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# Methylglyoxal bis(cyclohexylamidinohydrazone), a novel inhibitor of polyamine biosynthesis that simultaneously inhibits *S*-adenosylmethionine decarboxylase and spermidine synthase

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A large body of experimental evidence has clearly shown that inhibition of the biosynthesis of polyamines leads to the inhibition of cell growth and division [1–3]. The most widely used inhibitors of poylamine synthesis are difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC; EC 4.1.1.17) and methylglyoxal bis (guanylhydrazone) (MGBG), a potent inhibitor of putrescine-activated *S*-adenosylmethionine decarboxlase (AdoMetDC; EC 4.1.1.50). MGBG has been used very often in polyamine research [4, 5], but its severe side effects [6, 7] are compromising its usefulness. In view of the obvious advantages afforded by inhibitors of polyamine biosynthesis, we investigated new compounds and discovered that cyclohexylamine group had ability to inhibit spermidine synthase [8, 9]. Consequently, a compound having both methylglyoxalhydrazone and cyclohexylamine groups was expected to inhibit AdoMetDC and spermidine synthase activities simultaneously. In the present paper, we report a novel inhibitor of polyamine biosynthetic enzymes, methylglyoxal bis (cyclohexylamidinohydrazone) (MGBC), which exerts the inhibitory effects on AdoMetDC and spermidine synthase *in vitro*.

MGBC was synthesized in our laboratory as described previously [10]. DL-[1-$^{14}$C]Ornithine, *S*-adenosyl-L-[carboxy-$^{14}$C]methionine and *S*-adenosyl-L-[methyl-$^{14}$C]methionine were purchased from New England Nuclear (Boston, MA). Decarboxylated AdoMet, both unlabelled and labeled in the methyl group, was prepared by the action of AdoMetDC from *Escherichia coli* (strain B) and purified by chromatography on Dowex-50-H$^+$ and paper electrophoresis [11]. All other chemicals were products of Nakarai Chemicals Ltd. ODC from Ehrlich ascites tumor cells [12], AdoMetDC from rat liver [13], spermidine and spermine synthases from rat ventral prostate [8] were prepared as described in the original publications. Protein was determined by the method of Bradford [14] using bovine serum albumin as a standard. The activities of ODC [15], AdoMet DC [16], spermidine and spermine synthases [17] were measured as described earlier. Polyamines (putrescine, spermidine and spermine) were determined by HPLC (Shimazu LC-5A) as described previously [18].

MGBC showed the inhibition of AdoMetDC and spermidine synthase activities. The effect of the concentration of AdoMet on the inhibition of AdoMetDC by MGBC is shown in Fig. 1. This inhibition was competitive with AdoMet, and the calculated $K_i$ value for MGBC was 18.5 $\mu$M. The $K_m$ value for AdoMet was estimated to be 0.21 mM. On the other hand, the $K_i$ value for the parent inhibitor MGBG was 5 $\mu$M and the inhibition by MGBG was competitive with AdoMet using the same AdoMetDC (data not shown). Thus, MGBC was found to be a slightly weaker inhibitor of AdoMetDC than MGBG. Two substrates, putrescine and decarboxylated AdoMet, are involved in the reaction of spermidine synthase forming spermidine and methylthioadenosine. Figure 2 shows the effect of the concentrations of putrescine on the inhibition of spermidine synthase by MGBC. The inhibition was competitive with putrescine. The $K_i$ and $K_m$ values for MGBC and putrescine were 28.0 and 25.0 $\mu$M, respectively. On the other hand, the $K_i$ value for the parent inhibitor cyclohexylamine was 2 $\mu$M and the inhibition by cyclohexylamine was competitive with putrescine using the same

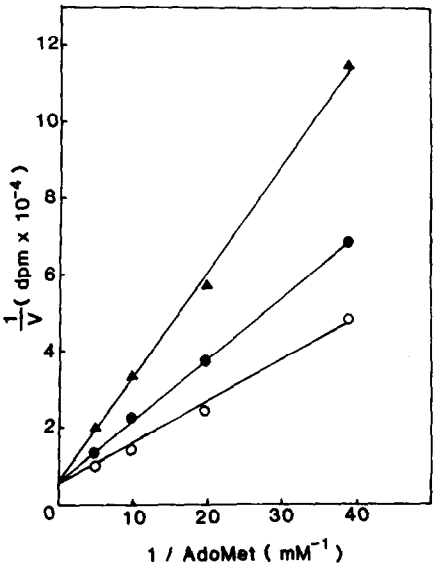


Fig. 1. Competitive inhibition of AdoMetDC by MGBC with AdoMet as the variable substrate. AdoMetDC activity was assayed in the absence (○) or presence of 20 $\mu$M (●) or 50 $\mu$M (▲) MGBC, with 0.026–0.207 mM AdoMet and 85 $\mu$g enzyme protein. Each point represents an average of duplicate assays.

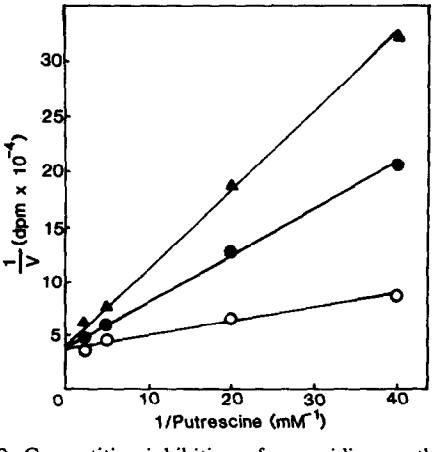


Fig. 2. Competitive inhibition of spermidine synthase by MGBC with putrescine as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 60 $\mu$M (●) or 100 $\mu$M (▲) MGBC, with 0.025–0.50 mM putrescine and 58 $\mu$g enzyme protein. Each point represents an average of duplicate assays.

Table 1. Effect of MGBC on polyamine contents and growth of human lymphoid Molt 4B leukemic cells

| Treatment | Putrescine | Spermidine (nmol/mg protein) | Spermine | Cell density ($\times 10^5$/ml) |
|---|---|---|---|---|
| Control | 5.9 (100) | 57.2 (100) | 92.9 (100) | 16.2 (100) |
| MGBC (10 μM) | 5.9 (100) | 36.8 (64) | 75.2 (81) | 10.8 (66) |
| MGBC (20 μM) | 5.8 (98) | 29.3 (51) | 71.5 (77) | 4.1 (25) |

Molt 4B cells were exposed to 10 or 20 μM MGBC for 5 days and harvested to determine cell number, polyamines and protein. The percent of the control (without treatment) is shown in parentheses. Each value is the mean of duplicate experiments.

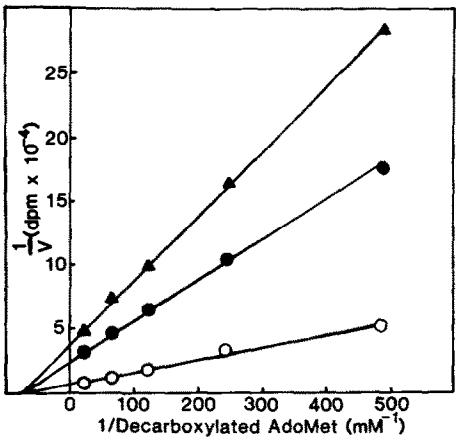


Fig. 3. Noncompetitive inhibition of spermidine synthase by MGBC with decarboxylated AdoMet as the variable substrate. Spermidine synthase activity was assayed in the absence (○) or presence of 60 μM (●) or 100 μM (▲) MGBC, with 0.0021–0.0412 mM decarboxylated AdoMet and 78 μg enzyme protein. Each point represents an average of duplicate assays.

spermidine synthase (data not shown). Thus, MGBC was found to be a slightly weaker inhibitor of spermidine synthase than cyclohexylamine. Figure 3 shows the effect of the concentration of decarboxylated AdoMet on MGBC inhibition of spermidine synthase. The plotting shows the noncompetitive inhibition in terms of decarboxylated AdoMet. The $K_i$ and $K_m$ values for MGBC and decarboxylated AdoMet were 30.2 and 13.3 μM, respectively. Removal of MGBC from the active form of spermidine synthase by dialysis restored the activity to that found in preparations treated similarly except for exposure to the inhibitor (data not shown). Thus, the inhibition of spermidine synthase by MGBC was reversible. The result that spermidine synthase was inhibited by MGBC competitively with putrescine and noncompetitively with decarboxylated AdoMet suggested that MGBC would bind to the putrescine-binding site. It is noteworthy that the hybrid compound MGBC retains an ability to inhibit AdoMetDC and spermidine synthase simultaneously.

As shown in Table 1, MGBC inhibited dose-dependently the growth of lymphoid leukemic Molt 4B cells at relatively low concentrations. The spermidine and spermine contents in the inhibitor-treated cells were also depressed dose-dependently, suggesting that these changes were resulted from the inhibitory effects of MGBC on AdoMetDC and spermidine synthase *in vivo*.

The $LD_{50}$ of 350 mg/kg and 115 mg/kg were obtained with MGBC and MGBG in DDY strain of mice, respectively. Thus, the former was about 3-fold higher than the latter.

*Department of Biochemistry*
*Mie University School of Medicine*
*Tsu, Mie 514, Japan*

HIROSHIGE HIBASAMI
TETSUYA TSUKADA
SATORU MAEKAWA
KUNIO NAKASHIMA

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