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Effects of α-Difluoromethylornithine and Methylglyoxal Bis(guanylhydrazone) on the Growth of Experimental Renal Adenocarcinoma in Mice[1]

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ABSTRACT

α-Difluoromethylornithine (DFMO) and methylglyoxal bis-(guanylhydrazone) (MGBG) were tested against a murine renal adenocarcinoma, because polyamines are necessary for neoplastic cell growth and because human renal adenocarcinomas contain higher levels of spermidine than do normal renal cells; MGBG inhibits spermidine synthesis and has some activity against human renal tumors; DFMO irreversibly inhibits ornithine decarboxylase, the first rate-limiting enzyme controlling polyamine biosynthesis; and DFMO promotes intracellular accumulation of MGBG in experimental tumor models and human leukemia. DFMO (2%) in drinking water, MGBG (15 mg/kg i.p.), or a combination of DFMO and MGBG was administered daily to BALB/c mice ($n = 80$) with intrarenal transplants of renal adenocarcinoma cells. At 28 days, renal carcinomas weighed 64 and 73% less, respectively, in DFMO- and DFMO-MGBG-teated mice than in control animals ($p < 0.01$). MGBG alone had no antigrowth effect. DFMO-MGBG reduced the total metastatic index (total number of metastases/total number of animals) to 1.2 *versus* 3.6 in control animals ($p < 0.01$) and increased survival by 12.3 ± 1.5 (S.E.) days, from 30.8 to 42.5 days ($p < 0.05$). Compared with control, DFMO-, or MGBG-treated animals, DFMO-MGBG exposure reduced tumor growth and the number of metastases, prevented metastases in some animals (47%), and increased survival of mice bearing renal adenocarcinomas. DFMO also appeared to selectively increase the uptake of [^{14}C]MGBG by tumor tissue, which may help to explain the enhanced synergistic antigrowth effect of DFMO and MGBG against this murine renal adenocarcinoma.

INTRODUCTION

Neoplastic cells contain high levels of polyamines (putrescine, spermidine, and spermine) necessary for their continued growth (6). Depletion of polyamines by inhibition of polyamine biosynthesis reduces growth of a number of experimental animal tumors (1, 8, 11), including a murine renal adenocarcinoma (7). Human renal adenocarcinomas contain high levels of spermidine (4), and MGBG,[3] a known inhibitor of spermidine synthesis (2), has been reported to cause regression of metastatic disease in some patients (14). DFMO, a nontoxic, enzyme-activated irreversible inhibitor of ODC, the first rate-limiting enzyme controlling polyamine biosynthesis (9), inhibits ODC activity, reduces putrescine levels, and retards tumor growth in mice bearing renal adenocarcinomas (7). Moreover, spermidine depletion by DFMO promotes selective uptake of MGBG within mouse and human leukemias (12, 13) and rat prostatic adenocarcinoma (5). These findings prompted us to investigate the effects of DFMO and MGBG, both as single agents and in combination, against experimental murine renal adenocarcinoma.

MATERIALS AND METHODS

Male BALB/c mice (6 weeks old; Charles River Breeding Laboratories, Wilmington, MA; initial weight, 18 to 20 g) were used as carriers of the renal adenocarcinoma. Mice were housed in groups of 4 to 6 in plastic cages, fed Purina chow, and allowed free access to tap water or a solution of 2% DFMO in water (MDL 71, 782A; Merrell Dow, Cincinnati, OH). Average daily intake was estimated from the total fluid intake per cage.

The adenocarcinoma originated spontaneously in the kidney of an inbred BALB/c mouse (10) and has been maintained by intrarenal passage approximately every 21 days in our laboratory since 1977. In the present study, the renal carcinoma was in its 189th transplant generation.

Donor BALB/c mice were ether anesthetized, and the tumors were excised. A single-cell suspension was prepared and was adjusted to a concentration of 1×10^6 live tumor cells/ml. Viability was determined by trypan blue exclusion. Mice ($n = 80$) received 0.1 ml (10^5 viable tumor cells) of the cell suspension injected through a 27-gauge needle under the renal capsule of the left kidney (Day 0). In prior extensive experiments, this method had resulted in 100% tumor engraftment palpable in 14 ± 3 (S.E.) days. Animals were randomly allocated to receive either plain drinking water (controls); 2% DFMO in drinking water starting on Day 0, MGBG (Lot 110F-0145; Sigma Chemical Co., St. Louis, MO) at a daily dose of 15 mg/kg i.p., beginning 14 days after intrarenal inoculation; or a combination of DFMO (on Day 0) and MGBG (on Day 14) and continued to the end of the experiment (tumors were present in each animal when MGBG was begun). As additional controls, DFMO alone (late DFMO) or DFMO-MGBG (late DFMO-late MGBG) was begun at 14 days after transplantation. At 28 days, the animals were killed, and the tumors were excised and weighed. The lungs, liver, spleen, right kidney, and peritoneum were dissected under a microscope, and metastases were counted. Since the transplantable murine renal adenocarcinoma often metastasizes only to the lungs as well as to multiple sites, both a lung metastatic index and a total metastatic index were calculated from the mean number of representative metastatic deposits in each group of mice. Student's *t* test for unpaired data was used for statistical analysis.

In the present protocol, MGBG was given as daily pulse doses after continuous DFMO had been administered for 2 weeks, since results in other experimental systems suggested that the synergistic effect of DFMO-MGBG was seen only after a period of "DFMO priming," which served to facilitate intracellular uptake of MGBG. To evaluate whether DFMO enhances the uptake of MGBG into murine renal adenocarcinoma cells, animals ($n = 10$) were given 0.2 μCi of [^{14}C]MGBG (specific activity, 19 mCi/mmol) by intracardiac injection after 4 days of therapy and sacrificed 60 min postinjection. Tissues were solubilized, and the distri-

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

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bution of [^{14}C]MGBG was determined by calculating the relative concentration of radioactivity in tumor, blood, and tissue samples (15), where

$$\text{Relative concentration} = \frac{\text{dpm/g tissue}}{\text{dpm injected/g animal mass}}.$$

Counting efficiencies were determined using the external standard method.

RESULTS

Effects of DFMO and MGBG on animal weights for 28 days are shown in Chart 1 and Table 1. At 10 and 20 days, the weights of DFMO-fed mice were decreased by 12.0 and 13.5%, respectively, than controls, whereas DFMO-MGBG-treated mice had respective decreases in weights of 16.5 and 18.5% at Days 10 and 20. Carcass weight (whole animal weight less excised tumor weight) of mice at 28 days, however, was the same in controls (15.6 g), DFMO-fed (16.5 g), MGBG (15.1 g), and DFMO-MGBG (14.7 g)-treated animals.

The mean daily fluid consumption of DFMO from 0 to 20 days was 2.7 ± 0.3 ml per animal (range, 2.5 to 2.9 ml) *versus* 3.0 ± 0.7 ml (range, 1.4 to 3.6 ml) in control animals fed plain tap water. The 2% solution of DFMO resulted in a daily intake of 2.5 g DFMO per kg body weight. From 20 to 28 days, mean DFMO-fluid intake was 2.1 ± 0.1 ml, yielding a daily intake of 2.3 g DFMO per kg body weight per animal (average weight, 16.2 g).

Table 1

DFMO intake and effect on carcass weight

Treatment	Daily fluid intake (ml)	Daily DFMO intake (g/kg)	Change in carcass wt (whole-animal wt less excised-tumor wt) after 28 days (g)
Control	2.9 ± 0.3[a]		−2.1
DFMO	2.9 ± 0.4	2.5 ± 0.3	−0.8
MGBG	1.8 ± 0.1		−1.6
DFMO-MGBG	2.1 ± 0.5	2.6 ± 0.2	−0.6

[a] Mean ± S.E.

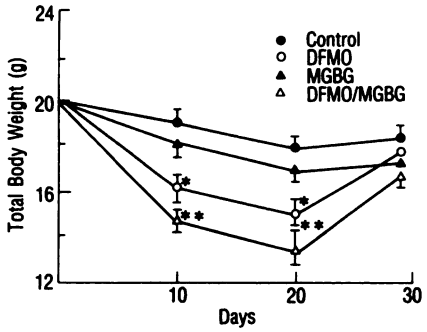

Chart 1. Host body weight after tumor transplantation. At Day 0, 10^5 renal adenocarcinoma cells were transplanted into recipient animals. *Points*, mean host body weight; *bars*, S.E. (n = 80 at Day 0). Significance *versus* control: *, $p < 0.01$; **, $p < 0.05$.

The effects of DFMO, MGBG, and DFMO-MGBG on specific tumor weights are shown in Table 2. Renal tumors weighed 64% less in DFMO-fed mice and 73% in DFMO-MGBG-treated mice than in controls at 28 days. Tumor growth was not significantly affected by MGBG alone.

Mortality of animals with tumors is also shown in Table 2. Similar numbers of animals in each group died before 28 days. These animals contributed to survival data, but they were unavailable for autopsy analysis. Mean host survival time was 4 ± 0.6 and 12.3 ± 1.5 days longer in DFMO- and DFMO-MGBG-treated mice, respectively, than in control mice (30.8 ± 0.7 days).

The mean number of lung and total metastases in control mice was 50 and 67% greater, respectively, than in DFMO-MGBG-treated mice. DFMO alone reduced the number of mice with multiple metastases (22%) *versus* controls (55%) but did not prevent metastases (35%) compared with untreated mice (20%). On the other hand, DFMO-MGBG not only reduced the total metastatic index to 1.2 (observed in 13% of the animals) compared with 3.6 in controls, but significantly ($p < 0.05$) prevented metastases in 9 (47%) animals. One animal fed DFMO and 2 treated with DFMO-MGBG had no histological evidence of either local or systemic disease at 28 days.

Correlation of primary tumor weight and frequency of metastases at 28 days is shown in Table 3. In both DFMO-fed and DFMO-MGBG-treated animals, local tumor weights were significantly ($p < 0.05$) less in mice without metastases than in those with metastases.

The tissue uptake of [^{14}C]MGBG at 60 min after therapy with

Table 3

Correlation of tumor weight and metastases

Treatment	Wt of animals	
	With metastases	Without metastases
Control	2.06[a] (0.60–5.00)[b]	2.14 (0.75–4.50)
DFMO	0.95 (0.25–1.85)	0.47 (0.00–0.80)
MGBG	1.60 (0.30–3.20)	1.40 (0.70–2.40)
DFMO-MGBG	0.93 (0.30–1.90)	0.53 (0.00–1.05)

[a] Mean weight (g) of local renal tumor at 28 days.
[b] Range of kidney tumor weights.

Table 2

Effect of DFMO and MGBG on renal tumor growth and host survival

Treatment	No. of animals dead before 28 days	Tumor wt (g) at 28 days	Inhibition of tumor growth (%)	Host survival time (days) (n = 20)	Lung metastatic index (total no. of lung metastases/ total no. of animals) (n = 46)	Metastatic index (total no. of metastatic deposits/total no. of animals) (n = 46)	No. of animals without metastases at 28 days
Control	5	2.08 ± 0.6[a]		30.8 ± 0.7	1.6	3.6	4 (20)[b]
DFMO	2	0.75 ± 0.1[c]	64	34.5 ± 1.1[d]	1.3	2.4	7 (35)
MGBG	4	1.40 ± 0.3	33	28.2 ± 0.4	1.9	3.8	3 (15)
DFMO-MGBG	3	0.57 ± 0.1[c]	73	42.5 ± 2.3[c]	0.8[c]	1.2[c]	9 (47)[d]
Late DFMO	3	0.95 ± 0.03[c]	54	33.8 ± 0.6	2.3	3.4	7 (35)
Late DFMO-late MGBG	2	0.70 ± 0.02[c]	67	40.7 ± 1.9[c]	0.9[c]	1.5[c]	8 (40)

[a] Mean ± S.E.
[b] Numbers in parentheses, percentage of animals without metastases at 28 days.
[c] Significance *versus* control, $p < 0.01$.
[d] Significance *versus* control, $p < 0.05$.

Table 4
Tissue uptake of [^{14}C]MGBG in mice bearing renal tumors after treatment with DFMO and MGBG

Tissue	Mean relative concentration [(dpm/g tissue)/(dpm injected/g animal mass)]			
	Control	DFMO	MGBG	DFMO-MGBG
Tumor	0.42 ± 0.11[a]	1.90 ± 0.13	0.53 ± 0.10	0.71 ± 0.19
Blood	0.03	0.03	0.03	0.03
Muscle	0.03	0.37	0.30	0.24
Intestine	2.68	2.85	2.29	2.53
Pancreas	0.25	0.29	0.26	0.26
Spleen	0.83	1.01	0.59	0.78
Liver	2.17	1.68	1.60	1.56
Kidney	1.84	2.11	1.99	1.39
Lung	1.08	1.05	1.15	1.04
Heart	1.25	1.36	1.31	1.32
Brain	0.02	0.03	0.03	0.02

[a] Mean ± S.E. from 3 separate experiments.

DFMO and MGBG is shown in Table 4. In 3 separate experiments, the relative concentration of [^{14}C]MGBG within tumor tissue was enhanced 4.5 times after 4 days of DFMO therapy, compared with the control animals and the MGBG- or DFMO-MGBG-treated mice. The reduced uptake of [^{14}C]MGBG in tumors exposed to both DFMO and MGBG is presumably due to competition between radiolabeled MGBG given once and unlabeled MGBG given as daily treatment for 4 days.

DISCUSSION

DFMO reduced the rate of primary tumor growth and metastatic spread of murine renal adenocarcinoma and increased host survival time, but such effects were not as significant as those reported in other studies using DFMO against the same tumor (3, 7). For example, although total metastases were marginally reduced by DFMO, the number of lung metastases was not in contrast with the prior report of 140% greater incidence of lung metastases in control mice *versus* mice receiving 2% DFMO (7).

Daily DFMO intake was 2.5 g/kg/animal and somewhat less (2.3 g/kg) during the terminal days of the experiment. This is considerably less than the average daily intake of 4.6 g of DFMO per kg associated with the reduction in the lung metastatic index noted above (7) and may have contributed to the reduced effect seen with DFMO alone in the present study.

Drug-related toxicity, which may have caused deaths before 28 days, was less with DFMO (10%) compared with controls (25%), MGBG (20%), or DFMO-MGBG (15%) (Table 2). Overall weight loss was also less in DFMO-fed mice (13.5%) than in those receiving MGBG (16.5%) or DFMO-MGBG (15.5%). Because macroscopic murine renal carcinoma appears only between Days 15 and 20 (10), weight change after Day 20 represents a combination of weight gain from tumor growth and weight loss induced by DFMO or tumor cachexia. Total body weight of both control mice (17.3 g) and DFMO-fed mice (16.4 g) was unchanged after Day 20, although tumors were 2.8 times heavier in controls. The large renal carcinomas appeared to produce cachexia while the tumors grew. Tumor-induced cachexia was not seen in DFMO-fed mice.

Animals (n = 20) started on DFMO on Day 0 weighed, on the average, 15.5 g at Day 20, and at 28 days, they weighed 17.3 g compared with 15.2 and 15.4 g on Days 20 and 28, respectively, in animals (n = 20) begun on DFMO on Day 14 and continued to Day 28 (Table 2). Average tumor size at 28 days was 0.75 ± 0.08 g in the early DFMO-treated mice *versus* 0.85 ± 0.03 g in the late-DFMO group. Thus, reduced DFMO intake in our study does not seem related to tumor cachexia or DFMO-induced toxicity.

The most significant observation was that a combination of DFMO, administered at the time of renal tumor transplant or after established growth of tumor (Day 14), and MGBG, begun 14 days later, reduced local tumor growth and the number of lung and total metastases, prevented metastases in some animals, and increased host survival to a greater extent than was achieved with either DFMO or MGBG alone. Complete suppression of both local and systemic disease occurred in 10% of the DFMO-MGBG-treated animals, and the data presented in Table 3 further suggest that control of primary tumor growth reduced the frequency of subsequent metastases. The antitumor effects of MGBG alone are disappointing, but they are consistent with recent results showing no significant objective responses or improved survival with MGBG against advanced human renal adenocarcinomas.[4]

Since DFMO has been reported to inhibit ODC activity by 75% and putrescine levels by 71% in renal carcinoma (7), polyamine depletion may facilitate uptake of MGBG into renal tumor cells and synergistically improve the antitumor effect. The radionuclide studies indeed suggest that DFMO enhanced the selective uptake of MGBG into renal tumor cells, since a similar major increase in the intracellular concentration of MGBG was not seen in the other tissues among the different treatment groups. Thus, the synergism of this combination may be explained, at least in part, by the growth-inhibiting effect of DFMO and its permissive effect in facilitating intracellular accumulation of the cytotoxic drug MGBG to an effective concentration not achieved when this drug is given alone. These data may provide a basis for clinical application of a combination of DFMO and MGBG in human renal adenocarcinoma.

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