Methylglyoxal bis(guanylhydrazone) stimulates the cellular transport system of the polyamines

Leena Alhonen-Hongisto, Pauli Seppänen and Juhani Jänne

Department of Biochemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

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1. INTRODUCTION

Methylglyoxal bis(guanylhydrazone) (MGBG), an inhibitor of polyamine biosynthesis [1], is apparently transported into the interior of the cell (by virtue of its structural resemblance to spermidine) via an inducible transport system used by the natural polyamines spermidine and spermine [2–5]. Cellular uptake of polyamines, and hence also of MGBG, is strikingly enhanced under conditions of intracellular putrescine and spermidine depletion, as a result of the use of inhibitors of ornithine decarboxylase (EC 4.1.1.17) [5]. Even though the latter phenomenon is obviously aimed at normalizing the reduced intracellular polyamine pools, it also offers a means of enhancing the cellular accumulation of the antiproliferatively acting MGBG when profound growth inhibition is desired [6].

Although intracellular polyamine depletion triggers a variety of compensatory mechanisms, such as induction and stabilization of ornithine and adenosylmethionine (EC 4.1.150) decarboxylases (for ref. see [7]), the greatly enhanced transport of extracellular polyamines can apparently alone abolish the antiproliferative effects of polyamine antimetabolites (inhibitors of polyamine biosynthesis) in the presence of only trace amounts of exogenous polyamines. This fact may be practically important since polyamine antimetabolites [MGBG and 2-difluoromethylornithine (DFMO)] have already been used alone (for ref. see [6]) or in combination [8] in the treatment of human malignancies.

We will show here that when cultured Ehrlich ascites carcinoma cells were exposed to MGBG for not longer than a few hours, the uptake of polyamines, especially that of diamines, was greatly enhanced. Exposure to MGBG likewise stimulated the uptake of polyamines by tumor cells that have been brought to the state of severe polyamine deprivation with DFMO, a condition characterized by strikingly increased uptake of polyamines [5]. The enhanced uptake of polyamines induced by the antimetabolites was apparently responsible for the appearance of substantial amounts of cadaverine (and putrescine) in L1210 leukemia cells harvested from mice treated with MGBG alone or in combination with DFMO.

2. MATERIALS AND METHODS

2.1. *Cells*

Ehrlich ascites carcinoma cells were grown in RPMI 1640 medium supplemented with 5% pooled human serum (Finnish Red Cross Transfusion Service, Helsinki, Finland) and antibiotics. Murine L1210 cells were maintained exclusively in the peritoneal cavity of female DBA/2J Bom mice. After harvesting the tumor cells were washed with physiological saline and disintegrated by ultrasonication for MGBG determinations or suspended in 0.2 N perchloric acid for polyamine measurements.

2.2. *Chemicals*

MGBG was synthesized by and obtained from Orion Pharmaceutical Company (Helsinki, Finland). DFMO was a generous gift from the Centre de Recherche Merrell International (Strasbourg, France). [^{3}H]putrescine (spec. act. 25.4 Ci/mmol),

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[^{14}C]cadaverine (spec. act. 106.3 mCi/mmol), [^{3}H]spermine (spec. act. 21.7 Ci/mmol) were purchased from the New England Nuclear Company (Dreieich, Federal Republic of Germany).

2.3. *Analytical methods*

Polyamines were measured from perchloric-acid extracts by the method of Seiler [10] with the modifications introduced by Dreyfuss et al. [11] using chloroform–*n*-butanol–dioxan (48:1:1, by vol.) as the solvent in the thin-layer chromatography of the dansylated amines. MGBG was determined by the method of Seppänen et al. [4] using adenosylmethionine decarboxylase from baker's yeast. Protein was measured by the method of Lowry et al. [12]. Cell densities were determined with the aid of an electronic particle counter (Coulter Electronics, Harpenden, England).

For the calculations of statistical differences, the two-tailed *t*-test was used.

3. RESULTS

An exposure of Ehrlich ascites carcinoma cells to MGBG for 6 h resulted in a distinct stimulation of polyamine uptake when measured after the transfer of the cells into drug-free medium (table 1). As also shown in the table, after 2 h the uptake of putrescine and cadaverine was more effectively (about 4-fold) stimulated than that of spermidine and spermine (about 2.5-fold). From the amines tested, net uptake of putrescine appeared to be most rapid after pretreatment with MGBG (table 1). Because of the unavailability of radiolabeled MGBG, we could not unambiguously show that a prior exposure to MGBG would also enhance its own uptake. The kinetics of MGBG uptake, in comparison with spermidine (see figs. 1 and 2), supported the idea of an autoinduction of the drug transport.

Treatment of tumor cells with DFMO rapidly results in a profound putrescine and spermidine deprivation, which in turn induces the polyamine transport system [5]. During severe polyamine depletion, all polyamines, including cadaverine, are taken up at a rate that is about 10-fold faster than in cells with normal intracellular polyamine pools [5,13].

In the experiment depicted in fig.1, tumor cells depleted of putrescine and spermidine by a previous treatment with DFMO, were exposed to MGBG for varying periods of time and the uptake of spermidine was then measured in drug-free medium. As shown in the figure, a prior treatment with MGBG, for any length of time, clearly enhanced the uptake of spermidine by these polyamine-depleted cells. Thus the loading with MGBG of tumor cells possessing greatly reduced intracellular putrescine and spermidine pools did not abolish the uptake of spermidine, on the contrary, a further stimulation occurred.

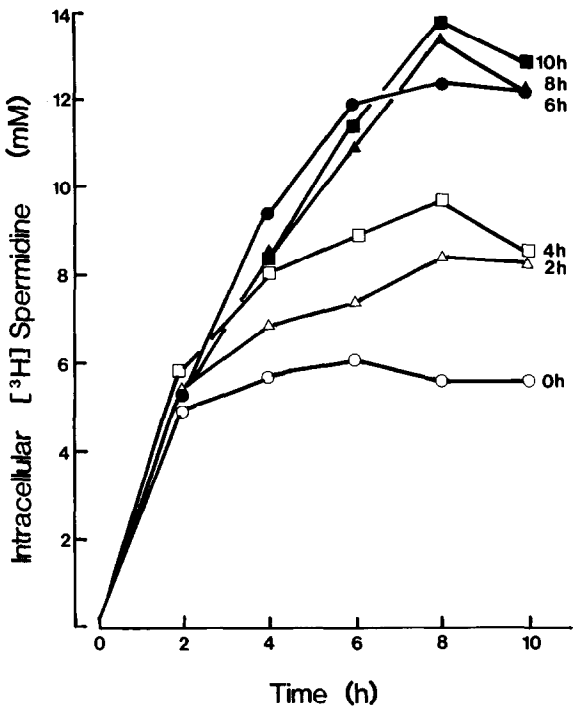

Fig.1. Effect of a prior exposure to MGBG on the uptake of spermidine in polyamine-depleted Ehrlich ascites cells. The cells were grown in the presence of 3 mM DFMO for 3 days whereafter 5 μM MGBG was added into the medium for various time periods (2 to 10 h). After MGBG treatment the cells were transferred into fresh medium containing 5 μM [^{3}H]spermidine and polyamine uptake was measured at the times indicated.

When the experiment was performed in the reverse order, i.e., the polyamine depleted cells were first loaded with spermidine (for 2 to 10 h) and the uptake of MGBG was measured thereafter (in the absence of spermidine), the situation (fig.2) was

Table 1

Effect of a prior exposure to MGBG on the uptake of polyamine in cultured Ehrlich ascites carcinoma cells

Duration of the uptake	MGBG exposure	Polyamine uptake (pmol/10^6 cells)			
		Put	Cad	Spd	Spm
2 h	–	287 (1.0)	131 (1.0)	354 (1.0)	214 (1.0)
2 h	+	1140 (4.0)	491 (3.8)	941 (2.7)	502 (2.4)
6 h	–	1150 (1.0)	471 (1.0)	1120 (1.0)	540 (1.0)
6 h	+	2620 (2.3)	1170 (2.5)	1600 (1.4)	878 (1.6)

Untreated cells and cells previously exposed to 5 μM MGBG for 6 h were transferred to fresh medium supplemented with 5 μM radiolabeled polyamines (0.05 μCi/ml). Polyamine uptake was measured at 2 h and 6 h after the medium change. Put, putrescine; Cad, cadaverine; Spd, spermidine; Spm, spermine

just the opposite of that shown in fig.1. An incubation of the DFMO-treated cells for 2 h in the presence of 5 μM spermidine was already sufficient to decrease the uptake of MGBG to low levels corresponding to that of cells with undepleted intracellular polyamines (fig.2). It thus appears that MGBG, though using the polyamine transport system, is not recognized as a natural polyamine, at least as regards the repression of the stimulated uptake system.

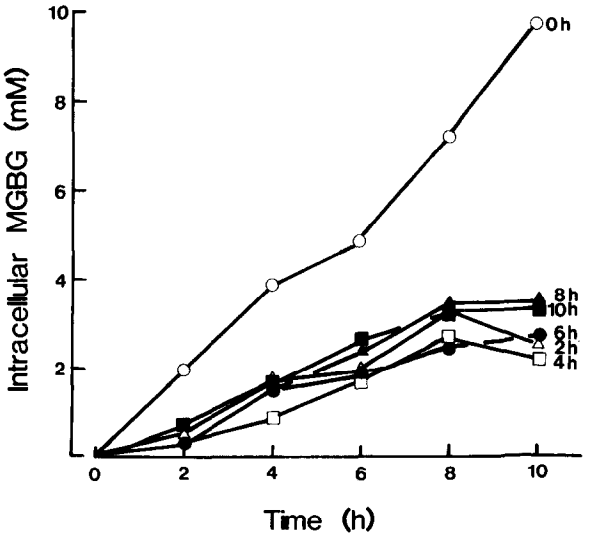

Fig.2. Effect of a prior exposure to spermidine on the uptake of MGBG in polyamine-depleted Ehrlich ascites cells. Experimental details as in fig.1, except that MGBG uptake was measured after loading the DFMO-treated cells with spermidine.

The practical consequences of the enhanced uptake induced by the antimetabolites of polyamines (DFMO, MGBG and their combination) in tumor-bearing animals are presented in table 2. In this experiment, L1210 tumor-bearing mice were treated with DFMO (2% in drinking water) or MGBG (50 mg/kg every other day) or their combination and the polyamine contents of L1210 cells were measured after 5 days. As shown in table 2, the treatment with DFMO alone resulted in a virtually complete disappearance of putrescine and spermidine, while the spermine content significantly increased and low amounts of *N*-aminopropylcadaverine (the cadaverine-based analog of spermidine) were detectable. Administration of MGBG alone decreased the level of spermine, whereas the concentration of spermidine, and most notably that of putrescine, were elevated, and now readily measurable amounts of cadaverine appeared (table 2). The changes of tumor polyamine pattern were even more striking when these two polyamine antimetabolites were administered together. Putrescine remained at a high level and cadaverine now represented one of the major amines in the leukemia cells (table 2). The concentration of sper-

Table 2

Effect of DFMO, MGBG and their combination on the polyamine content of L1210 cells in tumor-bearing mice

Treatment	Polyamines (nmol/mg protein ± SD)				
	Put	Cad	Spd	Apc	Spm
None	1.05 ± 0.16	n.d.	20.90 ± 0.50	n.d.	12.24 ± 0.62
DFMO	n.d.	n.d.	1.61 ± 0.44[b]	1.39 ± 0.41	18.34 ± 2.91[a]
MGBG	8.96 ± 3.33[a]	2.87 ± 0.86	25.92 ± 0.34[b]	n.d.	7.94 ± 1.00[b]
DFMO + MGBG	5.35 ± 1.50[a]	14.44 ± 1.76	16.83 ± 1.70[a]	n.d.	7.68 ± 0.75[b]

[a] $p < 0.01$
[b] $p < 0.001$

The DBA/2J mice (4 animals in a group) were inoculated with 0.25 ml of L1210 cell suspension and received DFMO (2% in drinking water), MGBG (50 mg/kg i.p. every 2 days) or the combination of the drugs until killed 5 days after the inoculation. Put, putrescine; Cad, cadaverine; Spd, spermidine; Apc, aminopropyl cadaverine; Spm, spermine; n.d., not detected

midine was more than 10 times higher in tumor cells collected from animals on the combined drug diet than that in cells obtained from animals receiving DFMO alone. It is tempting to speculate that these changes, i.e., high levels of putrescine, cadaverine and spermidine resulted, at least partly, from an enhanced polyamine uptake from extracellular ascitic fluid (which always contains micromolar concentrations of polyamines) rather than from endogenous synthesis that should have been blocked at two different sites.

4. DISCUSSION

The present findings may offer explanations for a variety of phenomena described in connection with the use of polyamine antimetabolites. The enhanced accumulation of putrescine in response to MGBG treatment is usually ascribed to a forward block of putrescine utilization (resulting from an inhibition of spermidine synthesis) together with a decrease in putrescine oxidation by diamine oxidase, since the latter enzyme is strongly inhibited by MGBG [14]. However, the possibility remains that the increased uptake of this diamine (in the presence of MGBG) has a major contribution to the enhanced accumulation. This view is supported by the fact that even in the presence of DFMO, an inhibitor of ornithine decarboxylase, putrescine continued to accumulate at a distinctly enhanced rate (table 2). The accumulation of cadaverine, which in animal tissues is most probably synthesized through the action of ornithine decarboxylase on lysine [15,16], likewise appears to result from an increased uptake from the extracellular compartment in the MGBG-treated animals.

Thus both DFMO and MGBG enhanced the uptake of polyamines, most notably of diamines and the combined use of these compounds results in an additive effect (table 2). Prakash et al. [17] recently reported that anti-tumor effects exerted by DFMO on a murine sarcoma were largely reversed when 1,1'-(methylethanediylidene)-dinitrilo bis(3-aminoguanidine) (MBAG, a close derivative of MGBG) was combined with DFMO. The latter compound was also reported to reverse DFMO-induced polyamine depletion in the tumor cells [17]. Bartholeyns and Kock-Weser [18] described a similar antagonism between DFMO and MGBG using the same murine sarcoma model. It is difficult to imagine other mechanisms than an enhanced uptake as being responsible for the normalization of intracellular polyamine pattern and the disappearance of the DFMO-induced tumor growth inhibition during the combined treatment. Not only can the natural polyamines (putrescine,

spermidine and spermine) abolish the growth-inhibitory effects of inhibitors of polyamine synthesis by enhanced uptake, but cadaverine and its aminopropyl derivatives have also to be taken into consideration. We recently found that replacement of putrescine and spermidine by cadaverine and aminopropylcadaverine is compatible with slow cell proliferation, at least in cultured Ehrlich ascites carcinoma cells [19]. Thus an accumulation of cadaverine from the extracellular space in response to DFMO and MGBG treatments is a possible reason for the resumption of cell growth, even though the intracellular levels of the natural polyamines would be greatly lowered by the use of polyamine antimetabolites.

A striking similarity between MGBG in animal cells and streptomycin in bacteria appears to exist. Höltje [20] recently reported that streptomycin induces a polyamine transport system in *E. coli* and utilizes the same system for its own transport. The author suggested, and also presented experimental evidence, that the binding of streptomycin to ribosomes induces the polyamine transport system. By considering the structural resemblance between spermidine and MGBG and the finding that under polyamine depletion MGBG is preferentially accumulated in the microsomal fraction of tumor cells [21], it may be justified to speculate that a similar mechanism for MGBG action is also operating in animal cells. The fact that MGBG is able to induce the polyamine transport system (that is also utilized by the drug itself) but, unlike spermidine, does not repress the stimulated uptake even at high intracellular concentration, would conceivably explain the 'suicide type' accumulation of the drug until cell death [22].

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