4'-O-Tetrahydropyranyladriamycin as a Potential New Antitumor Agent

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ABSTRACT

Chemotherapy with 4'-O-tetrahydropyranyladriamycin (THP-ADM), a new derivative of Adriamycin, was equally or more effective against several experimental mouse tumors than it was with Adriamycin (ADM). When mice with P388 leukemia were given i.p. injections of THP-ADM or ADM daily for 9 consecutive days, the maximum increases in life span (ILSs) of the mice were 190 and 175%, respectively. Eight of 24 mice treated with THP-ADM were free of tumor, while one of 24 mice treated with ADM was free of tumor. A single i.p. injection of either drug was also effective; maximum ILS was 170% for mice treated with THP-ADM and 240% for those treated with ADM. Nine of 12 mice were found to be free of tumor. THP-ADM was equally or slightly more effective against P388 leukemia than was ADM when either drug was given i.v. The maximum ILS was 106% with THP-ADM and 77% with ADM when the drug was given for 9 consecutive days. Single i.v. injections of THP-ADM or ADM were almost equally (ILS, 100%) effective. Chemotherapy with THP-ADM was also very effective against L1210 leukemia. THP-ADM administered i.p. five times, every other day starting from Day 1, was more effective than ADM was against Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 38 inoculated s.c. In the study with Lewis lung carcinoma, metastasis to the lungs was well suppressed by THP-ADM. ADM was more effective than was THP-ADM against colon adenocarcinoma 26. Because THP-ADM was more cytotoxic than or almost equally as cytotoxic as ADM against the established cell lines from the above mouse tumors, we suggest that THP-ADM is more efficiently transported into cultured cells.

INTRODUCTION

ADM (1) is one of the most widely used chemotherapeutic agents against various human neoplasias. ADM is being effectively used in the treatment of acute leukemia and malignant lymphoma, and it is also highly active against number of human solid tumors such as soft tissue and bone sarcomas, breast adenocarcinoma, bladder adenocarcinoma, bronchogenic carcinoma, testicular carcinoma, and thyroid carcinoma (2-4, 6). Although it has impressive antitumor activities, ADM possesses relatively strong side effects. Leukopenia, thrombocytopenia, and anemia are reversible side effects; however, myelosuppression is a major dose-limiting factor in clinical application and, most importantly, cardiomyopathy is cumulative and dose dependent and thus limits the use of ADM for long-term maintenance chemotherapy (8, 9).

Although a new anthracycline, aclacinomycin A, was found to have lower cardiotoxicity than did ADM (10), it does not necessarily have a greater chemotherapeutic effect than ADM against experimental mouse and human tumors (7). Thus, for several years, investigators have been striving to discover a new anthracycline that has lower toxicity but has antitumor activity similar to or superior to that of ADM. Recently, THP-ADM was synthesized by Umezawa et al. (12), who found that it is superior to ADM in its activity against L1210 leukemia (12). Although no animal experiment is generally accepted as predictive for human cardiotoxicity at the present time, it is important to note that THP-ADM possesses rather a weak cardiotoxicity among the anthracyclines used in animal tests (5).

In this study, we have examined the antitumor activities of THP-ADM against various experimental mouse tumors. THP-ADM had equal or slightly superior activity against P388 leukemia, L1210 leukemia, Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 38 than did ADM. Lung metastasis of Lewis lung carcinoma was well inhibited by THP-ADM. The growth-inhibitory effects of THP-ADM and ADM against cultured cell lines established from the mouse tumors used in this experiment were also examined. We believe that THP-ADM is a promising new candidate for clinical evaluation as an antitumor agent if the lower cardiotoxicity found in animals is also found in humans.

MATERIALS AND METHODS

Tumors and Animals. P388 leukemia, L1210 leukemia, Lewis lung carcinoma, B16 melanoma, and colon adenocarcinomas 26 and 38 were obtained from the National Cancer Institute, NIH, Bethesda, Md. DBA/2Cr, C57BL/6J, and BALB/c × DBA/2Cr F1 (hereafter called DB2F1) mice were supplied by Simonsen Laboratories, Gilroy, Calif., under the auspices of the National Cancer Institute, and BALB/c and male C57BL/6J × DBA/2Cr F1 (hereafter called BDF2F1) mice were obtained from Charles River Japan, Inc., Tokyo, Japan. DB2F1, mice, 24 to 27 g, were used for Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 38 studies. Female CD2F1 mice, 20 to 23 g, were used for P388 and L1210 leukemia and colon adenocarcinoma 26 studies. P388 and L1210 leukemia cells were maintained in male DBA/2Cr mice; adenocarcinoma 26 was maintained in female BALB/c mice; and Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 38 were maintained in male C57BL/6J mice.

Drugs. THP-ADM-HCl, formulated for preclinical use, was provided by Dr. H. Umezawa, Institute of Microbial Chemistry, Shinagawa, Tokyo, Japan. ADM, formulated for clinical use, is a product of Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Drugs were dissolved in 0.9% NaCl solution.

Evaluation of Antitumor Activity. One-tenth ml of cell suspension in HBSS containing 10⁶ P388 or 10⁶ L1210 leukemia cells was inoculated i.p. into CD2F1 mice. Cell suspensions in HBSS (20% v/v) of Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 26 were prepared from surgically removed corresponding tumors by
disaggregating the tumor pieces by gentle homogenization in a loosely fitted glass homogenizer. After the cell suspension was passed through a 40 mesh sieve, a volume (0.2 ml) of 5 x 10^5 viable cells of Lewis lung carcinoma and colon adenocarcinoma 26, as described by trypan blue dye exclusion, was implanted s.c. in the flank of BD2F, mice and i.p. into CD2F, mice, respectively. Tumor cell suspensions (0.25 ml) of B16 melanoma were inoculated s.c. into the flank of BD2F mice. Two-tenths ml of tumor brei of colon adenocarcinoma 38 diluted in HBSS (33%, w/v) was prepared by passing the tumor pieces through an 18-gauge needle; the suspension was then inoculated s.c. into the flanks of BD2F mice.

Mice were given ADM and THP-ADM at a constant rate of 0.01 ml/g body weight, in dose decrements of 0.67 or 0.5. Starting on Day 1, the drug was given i.p. or i.v. once or daily for 9 consecutive days, or every other day for a total of 5 injections.

Antitumor activity was determined by: (a) comparing the mean survival time of treated groups (7) with that of control groups (C) and expressed as an increase in life span [(T/C - 1) x 100%], Tumor-free survivors were excluded from these calculations; or (b) tumor mass growth inhibition.

Preparation of Tumor Cell Cultures. Solid tumor tissue was removed surgically from the mouse, trimmed from the necrotic portion, and finely minced using a sterile technique. One g of tissue was mixed with 3 ml of the fresh medium; the cells were further cultivated for 3 ml of the fresh medium. At 72 hr after the drug treatment, the cell layer was washed with PBS and trypsinized with 0.5 ml of 0.05% trypsin-EDTA (Grand Island Biological). PBS (2 ml) containing 2% fetal bovine serum was added to neutralize the trypsin. The cells were suspended in 2 ml of RPMI Medium 1640 containing 10% fetal bovine serum.

The cell suspension was centrifuged at 150 x g for 5 min at 4°. The cell pellet was washed with PBS and trypsinized with 0.5 ml of 0.05% trypsin-EDTA (Grand Island Biological). PBS (2 ml) containing 2% fetal bovine serum was added to neutralize the trypsin. The cells were suspended by pipetting and counted with a Model ZBI Coulter Counter as described previously (11).

For drug treatment of P388 leukemia cells, 2 x 10^6 P388 cells were suspended in 2 ml of RPMI Medium 1640 containing 10% fetal bovine serum, 20 µM 2-mercaptoethanol, and kanamycin (100 µg/ml) and were transferred to a Falcon No. 2054 culture tube (Falcon Plastics, Oxnard, Calif.). Two tubes were used for each drug concentration. The doubling time of the cells was approximately 14 hr. Twenty-four hr later, the cells were treated with THP-ADM and ADM as described above. At 1 hr after drug treatment, the medium containing the drug was removed by centrifugation at 150 x g for 5 min, and the cells were suspended in the fresh medium. After they were cultivated for an additional 72 hr, the cells were counted with a Coulter counter (11).

The concentration of each drug necessary to reduce growth by 50% was obtained by plotting the logarithm of the drug concentration versus the growth rate of the treated cells.

RESULTS

Antitumor Activity of THP-ADM and ADM against P388 Leukemia. The antitumor activity against i.p.-inoculated P388 leukemia of THP-ADM and ADM administered i.p. on Day 1 or on Days 1 to 9 was examined (Tables 1 and 2). The mean of the results from repeated experiments is illustrated in Chart 1. In repeated experiments, the maximum activity of THP-ADM and ADM ranged from 1.5 to 2.23 mg/kg, and ILSs of 170 to 190 and 150 to 175% were obtained for THP-ADM and ADM, respectively, when these drugs were given i.p. daily for 9 days (Table 1; Chart 1). Four and 8 of 24 mice given THP-ADM, 1.5 and 2.23 mg/kg, respectively, were tumor free when examined.
Antitumor effect of THP-ADM and ADM by single injection against P388 leukemia

Each group of 6 CD2F mice was given i.p. implants of $10^6$ P388 leukemia cells on Day 0, and drugs were given i.p. on Day 1.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>THP-ADM</th>
<th>ADM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>18.1 ± 1.6</td>
<td>18.6 ± 1.5</td>
</tr>
<tr>
<td>1.7</td>
<td>19.7 ± 1.1</td>
<td>19.9 ± 3.5</td>
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<tr>
<td>2.6</td>
<td>21.4 ± 2.5</td>
<td>20.5 ± 2.9</td>
</tr>
<tr>
<td>3.9</td>
<td>22.2 ± 0.4</td>
<td>20.4 ± 2.7</td>
</tr>
<tr>
<td>5.9</td>
<td>24.8 ± 4.5</td>
<td>26.4 ± 5.7</td>
</tr>
<tr>
<td>8.9</td>
<td>25.2 ± 3.0</td>
<td>33.0 ± 0</td>
</tr>
<tr>
<td>13.3</td>
<td>26.0 ± 2.4</td>
<td>31.0 ± 0.0</td>
</tr>
<tr>
<td>20.0</td>
<td>23.0 ± 1.0</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>30.0</td>
<td>5.0 ± 0.6</td>
<td>14.2 ± 3.3</td>
</tr>
<tr>
<td>Control</td>
<td>10.6 ± 0.9</td>
<td>10.6 ± 0.9</td>
</tr>
</tbody>
</table>

MST*: mean survival time of decreased mice.

*a All values showing more than 65% ILS were statistically significant (p < 0.05) by Student's t test as compared to that of the control value.

Antitumor Activity of THP-ADM and ADM against L1210 Leukemia. The maximum activity of THP-ADM against L1210 leukemia was 1.25 to 5 mg/kg, and an ILS of 71 to 149% was obtained; the maximum activity of ADM was 1.25 to 2.5 mg/kg, and an ILS of 78 to 118% was obtained (Table 3).

Antitumor Activity against Lewis Lung Carcinoma. Each drug was given i.p. 5 times every other day starting from Day 1 (early treatment) or Day 7 (delayed treatment) (Table 4). On Day 7, the mean tumor volume of the control group reached 290 cu mm. Maximum ILS of 58% was obtained with THP-ADM at 5 mg/kg in the early treatment regimen. THP-ADM at 7.5 mg/kg was toxic; however, 1 of 10 mice was tumor free on Day 90. ADM at 2.5 mg/kg was toxic, and a maximum ILS of 27% was obtained at 1.25 mg/kg. Treatments given every other day for a total of 5 treatments seemed to favor THP-ADM.

On Day 60, whereas only 1 of 24 mice given ADM at these doses was tumor free. The chemotherapeutic effect of a single i.p. injection of these drugs was comparable to the results described above (Table 2). In this case, the maximum activity of both drugs was in the range of 8.9 to 13.3 mg/kg, and ILSs of 150 to 100 and 100 to 120% were obtained for THP-ADM and ADM, respectively. No tumor-free survivors were noted.

Antitumor Activity of THP-ADM and ADM against L1210 Leukemia.

The maximum activity of THP-ADM against L1210 leukemia was 1.25 to 5 mg/kg, and an ILS of 71 to 149% was obtained; the maximum activity of ADM was 1.25 to 2.5 mg/kg, and an ILS of 78 to 118% was obtained (Table 3).

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Tumor-free survivors were not noted in these experiments. A single i.v. injection of the drugs also was therapeutically effective. Maximum activity of both drugs was 13.3 to 20 mg/kg, and ILSs of 100 to 140 and 100 to 120% were obtained for THP-ADM and ADM, respectively. No tumor-free survivors were noted.

Antitumor Activity of THP-ADM and ADM against L1210 Leukemia. The maximum activity of THP-ADM against L1210 leukemia was 1.25 to 5 mg/kg, and an ILS of 71 to 149% was obtained; the maximum activity of ADM was 1.25 to 2.5 mg/kg, and an ILS of 78 to 118% was obtained (Table 3).

Antitumor Activity against Lewis Lung Carcinoma. Each drug was given i.p. 5 times every other day starting from Day 1 (early treatment) or Day 7 (delayed treatment) (Table 4). On Day 7, the mean tumor volume of the control group reached 290 cu mm. Maximum ILS of 58% was obtained with THP-ADM at 5 mg/kg in the early treatment regimen. THP-ADM at 7.5 mg/kg was toxic; however, 1 of 10 mice was tumor free on Day 90. ADM at 2.5 mg/kg was toxic, and a maximum ILS of 27% was obtained at 1.25 mg/kg. Treatments given every other day for a total of 5 treatments seemed to favor THP-ADM.
with respect to the manifestation of toxicity. Almost equal tumor growth inhibition was observed at maximum effective doses of THP-ADM and ADM. In delayed treatment, THP-ADM showed a maximum ILS of 38% at 5.0 mg/kg; however, no significant therapeutic response was observed with ADM.

Inhibition of lung metastasis of Lewis lung carcinoma by THP-ADM was evident. In the control group, an average of 46.7 macroscopically countable tumor nodules was observed in the lungs on Day 21. In early treatment, THP-ADM at 5 mg/kg, a maximum effective dose, reduced the average number of lung metastases to less than one. The effect of ADM was not so prominent. THP-ADM still inhibited the lung metastasis in delayed treatment. At the most effective dose of 5 mg/kg, the number of tumor nodules in the lungs was reduced to 10% of that of the control value. Even at doses of 2.5 and 1.25 mg/kg, the lung metastases were reduced to 31 and 57% of that of control value. ADM, however, had only a marginal effect on lung metastases under this treatment schedule.

When the drugs were given 10 times, every other day from Day 1, inhibition of lung metastasis was greater than that observed in the above experiment; however, no significant improvement in survival time occurred.

### Table 3

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>MST (days)</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>9.1 ± 0.3</td>
<td>14</td>
</tr>
<tr>
<td>0.32</td>
<td>9.8 ± 0.4</td>
<td>23</td>
</tr>
<tr>
<td>0.63</td>
<td>10.4 ± 0.5</td>
<td>31</td>
</tr>
<tr>
<td>1.25</td>
<td>13.6 ± 3.7</td>
<td>73</td>
</tr>
<tr>
<td>2.5</td>
<td>19.8 ± 9.2</td>
<td>147 (1/12)</td>
</tr>
<tr>
<td>5.0</td>
<td>14.4 ± 1.4</td>
<td>79</td>
</tr>
<tr>
<td>Control</td>
<td>8.0 ± 0.7</td>
<td>0</td>
</tr>
</tbody>
</table>

**Antitumor effect of THP-ADM and ADM against L1210 leukemia**

Each group of 12 CD2F1 mice was given i.p. implants of 10⁵ L1210 leukemia cells on Day 0, and drugs were given i.p. daily from Day 1 to Day 9.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>MST (days)</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>9.5 ± 0.5</td>
<td>19</td>
</tr>
<tr>
<td>0.32</td>
<td>10.3 ± 0.6</td>
<td>26</td>
</tr>
<tr>
<td>0.63</td>
<td>11.0 ± 1.1</td>
<td>37</td>
</tr>
<tr>
<td>1.25</td>
<td>14.3 ± 6.7</td>
<td>78</td>
</tr>
<tr>
<td>2.5</td>
<td>17.4 ± 10.5</td>
<td>118</td>
</tr>
<tr>
<td>5.0</td>
<td>9.3 ± 1.5</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>8.0 ± 0.7</td>
<td>0</td>
</tr>
</tbody>
</table>

All values except that of ADM, 5.0 mg/kg, were statistically significant (p < 0.05) by Student's t test as compared to that of the control value.

### Table 4

**Inhibition of Lewis lung carcinoma by early or delayed treatment with THP-ADM and ADM**

Each group of 20 BD2F1 mice was given s.c. implants of 5 × 10⁵ cells of dissociated Lewis lung carcinoma on Day 0. Drugs were given i.p. every other day for 5 treatments starting from Day 1 or Day 7. Lung metastases of 10 mice from each group were counted on Day 21.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>MST (days)</th>
<th>ILS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>27.4 ± 7.0</td>
<td>10</td>
</tr>
<tr>
<td>2.5</td>
<td>20.8 ± 6.1</td>
<td>41</td>
</tr>
<tr>
<td>5.0</td>
<td>38.8 ± 3.8</td>
<td>66</td>
</tr>
<tr>
<td>7.5</td>
<td>20.1 ± 8.6</td>
<td>89</td>
</tr>
<tr>
<td>0.63</td>
<td>5.0 ± 5.0</td>
<td>5</td>
</tr>
<tr>
<td>1.25</td>
<td>31.2 ± 4.7</td>
<td>61</td>
</tr>
<tr>
<td>2.5</td>
<td>19.2 ± 11.2</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>24.8 ± 6.7</td>
<td>0</td>
</tr>
</tbody>
</table>

**Antitumor Activity of THP-ADM**

Number of tumor nodules in the lungs was reduced to 10% of that of the control value. Even at doses of 2.5 and 1.25 mg/kg, the lung metastases were reduced to 31 and 57% of that of control value. ADM, however, had only a marginal effect on lung metastases under this treatment schedule.

When the drugs were given 10 times, every other day from Day 1, inhibition of lung metastasis was greater than that observed in the above experiment; however, no significant improvement in survival time occurred.

### Table 5

**Antitumor Activities against B16 Melanoma and Colon Adenocarcinomas 26 and 38**

The results are summarized in Table 5. Against i.p.-implanted B16 melanoma, ADM did not show therapeutic effects when the drug was given every other day, 5 times starting from Day 1, whereas THP-ADM, 5.0 mg/kg, produced a significant therapeutic response (ILS, 39%). Both drugs showed good therapeutic effects against i.p.-inoculated colon adenocarcinoma 26. Eight of 10 mice given ADM, 2.5 mg/kg, and 5 of 10 mice given ADM, 1.25 mg/kg, became tumor free on Day 90, whereas 2 of 10 mice given THP-ADM, 5.0 or 2.5 mg/kg, were tumor free. At 2.5- and 5.0-mg/kg doses of THP-ADM, ILSs of 34 and 42% were obtained, respectively, against s.c.-implanted colon adenocarcinoma 38. Tumor growth inhibition of 90 and 50% was also observed for these doses. ADM, however, showed no effect on the prolongation of life span, although the drug inhibited tumor growth by 50 to 80%.

**Growth Inhibition of Cultured Mouse Cell Lines by THP-ADM and ADM**

Each of the established cultured cell lines from Lewis lung carcinoma, B16 melanoma, and colon adenocarcinomas 26 and 38 had almost the same sensitivity to ADM and THP-ADM (Table 6). However, P388 leukemia cells were more susceptible to THP-ADM than to ADM.
Table 5
Antitumor effect of THP-ADM and ADM against B16 melanoma and colon adenocarcinomas 26 and 38

B16 melanoma and colon adenocarcinoma 38 were implanted s.c. into the flank of BD2F mice. Colon adenocarcinoma 26 was implanted i.p. into CD2F mice. Ten mice per group were used. Drugs were given every other day for 5 treatments starting from the day after tumor implantation.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>MST*(days)</th>
<th>ILS (%)</th>
<th>Tumor inhibitionb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 25</td>
<td>Day 35</td>
</tr>
<tr>
<td>1. B16 melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THP-ADM</td>
<td>1.25</td>
<td>28.6 ± 8.6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>1.25</td>
<td>25.5 ± 3.9</td>
<td>-8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.0</td>
<td>25.5 ± 6.6</td>
<td>-3</td>
<td></td>
</tr>
<tr>
<td>2. Colon adenocarcinoma 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THP-ADM</td>
<td>1.25</td>
<td>23.9 ± 6.4</td>
<td>40*(1/10)</td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>1.25</td>
<td>32.8 ± 8.4</td>
<td>105*(5/10)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.0</td>
<td>10.8 ± 1.9</td>
<td>-32</td>
<td></td>
</tr>
<tr>
<td>3. Colon adenocarcinoma 38</td>
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</tr>
<tr>
<td>THP-ADM</td>
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<td>32 47</td>
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<tr>
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<td>41 49</td>
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<tr>
<td>Control</td>
<td>10.0</td>
<td>14.5 ± 3.2</td>
<td>-64</td>
<td></td>
</tr>
</tbody>
</table>

*a MST, mean survival time of deceased mice.
*b Tumor mass growth inhibition: (1 – T/C) x 100%. The average tumor masses of control mice were 2950 and 7213 cu mm on Days 25 and 35, respectively.
*c Mean ± S.D.
*d Statistically significant (p < 0.05) by Student’s t test as compared to that of control value.
* Statistically significant (p < 0.05) by U test as compared to that of control value.
+f Numbers in parentheses, 90-day survivors/number of treated mice.

Table 6
Effect of exposure to THP-ADM and ADM on in vitro growth of mouse tumor cells

Cultured cells were exposed to THP-ADM and ADM for 1 hr at 24 hr after seeding the cells. The cell number was counted on day 4 to estimate ICso.

<table>
<thead>
<tr>
<th>Tumor cell line</th>
<th>Doubling time (hr)</th>
<th>THP-ADM (ICso (nm))</th>
<th>ADM (ICso (nm))</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388</td>
<td>14</td>
<td>29.9</td>
<td>81.0</td>
</tr>
<tr>
<td>Lewis lung carcinoma</td>
<td>18</td>
<td>25.2</td>
<td>22.4</td>
</tr>
<tr>
<td>B16 melanoma</td>
<td>18</td>
<td>31.5</td>
<td>25.9</td>
</tr>
<tr>
<td>Colon adenocarcinoma 26</td>
<td>15</td>
<td>66.1</td>
<td>72.4</td>
</tr>
<tr>
<td>Colon adenocarcinoma 38</td>
<td>32</td>
<td>40.9</td>
<td>31.0</td>
</tr>
</tbody>
</table>

a ICso, concentration of drug necessary to reduce growth by 50%.

DISCUSSION

Using 6 tumor systems, we found that THP-ADM showed equal or superior activity against P388 leukemia, Lewis lung carcinoma, B16 melanoma, and colon adenocarcinoma 38 than did ADM. Against P388 leukemia, THP-ADM was almost equally effective as was ADM when the drug was given i.p. or i.v. THP-ADM was more effective than ADM in the inhibition of lung metastasis of Lewis lung carcinoma. Lung metastasis was almost completely inhibited at a 5.0-mg/kg dose of THP-ADM under the regimen used in this experiment. In an experimental model using golden hamsters, THP-ADM has been reported to possess lower cardiotoxicity (5). If this is also the case in humans, THP-ADM could be a promising candidate for clinical trials as a new anthracycline.

In in vitro experiments, THP-ADM had a cytotoxic effect similar to that of ADM against Lewis lung carcinoma, B16 melanoma, and colon adenocarcinomas 26 and 38. In in vivo experiments, however, the activity of THP-ADM was superior to that of ADM against s.c. inoculated B16 melanoma, Lewis lung carcinoma, and colon adenocarcinoma 38, suggesting that THP-ADM is better disposed to the s.c. inoculated tumors than ADM. THP-ADM also was effective in inhibiting lung metastasis of Lewis lung carcinoma, suggesting a better disposition of the drug to the lungs. One of the promising effects of THP-ADM is that, when inoculated i.p., it is more active than ADM against tumors implanted s.c. and lung tumors.

Tetrahydropyranyl residue may play an important role in the pharmacokinetics of THP-ADM, such as by permitting an effective disposition of the drug in the body. In in vitro experiments, THP-ADM showed cytotoxic effects higher than or almost equal to those of ADM. These results suggest that THP-ADM might be incorporated directly into cells, and in some cases THP-ADM might be more efficiently transported into cells than is ADM and it may inhibit the cellular DNA-dependent synthesis...
of nucleic acid. In our laboratory, we are examining more closely the mode of action of THP-ADM in cultured tumor cells.

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4′-O-Tetrahydropyranyladriamycin as a Potential New Antitumor Agent

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