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Modulation of the Tissue Disposition of Methylglyoxal Bis(guanylhydrazone) in Mice by Polyamine Depletion and by Polyamine Administration[1]

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ABSTRACT

Treatment of mice with DL-α-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17), produced a significant spermidine depletion in liver, small intestine, and bone marrow among eight tissues studied. The accumulation of methylglyoxal bis(guanylhydrazone) (MGBG) was selectively enhanced in small intestine and in bone marrow cells in response to a prior DFMO treatment. In other tissues studied, *i.e.*, brain, skeletal and cardiac muscle, liver, kidney, and spleen, a preceding treatment with DFMO had no effect on the accumulation of subsequently injected MGBG.

When mice, primed with DFMO and then treated with a single injection of MGBG, were given nontoxic doses of spermidine or putrescine through a gastric tube, high concentrations of MGBG in the small intestine and in bone marrow cells were effectively reduced. In spite of the route of administration, bone marrow cells appeared to be more sensitive than intestinal tissue as regards the prevention of the tissue accumulation of MGBG by the polyamines. The different sensitivity of various tissues to the natural polyamines in this respect may offer a means to develop a tissue-specific "polyamine rescue concept" to be used in connection with MGBG treatment.

INTRODUCTION

The findings that inhibitors of polyamine biosynthesis exert distinct antiproliferative effects in a variety of animal cells (8) have initiated a series of clinically oriented experimental studies (15) and even some pilot clinical trials (7, 9, 10, 24, 25) to test the possibility of treating human proliferative disorders with polyamine antimetabolites.

The oldest inhibitor of the polyamine biosynthesis is MGBG,[3] a compound known to be effective against experimental tumors and human neoplasms since the late fifties (18). Although strikingly effective in acute leukemia (6, 14), MGBG has not gained any widespread clinical use mainly because of its unacceptably narrow therapeutic index (18).

However, recent developments, including weekly or biweekly administration schedules (12, 27), introduction of assay methods for the drug (13, 19, 23, 24), and clinically established pharmacokinetic interaction between DFMO and MGBG (25), have apparently awakened new interest in this compound.

During the past few years, we have investigated the combined use of DFMO and MGBG in various experimental systems. Our initial finding indicated that in cultured tumor cells, brought to a stage of profound putrescine and spermidine depletion with the aid of DFMO, the uptake of both natural polyamines and MGBG was strikingly enhanced (1). Since then, we have extended our studies on the combined use of DFMO and MGBG to include various cultured tumor cells (21), tumor-bearing animals (22), mouse skin (11), and finally a pilot clinical trial in human leukemia (24). In every instance, a previous treatment with DFMO strikingly enhanced the uptake of MGBG added subsequently, resulting in a more profound antiproliferative (21, 22) and therapeutic (25) response.

In this paper, we have studied more systematically the effect of DFMO treatment on the cellular levels of polyamines and on the uptake of MGBG in various mouse tissues. With the possible exception of liver, there was a close correlation between the extent of DFMO-induced putrescine and spermidine depletion and the enhancement of MGBG uptake in the tissues studied. In small intestine and bone marrow cells, which responded to the DFMO treatment by enhancing the accumulation of MGBG, cellular concentrations of the latter drug could be effectively reduced by administration of spermidine or putrescine.

MATERIALS AND METHODS

Albino mice were used in all experiments. After decapitation, the tissues were removed and homogenized with either a Potter-Elvehjem or Ultra-Turrax homogenizer in 0.9% NaCl solution. For the collection of bone marrow cells, femurs and tibias were cut off, and the cells were washed out with the aid of a syringe filled with Roswell Park Memorial Institute Tissue Culture Medium 1640. The cells were pelleted by centrifugation and subjected to hypotonic treatment for 5 min at 37° in 0.155 M NH_4Cl:0.010 M $KHCO_3$:0.1 mM EDTA, pH 7.2.

Polyamines were determined from perchloric acid extracts by the method of Seiler (20) as modified by Dreyfuss *et al.* (5), using chloroform:dioxan:*n*-butyl alcohol (96:2:2, v/v) as solvent in the thin-layer chromatography. MGBG was determined by the method of Seppänen *et al.* (23).

DFMO was a generous gift from the Centre de Recherche Merrell International, Strasbourg, France. MGBG was obtained from and synthesized by Orion Pharmaceutical Company, Helsinki, Finland.

For statistical analyses, the 2-tailed Student *t* test was used.

RESULTS

Table 1 shows the changes of polyamine pattern in 8 different tissues in mice subjected to 3 days of treatment with DFMO (2% in drinking water). Putrescine content was lowered significantly in brain (indicating that the drug penetrates the blood-brain barrier?), kidney, spleen, small intestine, and bone marrow (Table 1). Spermidine concentration was decreased only in liver, small intestine, and bone marrow, whereas spermine

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[3] The abbreviations used are: MGBG, methylglyoxal bis(guanylhydrazone); DFMO, 2-difluoromethylornithine.

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content was significantly increased in the 2 last-mentioned tissues (Table 1).

Concentrations of MGBG after 24 hr of a single i.p. injection (according to previous experience, the tissue contents of the drug are stabilized by 24 hr) in the same tissues without or with a previous DFMO treatment (same as in Table 1 which presents the polyamine pattern at the time of MGBG administration) are depicted in Chart 1. The brain contained a low, hardly detectable MGBG concentration, whereas high drug concentrations were found in the small intestine and bone marrow cells (Chart 1). A prior treatment with DFMO enhanced MGBG accumulation significantly only in intestinal tissue and bone marrow cells. Even though an increase was evident also in spleen, this change did not reach the level of statistical significance (Chart 1).

The experiments presented in Table 2 were set up to elucidate whether small intestine and bone marrow, most actively concentrating MGBG, responded to concomitantly administered exogenous polyamines by decreasing the tissue levels of MGBG. The mice, first primed with DFMO, received a single i.p. injection of MGBG. Spermidine (Table 2A) or putrescine (Table 2B) was administered either 4 hr before MGBG or simultaneously with the latter drug through gastric tubing. As shown in Table 2, spermidine produced a distinct increase in spermidine level in small intestine, whereas tissue polyamine levels remained largely unaffected in bone marrow. It is also evident from Table 2 that spermidine administration, given at either time point, produced a substantial fall of bone marrow MGBG concentration, whereas only spermidine administered concomitantly with MGBG resulted in a decrease (that was less striking than that seen in bone marrow) in intestinal MGBG content.

A similar treatment with putrescine, causing only marginal changes in tissue polyamines, also powerfully inhibited the accumulation of MGBG in bone marrow but produced less pronounced changes in intestinal drug levels.

Table 1

Polyamine concentrations in mouse tissues in response in DFMO treatment

The mice (5 animals/group) received 2% DFMO in drinking water for 3 days, after which tissue polyamine concentrations were determined.

Tissue	Treatment	Polyamines (nmol/mg protein)		
		Putrescine	Spermidine	Spermine
Brain	None	0.22 ± 0.03[a]	5.15 ± 0.30	2.89 ± 0.16
	DFMO	0.14 ± 0.02[b]	4.58 ± 0.79	2.87 ± 0.11
Skeletal muscle	None	0.07 ± 0.01	0.42 ± 0.04	0.92 ± 0.06
	DFMO	0.07 ± 0.02	0.42 ± 0.04	0.81 ± 0.12
Cardiac muscle	None	0.19 ± 0.05	0.67 ± 0.10	1.29 ± 0.19
	DFMO	0.17 ± 0.05	0.78 ± 0.15	1.41 ± 0.21
Liver	None	0.08 ± 0.02	2.57 ± 0.17	1.82 ± 0.14
	DFMO	0.07 ± 0.02	1.73 ± 0.24[c]	1.80 ± 0.22
Kidney	None	0.23 ± 0.05	1.65 ± 0.17	3.36 ± 0.25
	DFMO	0.17 ± 0.01[d]	1.53 ± 0.50	3.35 ± 0.31
Spleen	None	0.48 ± 0.08	4.82 ± 0.24	2.83 ± 0.42
	DFMO	0.28 ± 0.03[c]	4.65 ± 0.71	2.83 ± 0.25
Small intestine	None	2.66 ± 0.72	17.76 ± 1.54	7.09 ± 0.43
	DFMO	0.63 ± 0.15[c]	8.20 ± 0.97[c]	9.01 ± 0.86[b]
Bone marrow	None	0.91 ± 0.18	6.57 ± 0.28	3.71 ± 0.37
	DFMO	0.61 ± 0.08[d]	4.97 ± 0.38[c]	4.34 ± 0.29[d]

[a] Mean ± S.D.
[b] $p < 0.01$.
[c] $p < 0.001$.
[d] $p < 0.05$.

DISCUSSION

The present results are in accordance with earlier data obtained largely with cultured tumor cells, indicating that rapidly dividing cells are most sensitive to DFMO as regards the development of putrescine and spermidine depletion (10, 21). These cells also take up MGBG more effectively than do resting cells (10, 24). Intestinal tissue and bone marrow cells obviously exhibit highest proliferative activity among the mouse tissues tested here; these tissues also developed distinct polyamine depletion in response to DFMO and most effectively accumu-

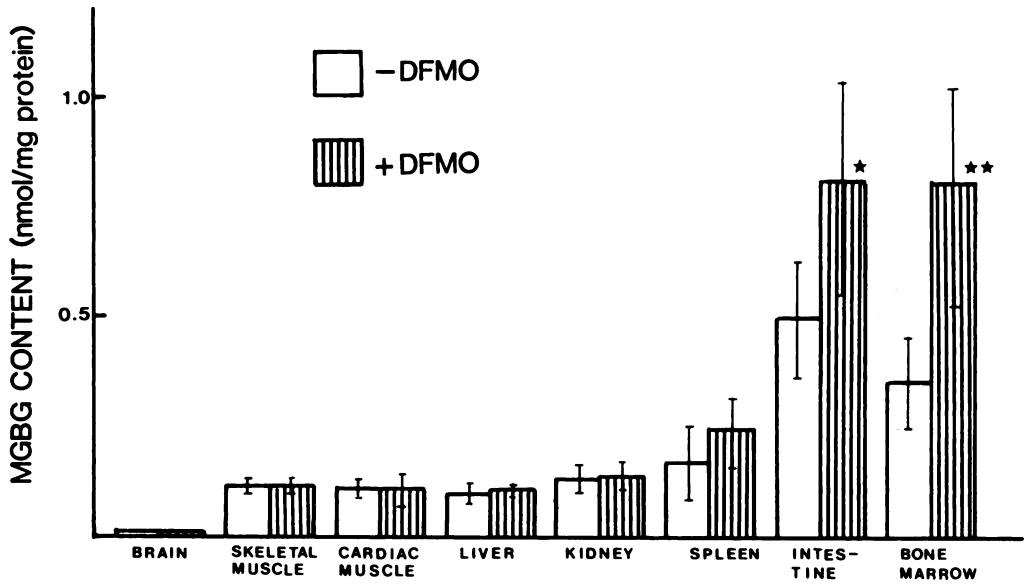

Chart 1. Effect of DFMO treatment on MGBG accumulation in mouse tissues. The mice received 2% DFMO in drinking water for 3 days, after which 75 mg of MGBG per kg were injected. The drug concentrations were determined 24 hr after the injection. *, $p < 0.05$; **, $p < 0.01$. *Bars*, S.D.

Table 2

Effect of spermidine or putrescine administration on polyamine and MGBG concentrations in small intestine and bone marrow cells from mice treated with DFMO

The mice (5 animals/group) received 2% DFMO in drinking water for 2 days, after which MGBG (75 mg/kg) was injected at the time point, 0. Spermidine or putrescine (750 mg/kg) was administered with the aid of gastric tubing 4 hr before or simultaneously with MGBG. Polyamines and MGBG were determined at 24 hr after MGBG administration. Concentrations are given in nmol/mg protein, except that of MGBG in bone marrow cells which is expressed as pmol/10^6 cells.

Tissue	Time of administration (hr)	Polyamine concentration			
		Putrescine	Spermidine	Spermine	MGBG
A. Spermidine					
Small intestine		4.35 ± 0.50[a]	5.94 ± 0.84	4.72 ± 0.36	1.15 ± 0.66
	−4	3.63 ± 0.71	15.77 ± 4.10[b]	5.93 ± 1.40	0.99 ± 0.27
	0	1.73 ± 0.50[b]	13.58 ± 3.77[c]	4.72 ± 0.60	0.63 ± 0.13[b]
Bone marrow		4.37 ± 0.32	8.95 ± 1.16	7.22 ± 1.45	85.7 ± 19.6
	−4	2.97 ± 0.52[c]	10.98 ± 1.81	5.99 ± 0.89	28.6 ± 3.4[b]
	0	2.02 ± 0.34[b]	12.08 ± 2.17	6.39 ± 1.26	21.7 ± 7.9[b]
B. Putrescine					
Small intestine		5.42 ± 2.13	11.81 ± 4.90	7.35 ± 1.83	2.95 ± 1.67
	−4	7.92 ± 2.37	9.28 ± 2.21	5.11 ± 1.00[d]	2.04 ± 0.95
	0	9.02 ± 1.44[d]	8.17 ± 2.30	4.77 ± 0.61[d]	1.07 ± 0.16[d]
Bone marrow		4.34 ± 0.97	6.21 ± 1.00	7.29 ± 0.99	91.8 ± 36.4
	−4	6.57 ± 0.62[c]	7.24 ± 1.18	6.15 ± 0.38[d]	30.7 ± 10.0[c]
	0	7.06 ± 2.52	7.41 ± 1.14	6.32 ± 0.63	23.2 ± 4.3[c]

[a] Mean ± S.D.
[b] $p < 0.001$.
[c] $p < 0.01$.
[d] $p < 0.05$.

lated MGBG without or with a prior DFMO treatment (Table 1; Chart 1). In fact, Danzin *et al.* (4) found substantial differences with respect to DFMO-induced inhibition of polyamine accumulation in some rat tissues. Among the tissues tested by the latter authors, ventral prostate, thymus, and testis were most sensitive to DFMO, whereas no or only marginal changes in polyamine concentrations were seen in spleen and liver (4). We have found similarly that in tumor-bearing mice, a prior treatment with DFMO rather selectively enhanced the accumulation of MGBG injected subsequently in the carcinoma cells with no changes in hepatic and insignificant increase in intestinal drug concentrations (22).

One of the purposes of the present experiments was also to elucidate whether some clinically "unique" side effects of MGBG, most notably commonly reported myalgia and muscle weakness (27), could be based upon a preferential tissue-specific accumulation of the drug. As shown here (Chart 1), muscle tissue (skeletal or cardiac) contained distinctly less MGBG than did actively proliferating tissues (intestine and bone marrow), and there was no enhancement of MGBG accumulation whatsoever in response to a prior treatment with DFMO (Chart 1). The muscle toxicity of MGBG may in fact be related to the antimitochondrial action exerted by the compounds (see Ref. 18), Nor was MGBG accumulation in parenchymal organs (kidney and liver) affected by the DFMO priming. Although not yet clinically apparent (small number of patients) (25), it is possible that a combined treatment with these 2 polyamine antimetabolites may enhance side effects of the antiproliferative type but not necessarily other types of unwanted effects. In a recent paper, Stewart *et al.* (26) found substantial accumulation of MGBG in liver in 2 patients receiving MGBG before death. The drug was also effectively concentrated by tissues infiltrated by leukemic cells (26).

We also tried to develop a "polyamine rescue concept," *i.e.*, with the aid of nontoxic putrescine or spermidine doses, to reduce tissue MGBG concentrations. As shown (Table 2), this concept worked in small intestine and bone marrow cells, yet slightly surprising was the finding that bone marrow cells seemed to be clearly more sensitive than was intestinal tissue to the inhibition of MGBG accumulation by exogenous polyamines. Thus, myelosuppression could possibly be reversed by exogenous polyamines; the clinical applicability of the concept remains to be tested. Our present results, involving actual tissue determinations of MGBG following DFMO priming, are in full agreement with the extensive toxicological studies of Mihich (16, 17), indicating that proliferative toxicities are prevented by spermidine. Whether also the antileukemic effect of MGBG obtained after DFMO priming is abolished upon spermidine administration remains to determined. The difference, at least under cell culture conditions, between the administration of MGBG as a single agent or after DFMO priming appears to be the fact that, in response to previous DFMO treatment, very high intracellular MGBG concentrations are reached, producing an irreversible effect and subsequent cell death (21).

In some respects, the present results are in slight disagreement with those obtained by Bennett *et al.* (2), who noticed that a reversal of MGBG-induced immunosuppression with spermidine was more difficult to achieve in bone marrow than in spleen.

It thus appears to us that rapidly proliferating cells are most sensitive to the growth-inhibitory action exerted by these polyamine antimetabolites both as single agents and especially in combination. In this respect, they closely resemble alkylating anticancer drugs possessing a carrier-mediated transport system and exerting a selective dose-dependent toxicity towards rapidly dividing cells (3). This is in contrast to carrier-independent, highly lipid-soluble compounds or X-rays, which exhibit equal toxicity to both proliferating and resting cells (3).

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