Pharmacokinetics of $[^{14}C]$Methylglyoxal-bis(guanylhydrazone) in Patients with Leukemia

Michael G. Rosenblum, Michael J. Keating, Boh Seng Yap, and Ti Li Loo

Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

Methylglyoxal-bis(guanylhydrazone) (MGBG; NSC 32946), a competitive inhibitor of S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50), is currently being reevaluated for its clinical antileukemic activity. MGBG labeled with $^{14}C$ in the guanylhydrazone moiety was administered i.v. (150 $\mu$Ci; specific activity, 1.9 $\mu$Ci/$\mu$mol; 20 mg total) to six patients with leukemia. All patients in the study had normal renal and hepatic function. $[^{14}C]$MGBG underwent no in vivo metabolism; it disappeared from the plasma with an average terminal t$_{1/2}$ of 4.1 hr. The 72-hr cumulative urinary excretion was only 14.5 ± 2.2% (S.E. M.) of the total radioactive dose. The apparent volume of distribution was 661 ml/kg and the total clearance rate was 21.2 ml/kg/min. The low urinary excretion rate and the relatively rapid plasma clearance suggest that MGBG may be sequestered in the body. Therefore, if MGBG is administered by a frequent treatment schedule, the prolonged biological half-life in humans may significantly contribute to its clinical toxicity.

INTRODUCTION

MGBG$^2$; NSC 32946 is a potent antineoplastic agent first shown to be effective against murine L1210 and P-388 leukemias in studies of guanylhydrazones by Freedlander and French (8). This antileukemic activity was confirmed in the ensuing Phase I and Phase II clinical trials with MGBG (9, 10). A 72% remission rate was obtained in acute myelogenous leukemia patients whose blast cells contained granules or were Auer rod positive (10).

The major toxic effects of MGBG in humans include severe mucositis, vasculitis, hypoglycemia, and weight loss (13, 14, 21, 22). However, myelosuppression was not a marked feature of MGBG toxicity in early clinical trials. Blood elements of patients with acute leukemia rapidly returned to normal after MGBG therapy (12).

Despite the observed antileukemic activity of MGBG in Phase I and Phase II clinical trials, further clinical use of MGBG was discontinued primarily because of severe toxicity which was not significantly attenuated by dose scheduling (15, 16). Recently, however, Knight et al. (11) reported that the dose-limiting toxicity of MGBG may be greatly diminished utilizing a weekly i.v. dose schedule. As a result, MGBG is currently being reevaluated for its ability to induce remission in patients with leukemia.

To facilitate the renewed clinical trials with MGBG, it is necessary to understand its pharmacokinetic behavior in humans. Accordingly, we have synthesized $[^{14}C]$MGBG and studied its pharmacokinetics and metabolism in patients with leukemia.

MATERIALS AND METHODS

Radioactive MGBG labeled with $^{14}C$ in the guanylhydrazone moiety was synthesized according to the procedure of Oliverio and Denham (18). Analysis of the product by both paper chromatography (18) and high-pressure liquid chromatography (23) showed that the product was greater than 99% pure chemically and radiochemically. Unlabeled MGBG was supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute.

Patient Studies. Patients with advanced and progressive leukemia taking part in the Phase I reevaluation of MGBG were selected for this study. None of the patients had received other chemotherapy for at least 2 weeks before the study, and informed written consent was obtained according to institutional guidelines. The results of the renal and hepatic function tests of these patients were within normal limits. Labeled MGBG (150 $\mu$Ci; 20 mg total; specific activity, 1.9 $\mu$Ci/$\mu$mol) was administered in 10 ml buffered 0.9% NaCl solution, pH 7, as an i.v. bolus. Venous blood (10 ml) was collected at designated intervals using heparin as the anticoagulant. A 1-ml aliquot of whole blood was centrifuged in a 5-ml plastic centrifuge tube at 5000 x g. The plasma was decanted and kept frozen until analysis. Urine specimens were collected for 72 hr after drug administration and kept frozen until analysis. For radioactivity determination, duplicate 0.1-ml portions of plasma or urine were added to 10 ml of Aquasol (New England Nuclear, Chicago, Ill.) in glass scintillation counting vials, and the samples were counted in a Packard Model 2650 Tri-Carb liquid scintillation spectrometer.

For the separation of leukocytes, a 4.0-ml aliquot of whole blood was diluted to 20.0 ml with PBS. A solution of 3.9 ml of 8% Ficoll and 1.25 ml of 5% Hypaque (1) was placed in a 50-ml glass centrifuge tube, and the diluted whole blood was layered on top. This solution was centrifuged for 35 min at 2000 x g, and the leukocyte layer was removed, washed twice with PBS, and diluted to 1.0 ml with PBS. The cells were counted with a Model ZBI Coulter counter, and radioactivity in duplicate 0.1-ml aliquots was determined by liquid scintillation in 10 ml Aquasol.

Plasma and urine from the patients were processed and chromatographed by high-pressure liquid chromatography as described previously (23) to determine the possible presence of metabolites. Briefly, plasma was deproteinized by the addition of 10 n perchloric acid. Excess perchloric acid was removed by neutralization with 10 n potassium hydroxide. Urine

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2. To whom requests for reprints should be addressed.
3. The abbreviations used are: MGBG, methylglyoxal-bis(guanylhydrazone); PBS, phosphate-buffered saline (0.005 M sodium phosphate in 0.9% NaCl solution).

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1748
samples were filtered and chromatographed with a Waters Associates (Milford, Mass.) Model 204 liquid chromatograph equipped with a Model M600A pump, a variable wavelength UV detector (Varian Var-Chrom; Varian Associates, Palo Alto, Calif.), and a Varian Model 1976 recorder. An analytical reverse-phase Cᵢ₈-Bondapak column (Waters; 4 mm x 30 cm) was used for separation. The mobile phase was 0.03 M sodium acetate buffer adjusted to pH 4.3 with glacial acetic acid and contained 5% methanol. Flow rate was 2 ml/min, and the column eluate was fractionated into 1-ml aliquots and assayed for ¹⁴C activity in scintillation vials containing 10 ml Aquasol scintillant.

RESULTS

Pharmacokinetic parameters were computed by standard techniques after nonlinear regression analysis of the plasma MGBG concentration versus time data. Fitting of the curves to the experimental data was excellent (r² = 0.92 to 1.00). Chart 1 shows the mean disappearance of [¹⁴C]MGBG from plasma of 6 patients with leukemia. Elimination of [¹⁴C]MGBG appeared to be triphasic; the calculated mean initial t₁/₂ was 1.5 min while the terminal t₁/₂ was 247 min (4.1 hr). The extrapolated apparent volume of distribution was 661 ml/kg, similar to the total body water or the antipyrine space in humans (4). Total clearance of MGBG from the plasma was 21.2 ml/kg/min. The 72-hr cumulative urinary excretion of MGBG (Chart 2) was only 14.5 ± 2.2% (S.E. M.) of the total dose administered. Analysis of the radiolabel in urine (Chart 3) and plasma by high-pressure liquid chromatography showed that the label was present as unchanged MGBG. Leukemic leukocytes of patients 4 hr after administration of [¹⁴C]MGBG showed no significant radiolabel present.

DISCUSSION

MGBG is the first clinically active antineoplastic agent which interrupts essential polyamine biosynthesis by the competitive inhibition of S-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) a key enzyme in the biosynthesis of spermidine and spermine (25). The polyamines putresine, spermidine, and spermine have been found to accumulate in mammalian tissues

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[Graphs and charts are described here, but the text does not contain them.]
M. G. Rosenblum et al.

with high rates of RNA and protein synthesis (2, 6, 24); subsequently, it was shown that the requirement for polyamines in macromolecular synthesis was absolute (5, 19). In addition, the intracellular biosynthesis and accumulation of polyamines appear to be linked to the biochemical events which control both normal and neoplastic growth (3, 20, 24).

The cellular penetration of MGBG into leukemic leukocytes in vitro was demonstrated by Field et al. (7) to occur via a carrier-mediated membrane transport system which is also responsible for polyamine transport. In the present study, no radioactivity was detected in leukocytes isolated from patients after [14C]MGBG administration. Many factors may account for the differences between in vitro and in vivo observations. However, it is likely that the high endogenous polyamine levels in plasma may inhibit MGBG penetration into WBC after a low dose.

The present study shows that in humans [14C]MGBG was rapidly cleared from the plasma but only slowly excreted in the urine. This suggests that MGBG may be sequestered in the body at a site outside the vasculature. However, it is possible that higher therapeutic doses may result in altered retention of drug in the body. Oliverio et al. (17) have shown that in mice and dogs MGBG is neither excreted into the bile nor metabolized to 14CO2 to a significant extent. The prolonged biological half-life of MGBG in humans may account for the cumulative toxicity with MGBG therapy observed earlier (15, 16). Although MGBG is an active antineoplastic agent, it is apparent from our pharmacological studies that frequent administration of this agent may result in accumulation of the drug in the body resulting in serious toxicity.

REFERENCES

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