Antitumor Properties of (2R,5R)-6-Heptyne-2,5-diamine, a New Potent Enzyme-activated Irreversible Inhibitor of Omithine Decarboxylase, in Rodents

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ABSTRACT

(2R,5R)-6-Heptyne-2,5-diamine hydrochloride (MDL 72175) is a new, potent, and selective inhibitor of mammalian omithine decarboxylase. MDL 72175 given p.o. in drinking fluid reduced by 80% the growth of EMT6 sarcoma in mice and of HTC hepatoma in rats. It prolonged the survival of mice bearing L1210 or P388 leukemias and inhibited the development of Lewis lung carcinoma in mice at doses 10- to 20-fold lower than those of α-difluoromethylomithine, the most widely used irreversible inhibitor of omithine decarboxylase. MDL 72175 depleted putrescine and spermidine levels in the tumors to the same extent as did α-difluoromethylomithine. In the EMT6 sarcoma, MDL 72175 achieved at low doses a greater maximal antitumor effect than did α-difluoromethylomithine.

In combination therapy, MDL 72175 plus Adriamycin gave at least additive antitumor effects on solid tumors and experimental leukemias in animals. The combination MDL 72175 plus methylglyoxal bis(guanylhydrazone) also gave additive antitumor effects on P388 leukemia, associated with an increased uptake of methylglyoxal bis(guanylhydrazone); in contrast, antagonistic effects were observed with this combination on EMT6 tumors in mice.

Since MDL 72175 did not present toxicity at effective antitumor doses, this new omithine decarboxylase inhibitor can be considered as a promising antitumor drug.

INTRODUCTION

It is well established that some macromolecular processes associated with abnormal and normal cellular growth and multiplication can be slowed by sufficient depletion of the polyamines, putrescine and spermidine (for a recent review, see Ref. 25). This can be achieved by DFMO, a specific enzyme-activated irreversible inhibitor of ODC (22), the rate-limiting enzyme of the polyamine-biosynthetic pathway. Antiproliferative effects of DFMO, first demonstrated in rat HTC cells (18), have been confirmed in many other cell lines of animal and human origin cultured in vitro (for review, see Ref. 25). In vivo, administration of DFMO markedly impaired the growth of chemically induced or implanted murine and rat tumors (4, 8, 10, 13, 16, 26, 27, 32, 35); DFMO also inhibits the appearance of metastases (2, 34) and the growth of human tumors transplanted into nude mice (9). Furthermore, DFMO potentiates or has additive antitumor effects in combination with known cytotoxic compounds such as alkylating agents (14, 21, 27), antimetabolites (8), an S-phase-specific drug (28), DNA-intercalating agents (4), microtubule-directed agents (4), less specific cytotoxic drugs (1, 5, 8, 11, 29, 30, 32), and immunomodulators (31, 33).

MDL 72175 was designed, synthesized, and shown to be a specific enzyme-activated irreversible inhibitor of ODC (6). MDL 72175 is about 10 times more potent than is DFMO in vitro and in vivo studies (6). Moreover, when used at concentrations 50 times lower than DFMO, it depletes the putrescine, the spermidine, and the spermine content of cultured HTC cells and leads to complete arrest of cell replication and ultimately to cell death (20). It might be expected, therefore, that this very potent ODC inhibitor would achieve at lower doses at least comparable or possibly more profound in vivo antitumor effects to those described for DFMO. We now report on the in vivo antitumor properties of MDL 72175 compared to those of DFMO in EMT6 sarcoma in mice, L1210 and P388 leukemias in mice, Lewis lung carcinoma in mice, and HTC hepatoma in rats. The antitumor effects observed with MDL 72175 used in combination therapy with MGBG or Adriamycin in the different models are also described.

MATERIALS AND METHODS

Animals. Female C57BL mice (18 to 20 g) were used for the transplantation of Lewis lung carcinoma. Female C57BL/6 × DBA/2 F1 mice (hereafter called BD2F1) mice (initial body weight, about 20 g; Centre National de la Recherche Scientifique, Orléans, France) were used for the leukemia models; female BALB/c mice (initial body weight, 18 to 22 g; Charles River Breeding Laboratories, France) were used for experiments with EMT6 tumor. Male Buffalo rats (initial body weight, 160 to 200 g) bred in our center were used for the experiments on solid tumors induced by HTC cells. The animals were housed in metal cages with free access to food and water or a solution of DFMO or MDL 72175. Fluid intake and body weight were measured at regular intervals. Room temperature (21–23°C), humidity (45 to 55%), and a 12-hr light cycle (beginning at 6 a.m.) were kept constant throughout the investigations.

Cells and Tumors. Lymphocytic P388 and L1210 leukemias were propagated and maintained in vivo in BD2F1 mice. Cells were transferred every week by i.p. transplantation of 106 L1210 cells or 5 × 105 P388 cells contained in 0.1 ml 0.15 M NaCl into new acceptor mice.

Mouse mammary EMT6 cells were grown as described previously (27) on Waymouth's medium supplemented with 15% fetal calf serum (Grand Island Biological Co., Grand Island, NY). Cell cultures were trypsinized and suspended in phosphate-buffered saline in g/liter: NaCl, 8; KCl, 0.2; NaH2PO4, 1.15; KH2PO4, 0.2) to a density of 105 cells/ml. BALB/c mice were inoculated s.c. in the interscapular region with 105 cells/mouse. Mice were sacrificed by cervical dislocation, and the tumors were removed and weighed.

HTC cells, derived from Morris hepatoma 7288C induced in Buffalo rats, were grown in spinner cultures according to the method of Herashk...
Antitumor Effect of MDL 72175, a New ODC Inhibitor

Antitumor Effect of MDL 72175 Compared to DFMO on EMT6 Tumors in Mice. Mice were inoculated s.c. with EMT6 tumor cells and were given DFMO or MDL 72175 in drinking water beginning 4 days following inoculation. The volume of fluid consumed by the mice was measured in each case to determine the dose of compound taken; it comprised between 2.5 and 4 ml/mouse/day yielding the drug intakes shown on Chart 1. MDL 72175 and DFMO treatment dramatically retarded tumor growth. When the animals were sacrificed on Day 18, the average tumor weight of the control mice was 2.3 g, and up to 80% reduction of the tumor mass was achieved after treatment with MDL 72175 (Chart 1). The maximal antitumor effects of MDL 72175 were greater than those of DFMO and were achieved by doses 10-fold lower than those of DFMO. MDL 72175 depleted putrescine and spermidine concentration in the tumors to a degree similar to that for DFMO used at a 10-fold higher dose (Table 1). Spermidine depletion was accompanied by an increase in SAMDC activity (Tables 1 and 2; see also Refs. 19 and 27). Treatment with MDL 72175 reduced tumor putrescine and spermidine levels and increased SAMDC activity in a dose-dependent manner, while spermine levels were unaffected or increased (Table 2). The marked dose-dependent cytostatic effects of MDL 72175 are apparently associated with the depletion of putrescine and spermidine in the tumor.

Effect of MDL 72175 on the Growth of HTC Hepatoma in Rats. The growth of HTC tumors induced i.m. in the leg of Buffalo rats was reduced by 70% in rats where minipumps delivering MDL 72175 continuously (20 mg/kg/day) were implanted (Table 3). This antitumor effect could be further enhanced to 80% inhibition when MDL 72175 was given at 2 g/liter in drinking water (drug intake, 0.2 g/kg/day).

When measured after 18 days of treatment at the end of the experiment, the tumor putrescine content was reduced by 60% in tumors treated with MDL 72175 (20 mg/kg/day) by minipump and by 94% after MDL 72175 given in drinking water (Table 3). The spermidine content was reduced after the MDL 72175 treatment, while the spermine content increased slightly. SAMDC activity was also increased in correlation with spermidine depletion (data not presented).

RESULTS

Antitumor Effets of MDL 72175 in Monotherapy

Administration of Drugs. MDL 72175 was infused continuously via an Alzet minipump (Alza, Palo Alto, CA) implanted s.c. in the middle of the back (control animals had similar minipumps containing saline) injected i.p. or, like DFMO, it was administered p.o. by offering a solution of the compound in tap water as the sole drinking fluid. Average daily fluid intake per animal was estimated from the total fluid intake per group. For the study of its i.v. toxicity, MDL 72175 was dissolved in saline, and the solution was adjusted to pH 7 with NaOH. MGBG dissolved in 0.15 M NaCl was injected twice weekly i.p. at a dose of 25 mg/kg. Adriamycin was administered similarly at a dose of 2.5 mg/kg weekly.

Biochemistry. ODC and SAMDC activities were measured according to published methods (27) on aliquots of freshly homogenized tumors in 9 volumes of ice-cold 0.1 M phosphate buffer (pH 7.2) containing 1 mM dithiothreitol, 0.1 mM disodium EDTA, and 10 μM pyridoxal phosphate. Further aliquots of the homogenate were deproteinized by mixing immediately with an equal volume of 0.4 M perchloric acid, centrifuged, and filtered to obtain a protein-free homogenate. Further aliquots of the homogenate were used for polyamine assay kit developed by Orion Diagnostica (Helsinki, Finland).

Hematological Measurements. Hematocrits were estimated by centrifugation of whole blood samples and subsequent measurement of the packed cell volume. Erythrocytes, leukocytes, and platelet counts of heparinized blood samples obtained from animals by the retroorbital method were determined using a Model ZBI Coulter Counter (Coultronics, Paris, France).

Drugs. MDL 72175 and DFMO (MDL 71782) were synthesized in the Centre de Recherche Merrell-Dow International, Strasbourg. Adriamycin (doxorubicin hydrochloride) was purchased from Roger Bellon Laboratories (Neuilly, France). MGBG was from Aldrich-Europe (Beerse, Belgium).

REFERENCES

and Tomkins (12). Cells were harvested from their culture medium, counted, and washed twice with 0.9% NaCl solution prior to use. The tumors were induced in Buffalo rats by i.m. injection of the left leg of a suspension of 2 × 10⁶ HTC cells in 0.2 ml of 0.15 M NaCl. Animals were examined every week for tumor growth, and the diameter of their legs at the tumor level was measured with callipers in 2 perpendicular directions (breadth and width). The tumor cross-section was considered an ellipse; the following formula was used to calculate its size:

\[ r_1 \times \sqrt{(r_2 - c_2^2)} \]

where \( r_1 \) and \( r_2 \) are the perpendicular axes of the tumorous left leg and \( c_1 \) and \( c_2 \) are the perpendicular axes of the control right leg.

Lewis lung carcinoma (3LL tumors, L557 B004-005) were propagated and maintained in vivo in C57BL mice. The tumors were cut into small pieces in 10 volumes of 0.9% NaCl solution (saline) homogenized by 2 strokes of a Potter-Elvehjem homogenizer at 500 rpm, and filtered on gauze. Tumors were induced in mice by injection of 5 × 10⁶ cells contained in 0.05 ml of 0.15 M NaCl into the thigh muscle. The tumors could be detected from Day 8. Animals were examined twice weekly for tumor growth as described for HTC cells. Mice bearing tumors were killed after 18 days, and their lungs were examined under a magnifying lens for macroscopic metastases.

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Antitumor Effects of MDL 72175 in Monotherapy

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Effect of ODC inhibitors on some biochemical parameters of EMT6 tumors induced in BALB/c mice

<table>
<thead>
<tr>
<th>Day after inoculation of EMT6 cells</th>
<th>Tumor wt. (% of control)</th>
<th>Putrescine (nmol/g)</th>
<th>Spermidine (nmol/g)</th>
<th>Spermine (nmol/g)</th>
<th>SAMDC activity (nmol/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>100 ± 11*</td>
<td>89 ± 10</td>
<td>688 ± 36</td>
<td>748 ± 60</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>100 ± 9</td>
<td>71 ± 5</td>
<td>810 ± 30</td>
<td>693 ± 25</td>
</tr>
<tr>
<td></td>
<td>DFMO, 20 g/liter in drinking water (3200 mg/kg/day)</td>
<td>7</td>
<td>50 ± 6*</td>
<td>10 ± 2*</td>
<td>393 ± 18*</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>42 ± 4*</td>
<td>8 ± 1*</td>
<td>256 ± 9*</td>
<td>653 ± 24</td>
</tr>
<tr>
<td></td>
<td>MDL 72175, 2 g/liter in drinking water (300 mg/kg/day)</td>
<td>7</td>
<td>25 ± 2*</td>
<td>24 ± 7*</td>
<td>325 ± 35*</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>23 ± 2*</td>
<td>11 ± 3*</td>
<td>248 ± 42*</td>
<td>640 ± 60</td>
</tr>
</tbody>
</table>

*p < 0.05
**p < 0.01
***p < 0.001

Table 2
Effect of increasing doses of MDL 72175 on SAMDC and polyamine levels in EMT6 tumors induced in BALB/c mice

<table>
<thead>
<tr>
<th>MDL 72175 given in drinking water at</th>
<th>Putrescine (nmol/g)</th>
<th>Spermidine (nmol/g)</th>
<th>Spermine (nmol/g)</th>
<th>SAMDC activity (nmol/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>229 ± 17*</td>
<td>1045 ± 45</td>
<td>689 ± 43</td>
<td>270 ± 44</td>
</tr>
<tr>
<td>0.05 g/liter</td>
<td>208 ± 24</td>
<td>920 ± 69</td>
<td>798 ± 53</td>
<td>296 ± 66</td>
</tr>
<tr>
<td>0.2 g/liter</td>
<td>119 ± 8</td>
<td>708 ± 55</td>
<td>967 ± 23</td>
<td>505 ± 81</td>
</tr>
<tr>
<td>0.5 g/liter</td>
<td>&lt;20</td>
<td>249 ± 26</td>
<td>860 ± 56</td>
<td>1112 ± 108</td>
</tr>
<tr>
<td>2 g/liter</td>
<td>&lt;20</td>
<td>222 ± 7</td>
<td>1010 ± 15</td>
<td>1861 ± 196</td>
</tr>
<tr>
<td>5 g/liter</td>
<td>&lt;20</td>
<td>186 ± 3</td>
<td>940 ± 40</td>
<td>2186 ± 160</td>
</tr>
</tbody>
</table>

*p < 0.05
**p < 0.01
***p < 0.001

Table 3
Biochemical effects of ODC inhibitors on HTC hepatoma in rats

<table>
<thead>
<tr>
<th>MDL 72175 given in drinking water at</th>
<th>Putrescine (nmol/g)</th>
<th>Spermidine (nmol/g)</th>
<th>Spermine (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 7*</td>
<td>167 ± 29</td>
<td>739 ± 68</td>
</tr>
<tr>
<td>20 mg/kg/day infused</td>
<td>30 ± 9*</td>
<td>66 ± 9</td>
<td>435 ± 70</td>
</tr>
<tr>
<td>2 g/liter</td>
<td>20 ± 4*</td>
<td>10 ± 2*</td>
<td>224 ± 50*</td>
</tr>
</tbody>
</table>

*p < 0.01
**p < 0.001

MDL 72175 could still inhibit tumor growth when treatment was started on rats bearing clearly detectable tumors (cross-section, 1 sq cm), a situation more relevant to the clinical situation. MDL 72175 was infused at 20 mg/kg/day via minipump implanted s.c. 2 weeks after the inoculation of HTC cells into Buffalo rats; the tumor cross-section measured 27 days after inoculation was 4.3 ± 0.6 sq cm for untreated animals and 2.1 ± 0.3 sq cm after treatment (n = 5).

Effect of MDL 72175 and DFMO on the Development of Primary Lewis Lung Carcinoma and on the Appearance of Lung Metastases in Mice. DFMO and MDL 72175 treatments were started 4 days after inoculation of Lewis lung carcinoma. MDL 72175 was effective either when given p.o. via drinking water or when injected i.p. every 6 hr at 20 mg/kg (Table 4). The p.o. treatments markedly reduced the development of lung metastases 18 days after i.m. inoculation of the carcinoma, DFMO (20 g/liter in water) being slightly more effective than MDL 72175 (2 g/liter). The weight of the lungs taken from mice treated with the ODC inhibitors was significantly reduced compared to that from control animals and remained in fact close to the value in animals not bearing tumors (142 ± 4 mg, n = 8). Putrescine and, to a lesser extent, spermidine levels were decreased in the primary tumor and in the lungs of the animals treated with the ODC inhibitors; spermine concentrations were not significantly affected (Table 4).

Effect of MDL 72175 and DFMO on the Survival of Mice Bearing L1210 or P388 Leukemia. In animals inoculated i.p. with leukemic cells on Day 0 and given drinking water containing MDL 72175 (2 g/liter) from Day 1 on, the increase in survival time was 1.26-fold for L1210 leukemia and 1.16-fold for P388 Leukemia respectively (Table 5). The corresponding values for DFMO (2 g/liter) were 1.18-fold for L1210 leukemia and 1.15-fold for P388 leukemia, respectively.

Antitumor Effects of MDL 72175 Combined in Therapy with Adriamycin or MGBG

L1210 Leukemia in Mice. MGBG injected i.p. at 25 mg/kg twice weekly increased the survival time of leukemic mice by 10% only. MDL 72175 (2 g/liter in the drinking fluid) increased survival by 28%. Following combination of MGBG and MDL 72175, small additive effects (39% increase in survival time) were observed (Table 5). The uptake of MGBG by the ascitic cells measured 3 hr after injection on Day 7 [34 ± 6 (S.E.) pmol MGBG/10^6 cells] was only slightly increased in animals treated with MDL 72175 [49 ± 5 pmol MGBG/10^6 cells]. Adriamycin given i.p. once a week prolonged survival time 2.7-fold. When this cytotoxic drug was given in combination with MDL 72175, a marked further increase in survival time was seen, and 30% of the mice were apparently cured [more than 50 days survival (Table 5)].

Effect on the Survival of Mice Inoculated with P388 Leukemia. MDL 72175 increased by 16% the mean survival time of P388 leukemia-bearing mice. MDL 72175 given in combination with MGBG produced a synergistic antitumor effect [55% increase in survival (see Table 5)] which was associated with an increased uptake of MGBG by the P388 ascitic cells. The uptake of MGBG 3 hr after injection on Day 7 was 15 ± 6 pmol/10^6 cells after MGBG alone and 64 ± 9 pmol/10^6 cells after combined
Table 4

Antitumor and biochemical effects of ODC inhibitors on Lewis lung carcinoma in mice

Tumors and lungs were removed 3 hr after the last injection and 18 days after i.m. inoculation of the carcinoma. Probability of significant difference from control is determined by Student’s t test.

<table>
<thead>
<tr>
<th>Tumor at Day 18</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-section</td>
<td>Putrescine</td>
</tr>
<tr>
<td>(sq cm)</td>
<td>(nmol/g)</td>
</tr>
<tr>
<td>Control</td>
<td>3.0 ± 0.2a</td>
</tr>
<tr>
<td>MDL 72175</td>
<td>20 mg/kg i.p. every 6 hr</td>
</tr>
<tr>
<td></td>
<td>2 g/liter in drinking water</td>
</tr>
<tr>
<td>DFMO</td>
<td>20 mg/kg i.p. every 6 hr</td>
</tr>
<tr>
<td></td>
<td>2 g/liter in drinking water</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E. of 8 animals.
<sup>b</sup> p < 0.05.
<sup>c</sup> p < 0.01.
<sup>d</sup> p < 0.001.

Table 5

Effect of MDL 72175 alone or combined with MGBG or Adriamycin on the survival of leukemia-bearing BD2F<sub>1</sub> mice

Female BD2F, mice inoculated with leukemic cells (10<sup>6</sup> for L1210, 5 × 10<sup>6</sup> for P388) were given MDL 72175 beginning 1 day after inoculation. Some animals were given i.p. injections of MGBG, 25 mg/kg twice weekly beginning on Day 3; some animals were given i.p. injections of Adriamycin, 2.5 mg/kg weekly beginning 1 day after inoculation. Each group contained 10 mice. Probability of significant difference from control is determined by Wilcoxon signed-rank test for nonparametric statistics.

<table>
<thead>
<tr>
<th>Mean survival (days)</th>
<th>L1210 leukemia</th>
<th>P388 leukemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.2 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.2 ± 0.3</td>
</tr>
<tr>
<td>MDL 72175, 2 g/liter in drinking water (0.3 g/kg)</td>
<td>10.3 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MGBG, 25 mg/kg i.p. twice weekly</td>
<td>9.0 ± 0.4</td>
<td>11.2 ± 0.2</td>
</tr>
<tr>
<td>MGBG + MDL 72175, 2 g/liter in drinking water</td>
<td>11.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.8 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adriamycin, 2.5 mg/kg i.p. weekly</td>
<td>22.4 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.6 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adriamycin + MDL 72175, 2 g/liter in drinking water</td>
<td>&gt;38.4 (30% cured)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;20.8 (10% cured)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± S.E.
<sup>b</sup> p < 0.05.
<sup>c</sup> p < 0.01.
<sup>d</sup> p < 0.001.

MDL 72175 and MGBG treatment (means ± S.E. of 5 animals). Combined therapy (MDL 72175 plus Adriamycin) resulted in a synergistic antitumor effect (Table 5).

EMT6 Sarcoma in Mice. BALB/c mice bearing EMT6 sarcoma received MDL 72175 p.o. in their drinking fluid from Day 4 onward, following tumor inoculation (drug intake, 0.3 g/kg). Some animals were then given i.p. injections of MGBG (25 mg/kg twice weekly) or Adriamycin (2.5 mg/kg weekly) or Adriamycin (2.5 mg/kg weekly).

Treatment with MDL 72175 or with MGBG inhibited the growth of EMT6 solid tumors by 60 and 30%, respectively, when measured on Day 12 after inoculation (Chart 2). In contrast, when MGBG was combined with MDL 72175 in therapy, only 12% inhibition of tumor growth was achieved. Thus, MGBG antagonized the cytostatic effects of MDL 72175. On the other hand, combination therapy of MDL 72175 with Adriamycin resulted in a small additive antitumor effect (Chart 2).

DISCUSSION

If treatment with MDL 72175 was begun 4 or 5 days after s.c. inoculation of EMT6 tumors in mice when the tumor just became palpable, the subsequent growth of the tumor was markedly retarded. In this model, the maximal antitumor effects were
somewhat greater than those seen after DFMO and were achieved at 10-fold lower doses (see Table 1). The results obtained in the other animal tumor models confirm this higher antitumor potency. The tumor growth retardation achieved in vivo is likely to be due to the depletion of the tumor concentrations of putrescine and spermidine. Thus, (a) increases in ODC activity and in polyamine synthesis are invariably associated with rapid cell proliferation (18, 25), and (b) depletion of intracellular polyamines achieved with inhibitors of polyamine biosynthesis leads to inhibition of proliferation as clearly established using DFMO (18, 31). The analogy between the antitumor properties of MDL 72175 and those of DFMO is striking; the greater rapid cell proliferation (18, 25), and (b) depletion of intracellular activity and in polyamine synthesis are invariably associated with

obtained in the other animal tumor models confirm this higher activity and in polyamine synthesis are invariably associated with inhibition of proliferation as clearly established using MDL 72175 can be used effectively in combined therapy with cytotoxic drugs. When MDL 72175 was combined with Adriamycin, tumor growth inhibition and/or prolongation of survival were greater than after treatment alone. Prior depletion of the tumor polyamine concentrations by DFMO is known to enhance the intracellular accumulation of MGBG (29), a known cytotoxic drug which is structurally related to spermidine. As a consequence, the sequential use of DFMO and MGBG produces more profound in vivo antitumor effects than those elicited by either drug used separately (5, 29, 30). Similar findings are now reported using MDL 72175 which also increases intracellular MGBG concentration in the P388 leukemia model and further increases the mean survival time of the tumor-bearing mice (the antitumor effects are additive at best). In L1210 leukemia, the marginal increases in MGBG accumulation achieved after treatment with MDL 72175 are accompanied by a moderate elevation of the animal mean survival time. In contrast, the antitumor effect achieved with MDL 72175 in murine EMT6 sarcoma is abolished by MGBG. This phenomenon has already been reported for the same tumor model where MGBG or its derivative 1,1’-(methylethanediyli)ditriino bis(3-aminoquandine) abolishes DFMO-induced growth inhibition and polyamine depletion (4, 27). Restoration of the tumor polyamine concentrations probably results from inhibition of diamine oxidase activity by these diguanidine derivatives as demonstrated in mice-bearing L1210 leukemia (15).

Antitumor synergism with MDL 72175 combined in therapy with bleomycin or ellipticine has also been observed. The most likely explanation for this sensitization to cytotoxic drugs by MDL 72175 is, as has been proposed for DFMO (4, 21), that MDL 72175 through depletion of the polyamines slows the growth rate of the tumors in the periods between the cytotoxic treatments. It may be envisaged that MDL 72175 would be effective in combination with diversified drugs in the treatment of tumors. However, additional studies will be necessary to establish the optimal combinations and the appropriate treatment schedules, since beneficial or adverse effects might depend on the cytotoxic drug used and the tumor type (4, 8, 11, 23, 24, 28).

Used alone, MDL 72175 caused no apparent toxicity at the effective dose in animals. The side effects seen occasionally after

DFMO treatment in rats (17), diarrhea or decrease in blood elements, were not observed after MDL 72175 given chronically at effective antitumor doses. Thus, MDL 72175 is a highly promising cytostatic drug which may prove to be a useful adjuvant in preventing regrowth of tumors or formation of metastases after radiotherapy, after chemotherapy, or after surgical excision of tumors.

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Antitumor Effect of MDL 72175, a New ODC Inhibitor

Antitumor Properties of (2R,5R)-6-Heptyne-2,5-diamine, a New Potent Enzyme-activated Irreversible Inhibitor of Ornithine Decarboxylase, in Rodents

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