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Antitumor effect of methylglyoxal *bis*(3-aminopropylamidinohydrazone), a new inhibitor of *S*-adenosylmethionine and ornithine decarboxylases, on human erythroid leukemia K562 cells

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Summary. Methylglyoxal *bis*(3-aminopropylamidinohydrazone (MGBA) inhibited *S*-adenosylmethionine decarboxylase (AdoMetDC) activity competitively with *S*-adenosylmethionine (AdoMet), showing a K_i value of 2.60×10^{-5} *M*. It also inhibited ornithine decarboxylase competitively with ornithine, showing a K_i value of 3.80×10^{-5} *M*. MGBA inhibited the growth of human erythroid leukemia K562 cells. Putrescine, spermidine, and spermine concentrations in MGBA-treated cells were depressed to 19%, 36%, and 66% of the values of control cells, respectively.

Introduction

A wide variety of experimental results has clearly shown that inhibition of the biosynthesis of polyamines (putrescine, spermidine, and spermine) leads to the inhibition of cellular growth and division both in vivo and in vitro [3, 7, 11, 16, 17]. The most widely used inhibitor of polyamine biosynthesis is difluoromethylornithine, and enzyme-activated irreversible inhibitor of ornithine decarboxylase (ODC; EC 4.1.1.17) [12]. Difluoromethylornithine has also been used in the treatment of cancer patients [8].

In view of the obvious advantages afford by inhibitors of polyamine biosynthesis, we discovered that cyclohexylamine groups could inhibit spermidine synthase and depressed polyamine concentrations in mammalian cells [4, 5]. Methylglyoxal *bis*(guanylhydrazone) (MGBG) is a potent inhibitor of putrescine-activated eukaryotic *S*-adenosylmethionine decarboxylase (AdoMetDC; EC 4.1.1.50) [22]. MGBG has been used very often in polyamine research [7, 11], but its severe side effects [8, 15] compromise its usefulness. In an effort to obtain inhibitors without such side effects, we synthesized methylglyoxal *bis*(3-aminopropylamidinohydrazone) (MGBA), replacing guanyl groups of MGBG with aminopropylamidino groups.

In the present paper the effect of MGBA on human erythroid leukemia K562 cells was investigated and the correlation between the antiproliferative effect and the polyamine concentrations in the leukemic cells was discussed.

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Abbreviations. MGBA, methylglyoxal bis(3-aminopropylamidinohydrazone); AdoMetDC, *S*-adenosylmethionine decarboxylase; AdoMet, *S*-adenosylmethionine; ODC, ornithine decarboxylase

Materials and methods

Animals. Male Wistar rats, 8 weeks old, were obtained from Shizuoka Agricultural Co-operative Association for Experimental Animals, Japan. The animals were given water and food ad libitum.

Chemicals. MGBA was synthesized as follows: 1,3-diaminopropane was reacted with acetic anhydride to yield monoacetyl-1,3-diaminopropane. Monoacetyl-1,3-diaminopropane was then reacted with *S*-methylisothiosemicarbazide hydroiodide to produce 3-(3-acetamidopropyl)-1-aminoguanidine hydrochloride. The product was refluxed with methylglyoxal 1-oxime. The resulting methylglyoxal *bis*(3-acetamidopropylamidinohydrazone) was refluxed again in 6 *N* HCl, yielding methylglyoxal *bis*(3-aminopropylamidinohydrazone). The detailed procedure for synthesis will be published elsewhere. Adenosyl-L-[carboxy-^{14}C]methionine (58 mCi/mmol) and DL-[l-^{14}C]ornithine (48.8 mCi/mmol) were obtained from New England Nuclear Corp. (USA). All other chemicals were products of Nakarai Chemicals Ltd (Japan).

Cell culture. Human erythroid leukemia K562 cells were grown at 37° C in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum, penicilin (50 IU/ml), and streptomycin (50 μg/ml) in a 95% air-5% CO_2 humidified incubator. Stock solutions of MGBA were made in RPMI 1640 medium and stored at 4° C.

Preparations of ODC and AdoMetDC enzyme extracts. Human erythroid leukemia K562 cells grown in 5-ml culture flasks were harvested by low-speed centrifugation (1000 *g* for 3 min), washed twice in 10 ml phosphate-buffered saline, and centrifuged at 1000 *g* for 3 min. To prepare ODC and AdoMetDC enzyme extracts, the cell pellets were suspended in 100 μl 50 m*M* TRIS-HCl buffer (pH 7.2) containing 10 m*M* dithiothreitol, 0.1 m*M* EDTA, and 50 μ*M* pyridoxal-5'-phosphate and in 100 μl 10 m*M* TRIS-HCl buffer (pH 7.5) containing 1 m*M* dithiothreitol, 0.1 m*M* EDTA, and 2.5 m*M* putrescine, respectively. The cell suspensions were frozen and thawed three times and centrifuged at 12,000 *g* for 10 min, and the supernatants were taken for ODC and AdoMetDC assays. Protein was determined by the method of Bradford [2], using bovine serum albumin as a standard.

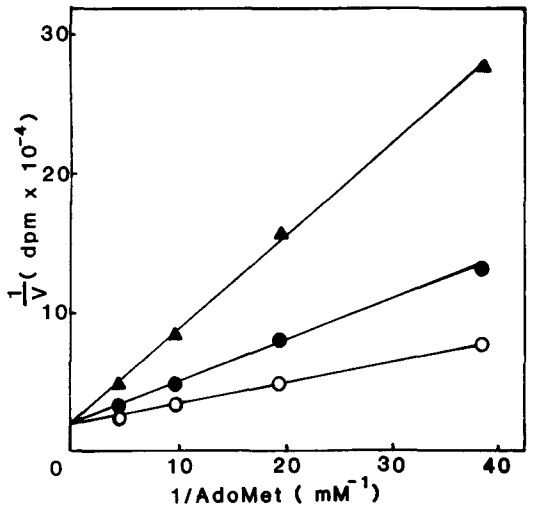

Fig. 1. Competitive inhibition of AdoMetDC by MGBA with AdoMet as the variable substrate. AdoMetDC activity was assayed in the absence (○) or presence of 25 μ*M* (●) or 100 μ*M* (▲) MGBA, with 0.026–0.207 m*M* AdoMet and 95 μg enzyme protein

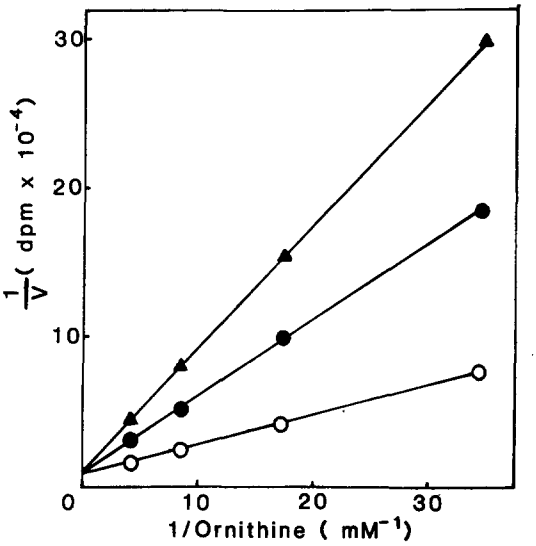

Fig. 2. Competitive inhibition of ODC by MGBA with ornithine as the varianle substrate. ODC activity was assayed in the absence (○) or presence of 50 μ*M* (●) or 100 μ*M* (▲) MGBA, with 0.029–0.232 m*M* ornithine and 97 μg enzyme protein

Assay for AdoMetDC activity. AdoMetDC activity was determined by the procedure developed by Pegg and Williams-Ashman [14]. The assay mixture contained 10 m*M* putrescine, 2 m*M* AdoMet (0.1 μCi), 10 m*M* dithiothreitol in 0.3 ml 10 m*M* TRIS-HCl buffer (pH 7.5), and 0.1 ml enzyme preparation.

Assay for ODC activity. ODC activity was determined as described by Seely et al. [21]. The assay mixture contained 0.1 m*M* DL-[l-^{14}C]ornithine (0.2 μCi), 20 μ*M* pyridoxal-5'-phosphate, 5 m*M* dithiothreitol in 0.2 ml 50 m*M* TRIS-HCl (pH 7.2), and 0.1 ml enzyme preparation.

Determination of polyamines. The cells were harvested by low-speed centrifugation (1,000 *g* for 3 min), suspended in 0.4 *N* perchloric acid, and disintegrated by freeze-thawing three times. After centrifugation for 30 min at 10,000 *g*, the resulting supernatant fractions were used for polyamine determination by HPLC (Shimazu LC-5A) as previously described [10].

Results

Inhibition of AdoMetDC and ODC activities by MGBA

MGBA showed the inhibition of AdoMetDC and ODC activities, inhibiting AdoMetDC activity more sensitively than ODC activity. The effect of the concentration of *S*-adenosylmethionine (AdoMet) on the inhibition of AdoMetDC activity by MGBA is shown in Fig. 1. This inhibition was competitive with AdoMet, and the calculated K_i value for MGBA was 2.60×10^{-5} *M*. The K_m value for AdoMet was estimated to be 8.00×10^{-5} *M*.

Figure 2 shows the effect of the concentration of ornithine on the inhibition of ODC activity by MGBA. The inhibition was competitive with ornithine; the K_i value for MGBA and the K_m value for ornithine were calculated to be 3.80×10^{-5} *M* and 2.40×10^{-4} *M*, respectively.

Stabilizing effect of MGBA on AdoMetDC in K562 cells

As shown in Fig. 3, AdoMetDC activity was induced by the addition of MGBG to the medium, whereas no increase of enzyme activity was observed in MGBA-treated cells. Thus, MGBA did not produce any stabilizing effect of AdoMetDC. This stabilizing effect of MGBG on AdoMetDC in K562 cells was consistent, with the result that a striking increase in AdoMetDC activity in Ehrlich ascites cells grown in the presence of MGBG was based on a dramatic prolongation of the half-life of the enzyme [1].

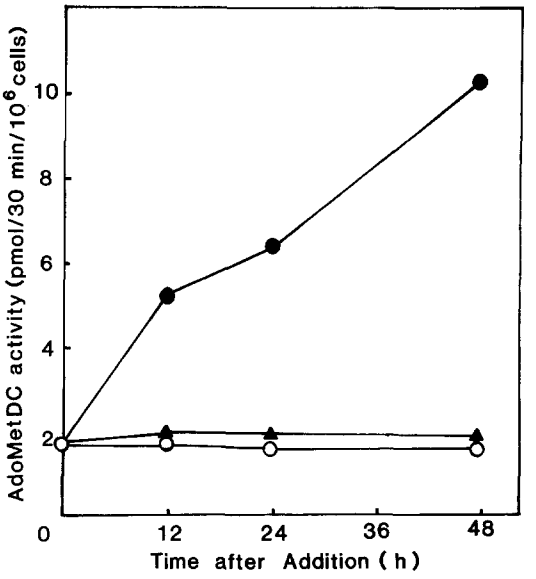

Fig. 3. Time-course of induction of AdoMetDC activity by MGBG or MGBA. Stock cultures were diluted with fresh medium to an initial density of 5×10^5 cells/ml and were grown in the absence (○) or presence of 10 μ*M* MGBG (●) or 10 μ*M* MGBA (▲). AdoMetDC activity was measured at the times shown. Each point is the mean of duplicate experiments

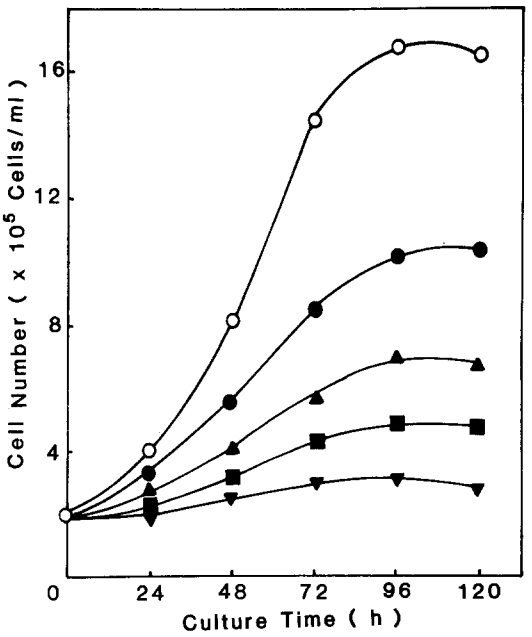

Fig. 4. Effect of MGBA on the growth of K562 cells. The stock cultures were diluted with fresh medium to an initial density of 2×10^5 cells/ml and were grown in the absence (○) or presence of 10 μ*M* (●), 25 μ*M* (▲), 50 μ*M* (■), or 100 μ*M* (▼) MGBA for the times indicated

Inhibition of growth of K562 cells by MGBA

Decreased rate of cell proliferation were observed in the presence of increasing concentrations of MGBA with human leukemia K562 cells (Fig. 4). The cells hardly grew at the concentration of 100 μ*M* MGBA.

Effect of MGBA on polyamine concentrations in K562 cells

At a concentration sufficient to inhibit the growth of K562 cells (50 μ*M*), MGBA depressed the intracellular levels of putrescine, spermidine, and spermine simultaneously, suggesting that both AdoMetDC and ODC activities were inhibited in the cells (Table 1). The decrease in putrescine concentration in MGBA-treated cells was noteworthy because this metabolite would markedly increase in MGBG-treated cells.

Discussion

Continuous polyamine biosynthesis appears to be required to maintain maximal rates of cell proliferation. An increased polyamine content in tumor-bearing tissues of animals or humans has been noted [9, 18, 20], and correlations between tumor growth rates and cellular polyamine contents have been demonstrated [19, 23].

Table 1. Effect of MGBA on intracellular levels of polyamines in human erythroid leukemia K562 cells[a]

Treatment	Putrescine	Spermidine	Spermine
None	174 (100)	2422 (100)	1819 (100)
MGBA (50 μ*M*)	33 (19)	872 (36)	1205 (66)

[a] K562 cells were grown in the absence or presence of 50 μ*M* MGBA for 72 h. Polyamine concentrations are shown in pmol per 10^6 cells; the percentage of control is shown in parentheses. Each value is the mean of duplicate experiments

Since MGBA was inhibitory to AdoMetDC (Fig. 1) and ODC (Fig. 2) but showed no AdoMetDC stabilizing effect (Fig. 3), it could present a simple experimental system to demonstrate the correlation between polyamine depletion and cellular function. MGBA showed a remarkable antiproliferative effect on K562 cells, and the inhibition of cell growth was associated with a significant depletion of intracellular polyamine levels (Fig. 4, Table 1). Particularly the concentration of putrescine was found to decrease, as well as that of spermidine and spermine, in contrast to MGBG, which dramatically increased the putrescine level [6]. One possible explanation is that MGBG induces ODC activity [6] and inhibits that of diamine oxidase [13] whereas MGBA may not, as has been observed with MGBB [6]. Investigations along this line are in progress. The findings obtained with MGBA suggested that the multifunctional inhibitors of polyamine biosynthesis would be very efficient for the depletion of polyamines in tumor cells. The further characterization of MGBA and optimal conditions in the search for a therapeutic possibility are now under investigation.

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