Reduction of Difluoromethylornithine-induced Thrombocytopenia in Rats with Ornithine while Maintaining Antitumor Activity

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INTRODUCTION

Polyamines are present in all mammalian cells and are required for cell growth and proliferation. Although their precise biological role is unclear, rapidly growing cells have higher levels of polyamines than slowly growing cells (1, 2). ODC catalyzes the initial rate-limiting reaction in polyamine biosynthesis, the decarboxylation of ornithine to form putrescine. DFMO is an irreversible suicide inhibitor of ODC. We have reported that a continuous 12-day infusion of DFMO to male Fischer 344 rats results in a reduction in the putrescine and spermidine levels of a transplantable fibrosarcoma and a dose- and time-dependent decrease in tumor growth (3). A reduction of polyamine levels has been reported to occur in blood and bone marrow mononuclear cells in patients with leukemia or multiple myeloma treated with i.v. DFMO (4–6). The toxicity of DFMO is thrombocytopenia, gastrointestinal, or hearing loss, depending on the route of administration (7) and type of neoplasm (5, 6). The effect of i.v. DFMO on platelet levels was not evaluated by Maddox et al. (4–6) due to the disease (5). These studies, however, did not evaluate the relative effect of ornithine or putrescine on the efficacy of DFMO in the tumor-bearing host. The objective of this study was to eliminate DFMO-induced thrombocytopenia while maintaining the inhibition of tumor growth. The results reported herein establish that, at specific molar ratios to DFMO, ornithine inhibits DFMO-induced platelet suppression while having no significant effect on the antiproliferative activity toward the tumor.

ABSTRACT

The purpose of this study was to evaluate the effect of a concomitant infusion of ornithine on the difluoromethylornithine (DFMO)-induced thrombocytopenia and antitumor activity. Male Fischer 344 rats with either a transplantable fibrosarcoma or Ward colon tumor were given a 12-day continuous infusion of DFMO (2000 mg/kg/day) alone or with ornithine. The dose of ornithine was defined as the molar ratio to DFMO. A continuous infusion of DFMO significantly reduced circulating platelet counts to 5–16% of the control. Concomitant ornithine treatment at a molar ratio of 0.2–0.5 resulted in protection of the rat from thrombocytopenia while the antiproliferative activity of DFMO against the fibrosarcoma or Ward colon tumor was unaffected. At a higher ornithine:DFMO molar ratio (0.7), the DFMO-induced inhibition of tumor growth was blocked. Tissue polyamine levels suggest a different sensitivity of tumor and normal tissue to DFMO. Concomitant ornithine resulted in a greater increase in the polyamine levels of normal tissues, compared with the tumor. These results suggest that ornithine can selectively inhibit DFMO-induced thrombocytopenia while not affecting the antitumor activity.

RESULTS

The effect of concomitant ornithine infusions on the antitumor activity and on DFMO-induced thrombocytopenia in fibrosarcoma-bearing rats is shown in Figs. 1–3. An ornithine:DFMO molar ratio of 0.35, but not 0.035, protected the host from thrombocytopenia (Fig. 1) while neither ratio ad-

MATERIALS AND METHODS

Male Fischer 344 rats obtained from Harlan-Sprague-Dawley (Indianapolis, IN) were used in all experiments. Rats were allowed a 7-day acclimation period with food and water ad libitum prior to tumor inoculation. The growth characteristics of the transplantable tumors used, a fibrosarcoma and Ward colon tumor, have previously been reported (13).

Groups of rats were inoculated s.c. in the right flank with a transplantable fibrosarcoma or a Ward colon tumor. After the tumors were at least 1.0 cm in width, 2-dimensional measurements (cm) were taken and the weight (g) calculated as length × width² × 1/2. When the tumor weights were at least 2 g, rats were anesthetized with Nembutal (50 mg/kg, i.p.) and a sterile central venous catheter was inserted (14). The rats were divided into groups by tumor weight and were allowed to recover overnight. Treatment was started the next morning. DFMO was given at 2000 mg/kg/day, because this dose has previously been reported to result in growth inhibition of the fibrosarcoma (3) and severe thrombocytopenia (8). DFMO and ornithine were formulated in 0.45% saline and administered by a continuous infusion in a single solution. The dose of ornithine given was calculated as mol of ornithine/mol of DFMO infused. This is referred to as the molar ratio. All rats were observed daily for signs of diarrhea.

At the end of the 12 days of treatment, rats were anesthetized with ketamine (i.m.) and the tumors were measured. Rats were exanguinated through the abdominal aorta, into a Vacutainer tube with heparin, and blood platelets were counted. The liver, spleen, kidneys, and tumor were removed and frozen at −70°C for determination of polyamine levels (15). No polyamine analysis was done for colon tumor tissue because of extensive center necrosis.

All data are reported as the mean ± SD. Means of the different treatment groups were initially compared with a one-way ANOVA analysis. Differences between individual means were then determined using the Student t test (two-tailed, P < 0.05).

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: ODC, ornithine decarboxylase; DFMO, α-difluoro-methylornithine.
experiments confirmed this protection from thrombocytopenia inversely affected the tumor response to DFMO. Subsequent experiments confirmed this protection from thrombocytopenia at a molar ratio of 0.35 (Fig. 2) and demonstrated that the same protection occurred when 0.2 and 0.5 molar ratios were infused (Fig. 3). At molar ratios of 0.5 or less, the final tumor weights were equal to those in rats treated with DFMO alone. The platelet counts at a molar ratio of 0.71 (580,000/mm³) were also equal to those in controls (676,000/mm³). At the molar ratio of 0.71 (Fig. 2), however, the final tumor weight (24.7 ± 5.8 g) was near the control weight (28.2 ± 2.8 g) and was significantly greater than that of the tumors of DFMO-treated rats (17.7 ± 1.5 g). At a molar ratio of 0.035 (Fig. 1), the final tumor weight and the platelet counts were similar to those for rats treated with DFMO alone. Hematocrit was not consistently affected by any of the treatments to fibrosarcoma-bearing rats (data not shown).

The effect of concomitant ornithine infusion on the toxicity and antitumor activity of DFMO in Ward colon tumor-bearing rats (Fig. 4) was similar to that seen for fibrosarcoma-bearing rats. A 0.035 molar ratio had no effect on DFMO-induced thrombocytopenia or antitumor activity. The 0.35 molar ratio, however, resulted in protection from DFMO-induced thrombocytopenia while not significantly affecting the antiproliferative effect toward the colon tumor. A trend toward an increase in final tumor weight was noted at the 0.70 ratio.

The polyamine levels of the fibrosarcoma were determined (Table 1); the tumor growth is depicted in Fig. 2. The putrescine and spermidine levels of the DFMO alone group (as percentage of controls) were reduced to 1.1 and 11%, respectively, while the tumor spermine levels were 165% of the respective control polyamine. The addition of ornithine resulted in a dose-dependent increase in the tumor putrescine and spermidine levels and a decline in tumor spermine levels.

The putrescine and spermidine levels of the liver, spleen, and kidney were decreased by DFMO (Table 2). The putrescine levels of kidney were decreased to 5% of the control. The putrescine levels of the liver and spleen were decreased to 30 and 40% of the controls, respectively. The spermidine levels of kidney were decreased to a greater degree (35% of controls) than those of either spleen (49% of control) or liver (59% of controls). The spermine levels were significantly altered only in the liver (127% of control). Concomitant ornithine resulted in an increase in the putrescine and spermidine levels of the spleen and kidney.

**DISCUSSION**

The dose level of cancer chemotherapy drugs is frequently limited by the toxicity to normal tissues; selective toxicity of a drug against the tumor has not yet been achieved. One strategy for increasing the activity of a drug toward the tumor is to increase the dose while rescuing the host tissues by the administration of another drug such as leukovorin (16, 17). Another
strategy is to selectively block or ameliorate the toxic effect to the normal tissues (18–20). In this study, concomitant ornithine treatment was used to protect against DFMO-induced thrombocytopenia while having no compromising effect on its anti-tumor activity. Ornithine is the natural substrate for ODC, the enzyme may exist between DFMO and ornithine. Which suggests that some competition for the binding sites on the enzyme is possible, and hence a high affinity of the drug for the ODC of normal tissue than for the ODC from epidermal papillomas. These data (21) would suggest that ornithine might better compete for the binding sites of the ODC of normal tissue than for the ODC from normal mouse epidermis was significantly greater than that for the ODC from epidermal papillomas. These data (21) would suggest that ornithine might better compete for the binding sites of the ODC of normal tissue than the tumor.

A differing sensitivity of polyamine biosynthesis in normal tissues and tumor to a continuous infusion of DFMO is suggested by the results reported herein. The putrescine levels of liver and spleen and the spermidine levels of liver, spleen, and kidney were suppressed to a lesser degree than were the respective polyamine levels of tumor tissue. Also, the spermine levels of bone marrow were not evaluated, a recent report demonstrates that spleen hematopoietic stem cells recover faster than does a similar population in bone marrow; the spleen demonstrates that spleen hematopoietic stem cells recover significantly faster than does a similar population in bone marrow; the spleen may be a good indicator for hematopoietic activity (22). Our data show that, when ornithine is administered with DFMO, the recovery of liver, spleen, and kidney polyamine levels (as percentage of the control levels) is greater than that for the tumor. An explanation for the differential response of the tumor growth and platelet synthesis can only be determined when the results of the studies reported herein demonstrate that concomitant administration of ornithine with DFMO selectively blocked the drug-induced thrombocytopenia when given at an ornithine:DFMO molar ratio of greater than 0.2. At a molar ratio of 0.2 to 0.5, the cytostatic effect of DFMO toward the tumor was not affected. These results demonstrate, therefore, that a selective process for blocking DFMO-induced thrombocytopenia exists. A possible explanation for these observations would be a difference in the polyamine biosynthesis of the normal tissue, as compared with that of the tumor. O'Brien et al. (21) demonstrated that the affinity of ornithine for the ODC from normal mouse epidermis was significantly greater than that for the ODC from epidermal papillomas. These data (21) would suggest that ornithine might better compete for the binding sites of the ODC of normal tissue than the tumor.

Table 1 Effect of concomitant ornithine and DFMO infusion on the polyamine levels of a transplantable fibrosarcoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.45% NaCl</td>
<td>70.0 ± 21.7</td>
<td>100</td>
<td>687 ± 180</td>
</tr>
<tr>
<td>DFMO</td>
<td>0.3 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>DFMO + 0.35 ornithine</td>
<td>1.7 ± 1.3</td>
<td>2.2 ± 1.6</td>
<td>93 ± 20</td>
</tr>
<tr>
<td>DFMO + 0.71 ornithine</td>
<td>1.9 ± 0.8</td>
<td>2.7 ± 0.3</td>
<td>135 ± 28</td>
</tr>
</tbody>
</table>

Table 2 Effect of concomitant ornithine and DFMO infusion on the polyamine levels of liver, spleen, and kidney

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Putrescine</th>
<th>Spermidine</th>
<th>Spermine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>8.0 ± 0.7</td>
<td>100</td>
<td>820 ± 109</td>
</tr>
<tr>
<td>DFMO</td>
<td>2.4 ± 0.2</td>
<td>30 ± 3</td>
<td>485 ± 114</td>
</tr>
<tr>
<td>DFMO + 0.35 ornithine</td>
<td>3.1 ± 0.2</td>
<td>39 ± 9</td>
<td>475 ± 41</td>
</tr>
<tr>
<td>DFMO + 0.71 ornithine</td>
<td>3.6 ± 0.5</td>
<td>45 ± 6</td>
<td>490 ± 59</td>
</tr>
<tr>
<td>Spleen</td>
<td>19.5 ± 1.6</td>
<td>100</td>
<td>758 ± 237</td>
</tr>
<tr>
<td>DFMO</td>
<td>7.7 ± 1.6</td>
<td>39 ± 8</td>
<td>373 ± 49</td>
</tr>
<tr>
<td>DFMO + 0.35 ornithine</td>
<td>10.0 ± 1.1</td>
<td>51 ± 6</td>
<td>441 ± 42</td>
</tr>
<tr>
<td>DFMO + 0.71 ornithine</td>
<td>11.1 ± 0.7</td>
<td>57 ± 4</td>
<td>507 ± 29</td>
</tr>
<tr>
<td>Kidney</td>
<td>20.2 ± 2.2</td>
<td>100</td>
<td>458 ± 27</td>
</tr>
<tr>
<td>DFMO</td>
<td>1.2 ± 0.1</td>
<td>5.9 ± 0.5</td>
<td>161 ± 10</td>
</tr>
<tr>
<td>DFMO + 0.35 ornithine</td>
<td>1.1 ± 0.2</td>
<td>5.5 ± 0.8</td>
<td>165 ± 13</td>
</tr>
<tr>
<td>DFMO + 0.71 ornithine</td>
<td>1.7 ± 0.5</td>
<td>8.5 ± 2.6</td>
<td>187 ± 7</td>
</tr>
</tbody>
</table>

See Table 1 for details on DFMO and ornithine doses and infusions. A differing sensitivity of polyamine biosynthesis in normal tissues and tumor to a continuous infusion of DFMO is suggested by the results reported herein. The putrescine levels of liver and spleen and the spermidine levels of liver, spleen, and kidney were suppressed to a lesser degree than were the respective polyamine levels of tumor tissue. Also, the spermine levels of bone marrow were not evaluated, a recent report demonstrates that spleen hematopoietic stem cells recover faster than does a similar population in bone marrow; the spleen demonstrates that spleen hematopoietic stem cells recover significantly faster than does a similar population in bone marrow; the spleen may be a good indicator for hematopoietic activity (22). Our data show that, when ornithine is administered with DFMO, the recovery of liver, spleen, and kidney polyamine levels (as percentage of the control levels) is greater than that for the tumor. An explanation for the differential response of the tumor growth and platelet synthesis can only be determined when the results of the studies reported herein demonstrate that concomitant administration of ornithine with DFMO selectively blocked the drug-induced thrombocytopenia when given at an ornithine:DFMO molar ratio of greater than 0.2. At a molar ratio of 0.2 to 0.5, the cytostatic effect of DFMO toward the tumor was not affected. These results demonstrate, therefore, that a selective process for blocking DFMO-induced thrombocytopenia exists. A possible explanation for these observations would be a difference in the polyamine biosynthesis of the normal tissue, as compared with that of the tumor. O'Brien et al. (21) demonstrated that the affinity of ornithine for the ODC from normal mouse epidermis was significantly greater than that for the ODC from epidermal papillomas. These data (21) would suggest that ornithine might better compete for the binding sites of the ODC of normal tissue than the tumor.
levels of putrescine and spermidine and the decreasing tumor levels of spermine in the DFMO plus ornithine group suggest that polyamine synthesis may return to normal at higher ratios of ornithine. This change in tumor polyamine levels correlates with an increasing final tumor weight in rats in which ornithine is infused concomitantly with DFMO. Although the trend toward an increased final tumor weight was confirmed with the Ward colon tumor, necrosis precludes evaluation of the effect of DFMO on polyamine levels in this tumor.

These results demonstrate that ornithine can selectively inhibit DFMO-induced thrombocytopenia while having a minimal effect on the antitumor activity toward two transplantable tumors of different origins and growth characteristics. By maintaining the ratio of ornithine to DFMO, a larger, more effective dose of DFMO may be possible.

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REFERENCES

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