Antitumor Activity of a Novel Antitumor Antibiotic, Quinocarmycin Citrate (KW2152)

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ABSTRACT

A novel antitumor antibiotic, 2a,3,4,5,6,6a,7,11b-octahydro-11-methoxy-12-methyl-3,6-imino-1H-2-oxa-11c-azanaphth(1,2-cf)azulen-5-carboxylic acid monocitrate (quinocarmycin citrate; KW2152) was selected for investigation in a number of experimental tumor systems because of its efficacy against P388 leukemia.

In the initial studies with P388 leukemia (i.p.-i.p.), KW2152 gave an increase in life span of &gt;80%. The activity was schedule dependent and daily administration was the most effective. KW2152 caused marginal activity against L1210 leukemia, B16 melanoma, and M5076 sarcoma.

The effect on cultured cells suggested that KW2152 was not cross-resistant to Adriamycin (ADM); however, KW2152 caused prolongation of life span against mice bearing P388/ADM or P388/MMC. In tests against human tumors xenografted s.c. in nude mice, KW2152 significantly inhibited the growth of MX-1 mammary carcinoma with all tumors cured at i.v. doses of 10 mg/kg/day and p.o. doses of 66 mg/kg/day given for 7 days. KW2152 also inhibited distinct human gastric carcinomas, St-4 and St-15 tumors, and colon carcinoma Co-3 by daily administration for 7 days. Against St-4, KW2152 gave a treated versus control percentage of 27%, compared to 52% for cis-diamminedichloroplatinum. Against Co-3, KW2152 was at least as effective as MMC, ADM, cis-diamminedichloroplatinum, and bleomycin, giving a treated versus control percentage of 18% at a dose of 8.6 mg/kg/day given daily for 7 days. KW2152 showed growth inhibitory activity against cultured murine tumors and human cells. The order of in vitro efficacy of KW2152 against murine tumors, P388 leukemia &gt; L1210 leukemia, B16 melanoma, correlated with the order of the sensitivity on the i.p.-i.p. systems of these tumors. The 50% inhibitory concentrations against P388 leukemia cells were 5.3 x 10^-4 and 1.1 x 10^-7 M after 1 and 72 h exposure, respectively. KW2152 caused significant inhibition of RNA synthesis after a short time exposure. In P388 leukemia cells exposed for 1 h with KW2152, the 50% inhibitory concentration for RNA synthesis was 10^-7 M, 30-fold less than that for DNA synthesis.

White blood cell depression or platelet depression was not significant after administration of the i.v. 10% lethal dose given daily for 7 days.

Because of its good activity against human mammary tumor MX-1 and some effectiveness against other gastric and colon carcinomas and its water solubility, a novel antitumor antibiotic, KW2152, is being developed as a Phase I anticancer agent.

INTRODUCTION

Quinocarmycin citrate (KW2152), a citrate salt of quinocarcin (DC52) (1, 2) which contains a novel skeleton with the isocoumarin structure in the molecule, was found in the culture broth of Streptomyces melanojacins nov. sp. Quinocarmycin citrate was active against Bacillus subtilis, Staphylococcus aureus, and Klebsiella pneumoniae but not against Gram negative bacteria. The antitumor activity of quinocarcin has not been reported.

However, since quinocarcin is not stable in aqueous solution for the purpose of further evaluation including clinical trials, a citrate salt of quinocarcin was synthesized with higher stability, and more than 95% activity remained after 72 h in phosphate buffer (pH 7.2) at 37°C.

Saframycins (3) and naphthyridinomycin (4) were antitumor antibiotics with common pyrazinoisoquinoline ring systems in their molecule. However, quinocarcin also has a pyrazinoisoquinoline structure completely different from both compounds in the structure, because quinocarcin has an aromatic ring in place of the quinone ring of saframycin and naphthyridinomycin, and interestingly it has a carboxylic acid group that is absent in the other two compounds. Saframycin (5) was reported to be highly active against P388 leukemia and moderately active against L1210 leukemia and B16 melanoma, but antitumor activity against human tumor cells transplanted to nude mice were not reported. Naphthyridinomycin was reported to have only marginal activity (6).

This paper describes the spectrum of activity of KW2152 against murine tumors and human carcinomas xenografted in nude mice. We also report its growth inhibitory activity against cultured cells and its effect on macromolecular synthesis.

MATERIALS AND METHODS

Drugs. Quinocarmycin citrate (KW2152; M, 522.5) was isolated from S. melanojacins in our laboratory. The structure is shown in Fig. 1. Its isolation procedure and chemical nature have been reported previously (2). Mitomycin C and Adriamycin were obtained from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Bleomycin, formulated for clinical use, was obtained from Nippon Kayaku Co., Ltd., Tokyo, Japan. cis-Diamminedichloroplatinum was synthesized in our laboratory. [6-3H]Thymidine (22 Ci/mm), [5-3H]uridine (25 Ci/mm), and [L-4,5-3H]leucine (54 Ci/mm) were purchased from Amersham International, Ltd., Amersham, England.

Animal and Tumor Cells. Adult male BALB/c × DBA/2 F, (hereafter called CD2F) mice weighing 20-25 g, male C57BL/6 × DBA/2 F (hereafter called B6D2F) mice weighing 20-25 g, male ddY mice weighing 18-20 g were obtained from Shizuoka Agricultural Co-operative Association for Laboratory Animals, Hamamatsu, Japan. Adult male BALB/c × nu/nu mice weighing 20-25 g were supplied by Nihon Clea Co., Ltd., Tokyo, Japan. P388 leukemia, L1210 leukemia, B16 melanoma, M5076 sarcoma, Lewis lung carcinoma, Sarcoma 180, and human mammary carcinoma MX-1 cells were supplied by Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan. P388/ADM² leukemia cells (7) and P388/MMC leukemia cells obtained by serial passage in mice bearing P388 with MMC or ADM were kindly supplied by Dr. Inaba, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Co-3 (8), well differentiated adenocarcinoma of colon; St-4 (8), poorly differentiated gastric and colon carcinomas and its water solubility, a novel antitumor antibiotic, KW2152, is being developed as a Phase I anticancer agent.

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²To whom requests for reprints should be addressed.
and sensitivity to several antitumor agents in our laboratory.

Evaluation of Antitumor Activity. LI210 (IO5), P388 (IO6), P388/ADM (IO7), or P388/MMC (IO6) cells were transplanted i.p. into CD2F1 mice and i.p. administration of drugs was started the day after tumor transplantation. B16 melanoma was introduced into B6D2F mice by i.p. administration of 0.5 ml of a 10% homogenate of tumor brei and drug administration was started the day after tumor transplantation. M5076 (IO6) was transplanted i.p. into B6D2F mice. P388 cells (IO7) transplanted i.v. were treated with i.v. drugs. Lewis lung carcinoma was transplanted s.c. at 8 mm³ by trocar to B6D2F mice and drug administration was started the day after tumor inoculation and the tumor volume was measured on day 7. Efficacy was based on mean tumor volume as derived from caliper measurements of the length and width of the tumor volume calculated using the formula for a prolate ellipsoid

\[
\text{Tumor volume} = \frac{L \times W^2}{2}
\]

in which \(L\) is the length of the major axis and \(W\) is the length of the minor axis (12). The T/C percentage of mean tumor volume was calculated. BALB/c-nu/nu mice were given s.c. tumor fragment equivalent to 8 mm³ of MX-1, Co-3, St-4, or St-15 tumor passaged in nude mice. When tumor volume reached to 100-300 mm³, the mice were pair matched in groups of 6 mice each and drug or vehicle was administered i.v. according to the protocol. Tumor volume was calculated as shown for Sarcoma 180 cells. Drug efficacy was expressed as the percentage of the \(V/V_\text{control}\) of the control group, where \(V\) is the mean tumor volume at the day of evaluation and \(V_\text{control}\) is the mean tumor volume at the day of initial administration of drug.

Cell Culture. P388, P388/MMC, P388/ADM, L1210, and Sarcoma 180 cells were transplanted to the culture medium with Roswell Park Memorial Institute Medium 1640 (GIBCO) supplemented with 10% fetal bovine serum (GIBCO), 100 units/ml of penicillin, and 100 µg/ml of streptomycin. B16, HeLa S3 (9), KB (10), and SK-MEL-28 (11) cells were cultured in Eagle's minimum essential medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% fetal bovine serum (GIBCO) and 60 µg/ml of kanamycin.

The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂. For the drug treatment experiments, P388, P388/ADM, P388/MMC, L1210, and Sarcoma 180 cells (2 × 10⁵ cells) were cultured in Falcon 2058 culture tubes (Falcon Plastics, Oxnard, CA) containing 1 ml of growth medium. B16, HeLa S3, KB, and SK-MEL-28 cells were cultured in Nunc 24-well multidishes (Nunc, Roskilde, Denmark) containing 1 ml of growth medium. For the short exposure time experiments (1 h exposure) cells were transferred to the fresh growth medium and incubated for another 71 h. The monolayer cells were washed with phosphate buffered saline solution (Flow Laboratories, United Kingdom) and incubated with 0.05% trypsin (Difco Laboratories, Detroit, MI)-0.02% EDTA (Wako Pure Chemical Industries Co., Ltd., Osaka, Japan). Monolayer cells treated as described above or suspension cells were counted with a Toa Micro-Cell Counter (Toa Medical Electronics Co., Ltd., Kobe, Japan).

The growth inhibitory activity was expressed at the IC₅₀.

Effect of the Incorporation of Labeled Precursors into Nucleic Acids and Proteins. For the time response studies, P388 cells (10⁵/ml) in exponential phase were cultured simultaneously with KW2152 and [6-³H]thymidine (1 µCi/ml) or [5-³H]uridine (1 µCi/ml) or L-[4,5-³H]leucine (1 µCi/ml) for various time periods. The radioactivity incorporated into the trichloroacetic acid insoluble fraction was counted. The average radioactivities of 3 tubes at each point were calculated and a percentage of the radioactivity of the control group was determined.

Effect of KW2152 on Murine Tumors. From the schedule dependency test on P388, antitumor test against murine tumors was evaluated by daily injection. KW2152 showed marginal activity against i.p. L1210 leukemia in mice and i.p. B16 melanoma in mice with T/C percentages of 139 (7.3 mg/kg/day) and 140 (3.7 mg/kg/day), respectively.

Against the M5076 sarcoma, KW2152 showed marginal activity (T/C percentage, 125 at 3.7 mg/kg/day for 10 days). KW2152 showed activity against i.p. P388 leukemia in mice. Daily i.v. administration for 7 days (5.7 mg/kg/day) gave a T/C percentage of 157 in one test.

Effect of KW2152 on Survival of Mice with P388 Leukemia

The effects of dose and schedule on the efficacy of KW2152 against P388 leukemia were determined. P388 cells (1 × 10⁵/mouse) were implanted into CD2F1 mice (6 mice/group) on day 0 and i.p. treatment was initiated on day 1 according to the schedules shown below.

<table>
<thead>
<tr>
<th>Day</th>
<th>Dose (mg/kg/day)</th>
<th>Mean survival days ± SD</th>
<th>T/C (%)</th>
<th>Mean survival days ± SD</th>
<th>T/C (%)</th>
<th>Mean survival days ± SD</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10.0 ± 0.8</td>
<td>10.0 ± 0.8</td>
<td>10.0 ± 0.8</td>
<td>8.1 NT</td>
<td>11.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>24.6</td>
<td>130 ± 3.5</td>
<td>130 ± NT</td>
<td>130 ± NT</td>
<td>130 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>16.4</td>
<td>130 ± NT</td>
<td>130 ± NT</td>
<td>130 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>12.3</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>0.26</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>118 ± NT</td>
<td>11.7 ± 0.5</td>
<td>117 13.8 ± 1.7</td>
<td>138 18.0 ± 2.3</td>
</tr>
</tbody>
</table>

* NT, not tested.
KW2152 was not effective against s.c. Lewis lung carcinoma in mice on the prolongation of life span. KW2152 also did not show activity against s.c. Sarcoma 180 by daily i.v. administration (Table 2).

KW2152 was active against i.p. P388/MMC in mice with a T/C percentage of 168 at a dose of 5.4 mg/kg/day. It was also effective against i.p. P388/ADM in mice with a T/C percentage of 168 at a dose of 3.6 mg/kg/day by daily administration for 7 days (Table 3).

The growth inhibitory effects of KW2152 on P388 leukemia and the above resistant cell lines were examined in culture. KW2152 gave IC50 value similar to those P388 leukemia cells and P388/ADM cells after 1 and 72 h exposure; however, KW2152 was 6- and 45-fold less effective on P388/MMC cells than P388 leukemia cells, after 72 and 1 h exposure, respectively (Table 4).

Although KW2152 showed similar T/C percentages against P388/ADM and P388/MMC, efficacy against cells in culture suggested that KW2152 might not show cross-resistance to ADM but might show cross-resistance to MMC.

### Table 2. Effect of KW2152 on murine tumors

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Drug route</th>
<th>Schedule of treatment days</th>
<th>Dose (mg/ Kg/day)</th>
<th>Mean survival T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210</td>
<td>i.p.</td>
<td>7</td>
<td>7.2 ± 0.7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1</td>
<td>147</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>7.3 ± 0.6</td>
<td>139</td>
</tr>
<tr>
<td>B16</td>
<td>i.p.</td>
<td>7</td>
<td>15 ± 2.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1</td>
<td>147</td>
<td>116</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–10</td>
<td>3.7 ± 0.2</td>
<td>140</td>
</tr>
<tr>
<td>M5076</td>
<td>i.p.</td>
<td>1</td>
<td>23 ± 3.2</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–10</td>
<td>3.7 ± 0.2</td>
<td>125</td>
</tr>
<tr>
<td>P388</td>
<td>i.v.</td>
<td>1</td>
<td>11 ± 0.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>1–7</td>
<td>11.7 ± 0.5</td>
<td>112</td>
</tr>
<tr>
<td>Lewis lung</td>
<td>i.v.</td>
<td>1–10</td>
<td>5.9 ± 0.8</td>
<td>157</td>
</tr>
<tr>
<td>carcinoma</td>
<td>i.v.</td>
<td>1</td>
<td>25.5 ± 3.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>1–10</td>
<td>5.7 ± 0.0</td>
<td>111</td>
</tr>
<tr>
<td>S180</td>
<td>s.c.</td>
<td>1</td>
<td>11.7 ± 0.7</td>
<td>71*</td>
</tr>
<tr>
<td></td>
<td>s.c.</td>
<td>1–5</td>
<td>5.9 ± 0.7</td>
<td>59</td>
</tr>
</tbody>
</table>

* Ratio of tumor volume of treated group to that of control group.

Effect of KW2152 on Human Tumors. KW2152 was evaluated against MX-1 breast, Co-3 colon, St-4 gastric, and St-15 gastric carcinomas under the growth of tumor cells transplanted to nude mice. Against the MX-1 human mammary carcinoma, KW2152 demonstrated significantly good activity after i.v. administration. Daily administration gave significant reduction of tumor volume and all tumors were not palpable on day 13 at 5.9 and 4.4 mg/kg/day, respectively. The regrowth of tumor was not observed until day 25 after starting drug administration. Single injection of KW2152 also gave temporary tumor regression, but tumor growth started with almost the same growth rate in the untreated control. MMC used as a positive control produced significant regression of tumor with all tumors cured at a dose of 6.0 mg/kg i.v., but the regression of tumor was more rapid with KW2152 than with MMC (Fig. 24). KW2152 was also significantly effective against MX-1 p.o. Because a dose of 26.2 mg/kg/day cured all mice, an approximately 5-fold higher dose was required p.o. than i.v. to obtain the same extent of effect. MMC was also effective p.o. with an approximately 5-fold dose i.v. needed (Fig. 2B).

Against St-4 human gastric carcinoma, KW2152 demonstrated moderate activity. A dose of 8.6 mg/kg/day with daily i.v. administration produced the decrease in tumor size and T/C percentage value was 27 on day 10, but daily administration of 5.7 mg/kg/day or a single administration (17.1 mg/kg) was not effective. MMC showed a growth inhibiting effect with a T/C percentage value of 32 after a single i.v. administration. KW2152 did not show a significant duration of activity, giving maximum effect on day 10 and rapid growth of tumor thereafter with daily administration for 7 days starting from day 0 (Fig. 3).

Against St-15 human gastric carcinoma, KW2152 showed moderate activity. Tumor growth was not observed until day 8 by daily administration and T/C percentages were 29 and 35 on day 13 at doses of 8.6 and 5.7 mg/kg/day, respectively. However, the regrowth of tumor was shown thereafter. Single i.v. injection of MMC (6.0 mg/kg) was more effective with no tumor growth until day 26 (Fig. 4).

Against Co-3, human colon carcinoma, KW2152 showed moderate activity at doses of 5.7–8.6 mg/kg/day and was more active than MMC. T/C percentages for KW2152 were 18 (8.6 mg/kg/day) and 30 (5.7 mg/kg/day) on day 11, and those for MMC were 41 and 39 on days 11 and 21, respectively (Fig. 5).

The antitumor activity of KW2152 against 4 human tumor xenografts was compared with that of established antitumor agents (Table 5). MMC, ADM, cis-DDP, and BLM were administered at their optimal doses and schedules as determined from reported values and preliminary lethality tests in nude mice conducted in our laboratory. As reported previously (14), MX-1 was relatively sensitive to various anticancer agents, but not as many compounds cured all mice. MMC and cis-DDP cured all mice, but ADM did not give significant growth inhibition against MX-1. The activity of KW2152 against MX-1 was comparable to those of MMC and cis-DDP. Against Co-3, only KW2152 showed tumor reduction with a T/C percentage of 18 and was more effective than MMC, ADM, cis-DDP, and BLM which gave T/C percentages of 30–47.

Against St-4, MMC produced an effective response, but cis-DDP showed a marginal response with a T/C percentage of 52; however, KW2152 caused significant growth inhibition of St-4. MMC and cis-DDP caused a significant tumor growth inhibition (T/C percentage = 7 for MMC and 9 for cis-DDP).

### Table 3. Effect of KW2152 against CD2F, mice bearing P388/MMC and P388/ADM

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Drug route</th>
<th>Schedule of treatment days</th>
<th>Dose (mg/kg/day)</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388</td>
<td>i.p.</td>
<td>1</td>
<td>7.1</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>5.4 ± 0.8</td>
<td>198</td>
</tr>
<tr>
<td>MMC</td>
<td>i.p.</td>
<td>1</td>
<td>6.0 ± 0.6</td>
<td>164</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>6.0 ± 0.6</td>
<td>164</td>
</tr>
<tr>
<td>ADM</td>
<td>i.p.</td>
<td>1</td>
<td>12.0 ± 0.1</td>
<td>215</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>12.0 ± 0.1</td>
<td>215</td>
</tr>
<tr>
<td>P388/MMC</td>
<td>i.p.</td>
<td>1</td>
<td>7.1 ± 0.6</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>5.4 ± 0.6</td>
<td>168</td>
</tr>
<tr>
<td>MMC</td>
<td>i.p.</td>
<td>1</td>
<td>2.0 ± 0.3</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>2.0 ± 0.3</td>
<td>104</td>
</tr>
<tr>
<td>P388/ADM</td>
<td>i.p.</td>
<td>1</td>
<td>14.3 ± 1.0</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1–7</td>
<td>3.6 ± 0.5</td>
<td>168</td>
</tr>
<tr>
<td>ADM</td>
<td>i.p.</td>
<td>1</td>
<td>8.0 ± 0.5</td>
<td>108</td>
</tr>
</tbody>
</table>

3 Unpublished data.
Table 4 Growth inhibitory activity of KW 2152 in P388, P388/MMC, and P388/ADM leukemia cells in culture

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC_{50} (1 h x 10^{-9})</th>
<th>IC_{50} (72 h x 10^{-9})</th>
</tr>
</thead>
<tbody>
<tr>
<td>P388</td>
<td>1.1 ± 0.6*</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>P388/MMC</td>
<td>49.4 ± 13.7*</td>
<td>10.1 ± 5.1†</td>
</tr>
<tr>
<td>P388/ADM</td>
<td>4.9 ± 1.7*</td>
<td>1.9 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± SD of four to six experiments.
† P < 0.05 versus P388 leukemia cells by Student's t test.

Fig. 2. Effect of KW2152 on human mammary carcinoma MX-1. MX-1 cells (8-mm³ fragment) were implanted s.c. into male BALB/c nu/nu mice (6 mice/group). In A, when tumor volume reached to 217 ± 69 (SD) mm³, i.v. treatment was initiated. Untreated control (●); KW2152, 17.6 mg/kg on day 0 (●); KW2152, 5.9 mg/kg/day daily administration for 7 days from day 0 (●); KW2152, 4.4 mg/kg/day daily i.v. administration for 7 days from day 0 (●); MMC, 6.0 mg/kg on day 0 (●). In B, p.o. treatment was initiated when tumor volume reached to 184 ± 51 mm³. Untreated control (●); KW2152, 65.5 mg/kg on day 0 (●); KW2152, 26.2 mg/kg/day daily administration for 7 days from day 0 (●); KW2152, 19.7 mg/kg/day daily administration for 7 days from day 0 (●); MMC, 30 mg/kg on day 0 (●).

Fig. 3. Effect of KW2152 on the growth of s.c. implanted human gastric carcinoma St-15 (8-mm³ fragment/mouse). Drug administration (i.v.) was started on day 0 when tumor volume reached 195 ± 49 mm³. Untreated control (●); KW2152, 20 mg/kg on day 0 (●); KW2152, 8.6 mg/kg/day daily for 7 days (●); KW2152, 5.7 mg/kg/day daily for 7 days (●); MMC, 6.0 mg/kg on day 0 (●).

Fig. 4. Effect of KW2152 on the growth of s.c. implanted human gastric carcinoma Co-3 (8-mm³ fragment/mouse). Drug administration (i.v.) was started on day 0 when tumor volume reached 189 ± 53 mm³. Untreated control (●); KW2152, 20 mg/kg on day 0 (●); KW2152, 8.6 mg/kg/day daily for 7 days (●); KW2152, 5.7 mg/kg/day daily for 7 days (●); MMC, 6.0 mg/kg on day 0 (●).

Fig. 5. Effect of KW2152 on the growth of s.c. implanted human colon carcinoma Co-3 (8-mm³ fragment/mouse). Drug administration (i.v.) was started on day 0 when tumor volume reached 162 ± 41 mm³. Untreated control (●); KW2152, 20 mg/kg on day 0 (●); KW2152, 8.6 mg/kg/day daily for 7 days (●); KW2152, 5.7 mg/kg/day daily for 7 days (●); MMC, 6.0 mg/kg on day 0 (●).

Against St-15, KW2152 was efficacious with T/C percentage value of 29, but less effective than MMC and cis-DDP.

Growth Inhibitory Effect of KW2152 on Murine and Human Tumor Cell Lines in Culture. The growth inhibitory effect of KW2152 against 4 murine tumor cell lines and 3 human tumor cell lines was tested with 1 and 72 h exposures. KW2152 showed comparable potency against the murine tumors P388 leukemia, L1210 leukemia, B16 melanoma, and Sarcoma 180 with 72 h exposure; IC_{50} ranged from 0.11 to 3.4 μM. After 1 h exposure, the IC_{50} was 5.3 x 10^{-6} M for P388 leukemia but was 30- and 40-fold greater for L1210 and B16, respectively. The ratio of
IC₅₀ values of 1 h exposure to that of 72 h was about 50 for P388 but was in the range of 200–600 for other 3 murine tumor cells. Against human tumor cell lines, KW2152 also showed significant potency comparable to that against murine tumor cell lines by 72 h exposure; IC₅₀ values were 0.10–0.16 μM. After 1 h exposure, IC₅₀ for HeLa S3 and KB cells were 10-fold smaller than that for SK-MEL-28 cells (Table 6).

DNA, RNA, and Protein Synthesis. The effect on the synthesis of DNA, RNA, and protein of P388 leukemia cells was measured by the incorporation of [6-³H]thymidine, [5-³H]uridine, and L-[4,5-³H]leucine, respectively. Change was observed in synthesis of macromolecules exposed continuously to 10⁻⁴ M or 10⁻³ M KW2152 and radioactive precursors for various times. Rapid inhibition of RNA synthesis was observed after slight increase of RNA synthesis at 15 min. At 30 min RNA synthesis was inhibited to 50% of control with 10⁻⁵ M KW2152. On the other hand, DNA and protein synthesis were not inhibited at 60 min with 10⁻⁴–10⁻³ M KW2152 (Fig. 6A).

A dose-response relationship was measured after 1 h exposure to various concentrations of KW2152 and with radioactive precursors. Significant RNA selective inhibition was observed in which inhibition of RNA synthesis gradually increased as the concentration of KW2152 increased from 3 × 10⁻⁴ to 10⁻³ M at an IC₅₀ of 1.2 × 10⁻⁵ M which was comparable to the concentration that inhibited 50% of in vitro cell growth for KW2152 with 1 h exposure. DNA synthesis was not significantly inhibited, as compared with RNA synthesis. The IC₅₀ of KW2152 for DNA synthesis was 3.4 × 10⁻⁴ M after 1 h exposure. Protein synthesis was not significantly inhibited with less than 20% inhibition after exposure of the cells to a KW2152 concentration of 3 × 10⁻⁴ M, the concentration that inhibits 50% of DNA synthesis (Fig. 6B).

General Toxicity of KW2152 in Mice. LD₅₀ was 13.9 and 6.5 mg/kg/day by an i.v. single administration and an i.v. daily administration for 7 days, respectively. Successive administration could attain higher total dose than a single administration.

Table 6 Evaluation of the cellular cytotoxicity of KW2152 in vitro

<table>
<thead>
<tr>
<th>T/C % (dose, schedule)</th>
<th>Drug</th>
<th>MX-1</th>
<th>Co-3</th>
<th>St-4</th>
<th>St-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>KW2152</td>
<td>0 (4.4 mg/kg, days 0–6)</td>
<td>18 (8.6 mg/kg, days 0–6)</td>
<td>27 (8.6 mg/kg, days 0–6)</td>
<td>29 (8.6 mg/kg, days 0–6)</td>
<td></td>
</tr>
<tr>
<td>MMC</td>
<td>0 (6.0 mg/kg, day 0)</td>
<td>38 (6.0 mg/kg, day 0)</td>
<td>32 (6.0 mg/kg, day 0)</td>
<td>NT*</td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>77 (10.0 mg/kg, day 0)</td>
<td>47 (15.0 mg/kg, day 0)</td>
<td>52 (7.1 mg/kg, days 0, 4, 8)</td>
<td>9 (7.1 mg/kg, days 0, 4, 8)</td>
<td></td>
</tr>
<tr>
<td>cis-DDP</td>
<td>0 (7.1 mg/kg, days 0, 4, 8)</td>
<td>30 (7.1 mg/kg, days 0, 4, 8)</td>
<td>34 (80.0 mg/kg, days 0, 4, 8)</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>BLM</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*T/C %, percentage of treated versus control tumor volume.

**NT, not tested.

WBC and platelets were measured periodically after i.v. administration of LD₁₀ and 1/1.57 × LD₅₀ doses in CD2F, mice. WBC nadir occurred on day 3 with 66% of mean control radioactivity was counted after pulse labeling for 1 h.
WBC by successive administration of LD_{10} dose (6.5 mg/kg/day). The WBC of mice treated with a single administration of MMC (5.6 mg/kg) reached nadir with 57% of control on day 3 and recovered on day 14. Platelets decreased to 541 x 10^3 cells/mm^3 on day 3 after an LD_{10} of daily administration for 7 days. On the other hand, MMC gave a significant and prolonged suppression of platelets with 159 x 10^3 cells/mm^3 on day 10 and the suppression did not recover until day 23 (Fig. 7). The leukopenia and thrombocytopenia caused by KW2152 were not significant as compared with those caused by MMC. KW2152 caused no abnormal values in erythrocyte counts after daily administration for 7 days (data not shown).

The main toxic signs observed in a single LD_{10} dose were failure to thrive, diarrhea, and decrease of thymus weight. These main toxic signs of KW2152 were not clearly observed by a daily administration for 7 days except the decrease in thymus weight (data not shown).

**DISCUSSION**

KW2152 showed marked activity against P388 leukemia but marginal activity against L1210 leukemia, B16 melanoma, and M5076 sarcoma in the i.p.-i.p. system. These results suggested that antitumor spectrum of KW2152 against murine tumor system tested was not remarkable among established antitumor agents. The growth inhibiting activity against cultured cells showed that P388 leukemia cells were sensitive to KW2152 with an IC_{50} of 5.3 x 10^{-4} M by 1 h exposure, but L1210 leukemia and B16 melanoma cells required 30- to 40-fold high concentration to obtain 50% inhibition of cell growth. Growth inhibitory activity of KW2152 against cultured cells correlated with the in vivo antitumor activity in the i.p.-i.p. system with the same murine tumor cells.

In spite of its narrow spectrum against murine tumor models, KW2152 showed marked activity against s.c. human tumors transplanted to nude mice. At optimal doses, KW2152 suppressed the growth of MX-1 human mammary carcinoma with all mice cured by daily administration for 7 days. Both i.v. and p.o. administration were markedly effective against MX-1; however, a 5- to 6-fold dose was required by p.o. administration to obtain the same extent of tumor growth inhibition produced by i.v. administration. It was active against Co-3 human colon carcinoma and St-4 gastric carcinoma which had been reported to belong to the insensitive human tumors against established anticancer drugs on the schedule that s.c. transplanted tumors were treated when tumor volume was 100-300 mm^3 (15-17). From our studies, MMC, ADM, cis-DDP, and BLM gave a moderate effect with T/C percentage of 30-47 against Co-3. Against St-4, KW2152 was active, with a T/C percentage of 27 comparable to that of MMC, and more effective than cis-DDP. KW2152 produced moderate effectiveness against St-15 human stomach carcinoma which was significantly sensitive to MMC and cis-DDP. The predictive value of s.c. human tumor xenografts for clinical efficacy is not well established, but the characteristics of the antitumor spectrum of KW2152 which is rather effective to human tumor xenografts as compared with murine tumors, are interesting in the further evaluation of this compound. Because KW2152 showed a significant effect on P388/ADM leukemia in vitro and in vivo, the pleiotropically resistant line of P388 leukemia (18) was suggested not to be cross-resistant to KW2152. KW2152 was 45 times less effective to P388/MMC than to P388; however, it gave a significant prolongation of life span of mice bearing P388/MMC compatible to that of mice bearing P388. The reason of the discrepancy between in vivo and in vitro was not certain, but the distribution of i.p. P388/MMC cells in mice might be different from that of P388 cells.

The schedule dependency of KW2152 suggested that it might act like an antimetabolite; however, the competition of various nucleosides to the growth inhibitory activity of KW2152 on HeLa S_2 cells were not observed in our preliminary study. From these results, antimetabolite activity of KW2152 could not be proved.

**Synthesis of RNA is sensitive to KW2152 in P388 leukemia cells.** DNA synthesis was shown to be almost 30-fold less sensitive to KW2152 than RNA synthesis from dose-response experiments. In B. subtilis, DNA synthesis was significantly inhibited at concentrations which did not inhibit RNA or protein synthesis, suggesting primary inhibition of DNA synthesis by quinocarcin in bacteria (19). Although at present there is no way to explain this different effect, some drugs such as ADM affect DNA and RNA synthesis to a different degree dependent on the species and system tested (20-22).

Saframycin was reported to inhibit RNA synthesis more significantly than DNA synthesis in L1210, and vice versa in B. subtilis (23). These results might suggest that the isoquinoline moiety of KW2152 and saframycin was important for inhibition of RNA synthesis. From the preliminary flow cytometry analysis of DNA distribution of HeLa S_2 cells treated with KW2152, delay of S phase progression was observed. Preliminary toxicology studies indicate that bone marrow toxicity is not significant for this compounds. Gastrointestinal toxicity and bone marrow toxicity are more significant after a single injection than after daily administration for 7 days. We are currently investigating the biochemical mechanism of the antitumor effect of KW2152 as well as its tissue distribution pattern and its toxicity profile. Such information will be important in designing clinical protocols with this compound, which is being developed as a Phase I agent.

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Antitumor Activity of a Novel Antitumor Antibiotic, Quinocarmycin Citrate (KW2152)

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