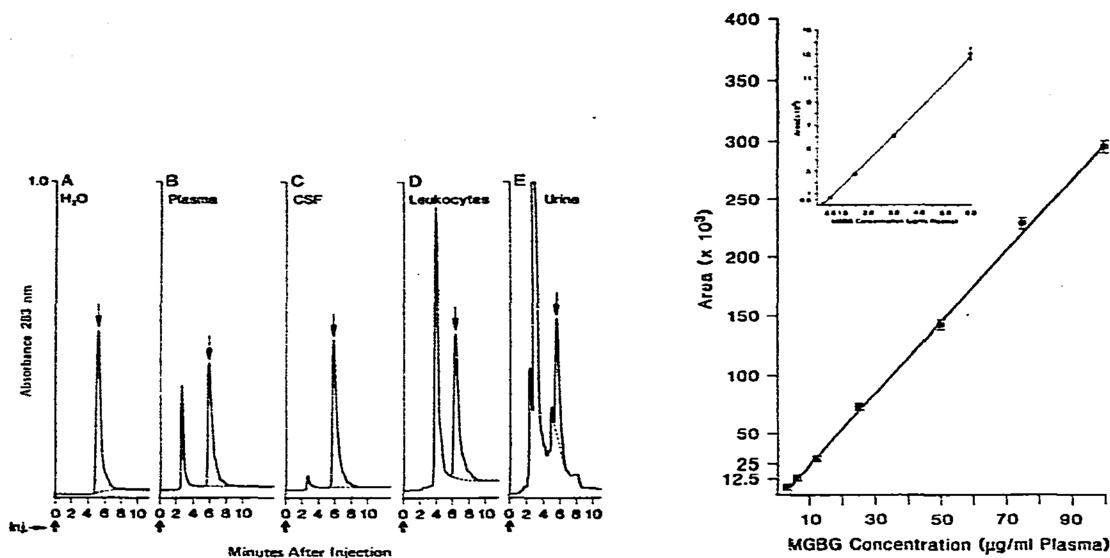


Fig. 2. Chromatograms of MGBG (peaks indicated by arrows) in various media and biological fluids. Broken line under the MGBG peak is the chromatographic pattern of the same biological fluid without MGBG. In all cases, MGBG concentration was 50 μg/ml.

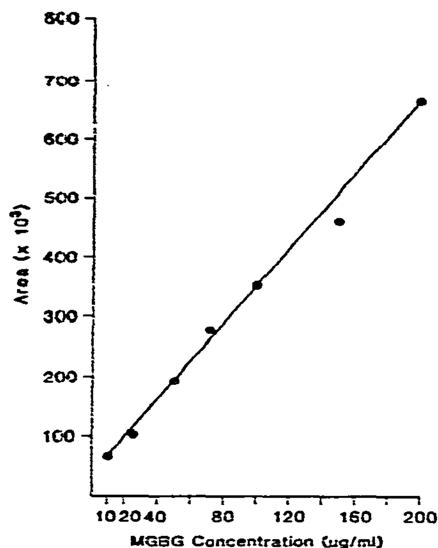
Fig. 3. Standard curve for MGBG in plasma. Values shown are means ± S.E.M. for duplicates of three separate determinations. Insert shows the linearity of the assay over the concentration range 0.25—6 pg MGBG per ml plasma.

Perchloric acid extraction of MGBG from plasma resulted in the loss of between 6% and 22% of the drug at various concentrations. The mean recovery rate was 84 ± 2% for duplicate determinations on nine drug concentrations between 0.25 and 100 μg MGBG/ml plasma. No change in recovery rate was noted when plasma and MGBG were incubated at 37°C for periods of up to 4 h (Table I). Slightly higher recovery rates were found for CSF and for a suspension of leukocytes (88% and 84% respectively).

TABLE I

RECOVERY OF MGBG FROM VARIOUS BIOLOGICAL FLUIDS

Medium	MGBG concentration (μg/ml)	Peak area (× 10^3) (Mean ± S.E.M.)	Recovery (%)
Water	50	181 ± 0.4	100
Plasma	50	138 ± 1.2	76
Plasma, incubated 2 h at 37°C	50	138 ± 0.7	76
Plasma, incubated 4 h at 37°C	50	138 ± 1.5	77
CSF	50	159 ± 0.9	88
Phosphate buffered saline containing 1 · 10^8 leukocytes	50	152 ± 0.7	84



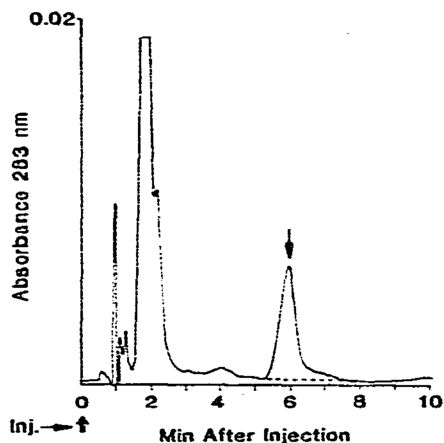


Fig. 4. Standard curve for MGBG in urine. Values are averages for duplicate determinations.

Fig. 5. Chromatogram of plasma from a cancer patient 3 h after intravenous MGBG (200 mg/m²). Broken line indicates the chromatographic pattern of plasma from the same patient prior to MGBG therapy.

A standard curve for MGBG in plasma (Fig. 3) shows that this HPLC assay has a lower limit of detection of approximately 250 ng and is linear over the concentration range 0.25 to 100 μg/ml. In urine (Fig. 4), this assay has a detection limit of 10 μg/ml due to interfering substances in urine.

MGBG was readily detected in the plasma of a patient who received MGBG therapy (Fig. 5). This assay method will therefore be useful for monitoring patients who receive MGBG therapy and for determining the pharmacokinetics of MGBG in patients with cancer.

ACKNOWLEDGEMENT

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