Enhancement of Antineoplastic Effects of Cisplatin by Calmodulin Antagonists in Nude Mice Bearing Human Ovarian Carcinoma

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ABSTRACT

The present study was designed to potentiate the antineoplastic effects of cisplatin by combination with calmodulin antagonists [N-(6-amino-5-chloro-1-naphthalenesulfonamido (W-7) and N-(6-amino-1-naphthalenesulfonamido (W-5)] in nude mice bearing human ovarian carcinoma. Tumor growth of nude mice treated with W-7 or W-5 combined with cisplatin was significantly inhibited, compared to that of nude mice treated with W-7 alone, W-5 alone, or cisplatin alone. Although treatment with cisplatin alone markedly inhibited lytic activity of spleen cells from tumor-bearing nude mice against the tumor cells, the inhibitory effect was eliminated by combination with W-7 or W-5. There was no significant difference in survival time among untreated, cisplatin-treated, W-7-treated, and W-5-treated groups. Only when cisplatin was followed by W-7 or W-5 was a significant enhancement by W-7 or W-5 of the antitumor effect of cisplatin observed with respect to inhibition of tumor growth as well as prolongation of survival time.

INTRODUCTION

Since the discovery of the antineoplastic effects of cisplatin by Rosenberg et al. (1), cisplatin-based combination chemotherapy has been undertaken for ovarian cancer patients and improvement in the survival and response rates has been reported (2–4). However, the relatively marked side effects of cisplatin are one of dose-limiting factors. We have reported previously that combinations of calmodulin antagonists and anticancer drugs resulted in adjuvant effects with regard to the inhibition of tumor cell proliferation in vitro (5). In addition, we have demonstrated that in the in vitro study timing of administration of calmodulin antagonists is important to obtain optimum adjuvant effects to anticancer drugs (6). On the basis of these previous observations, we attempted to determine timing of administration of calmodulin antagonists (W-7 and W-5) to enhance antitumor effects of cisplatin, using nude mice bearing human ovarian carcinoma.

MATERIALS AND METHODS

Agent. W-7 and W-5 were obtained from Rikaken Co., Ltd., Nagoya, Japan.

Cells. SN cells derived from a patient with clear cell carcinoma of the ovary were used exclusively in this study. The passage number is about 105. The tumorigenicity of the SN cells in the nude mice was 100%. Doubling time and plating efficiency were 26 h and 30%, respectively. The cells were cultured as described previously (7). Briefly, cells were incubated in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 µg/ml; Grand Island Biological Co.) in a 5% CO2 atmosphere at 37°C. The medium was changed every 3 days, and the cells were passed when confluency was achieved.

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1 Supported in part by a grant from the Special Scientific Research Program of the Defense Agency in Japan.

2 To whom requests for reprints should be addressed.

3 The abbreviations used are: W-7, N-(6-amino-5-chloro-1-naphthalenesulfonamido; W-5, N-(6-amino-1-naphthalenesulfonamido.

RESULTS

Adjuvant Effects of Calmodulin Antagonists to Cisplatin on Tumor Growth. When 5 × 104 SN cells were inoculated s.c. to the right flank of nude mice, all mice developed palpable tumor on Day 21. Tumor volumes in a cisplatin-treated group were significantly smaller than that in an untreated group but not in the cisplatin-treated group. When treatment with W-7 or W-5 alone did not result in inhibitory effects on tumor volumes. When treatment with W-7 or W-5 was followed by cisplatin, tumor growth was significantly inhibited 42 and 49 days after tumor inoculation, compared to that in untreated group but not in the cisplatin-treated group. If cisplatin was administered before injection of W-7 or W-5, the tumor volumes were significantly smaller on both Days 42 and 49 than those not only in the untreated group but also in the cisplatin-treated group, suggesting the importance of timing of W-7 or W-5 treatment to

Mice. Approximately 8-week-old female BALB/c nude mice were purchased from Japan Clea Laboratories, Tokyo, Japan, and maintained in a pathogen-free environment.

In Vivo Treatment. SN (5 × 104) cells were inoculated s.c. to right flank of nude mice. After 7 days of tumor inoculation, 2 mg/kg cisplatin and 15 mg/kg W-7 or W-5 were administered i.p. every week for 5 weeks. The mice were inspected daily, and tumor growth was determined by the measurement of diameters in two dimensions of the tumor nodule with a caliper once a week. Tumor volume (cm3) was calculated according to the formula

\[ V = \frac{4}{3} \pi (r_1 + r_2)^2 \frac{r_1}{8} \]

where \( r_1 \) is the longitudinal radius and \( r_2 \) is the transverse radius. The values were presented as mean ± SD. Blood from tail vein was collected to hematocrit tubes every week and hematocrit values and body weight were measured for monitoring of side effects of drugs.

Measurement of Lytic Activity. Spleen cells of nude mice bearing SN cells or intact nude mice were used as effector cells. The spleen cells were prepared as described previously (8). SN cells were used as target cells. Aliquots containing 104 target cells were labeled with 100 µCi of sodium [51Cr]chromate solution (New England Nuclear, Boston, MA) for 1 h in 1 ml of medium. After three washings, 104 cells in 0.1 ml of medium were pipetted into microtiter plates (Linbro Scientific, Inc., Hamden, CT). Various concentrations of effector cells in 0.1 ml of medium were added in triplicate to give effector:target cell ratios of 100:1 and 50:1, respectively. After incubation for 18 h at 37°C in a humidified atmosphere of 5% CO2 in air, supernatants were collected with a Titertek collection system (Flow Laboratories, Inc., Rockville, MD) and counted in a gamma counter. The specific 51Cr release was calculated as

\[ \frac{\text{cpm test release} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100 \]

Spontaneous and maximum releases are cpm releases from target cells incubated in medium and in medium to which 1 N HCl was added, respectively.

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ENHANCEMENT OF ANTITUMOR EFFECTS BY W-7 AND W-5

Table 1  Adjuvant effects of W-7 or W-5 to cisplatin on tumor growth on SN cells grown in nude mice

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Untreated group</th>
<th>Cisplatin-treated group</th>
<th>W-7→cisplatin-treated group</th>
<th>Cisplatin→W-7-treated group</th>
<th>W-5→cisplatin-treated group</th>
<th>Cisplatin→W-5-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>Palpable</td>
<td>Palpable</td>
<td>Palpable</td>
<td>Palpable</td>
<td>Palpable</td>
<td>Palpable</td>
</tr>
<tr>
<td>21</td>
<td>0.48 ± 0.52a</td>
<td>0.13 ± 0.15</td>
<td>0.38 ± 0.41</td>
<td>0.15 ± 0.11</td>
<td>0.04 ± 0.05</td>
<td>0.35 ± 0.32</td>
</tr>
<tr>
<td>28</td>
<td>1.36 ± 1.22</td>
<td>0.52 ± 0.35</td>
<td>1.06 ± 0.98</td>
<td>0.55 ± 0.37</td>
<td>0.34 ± 0.34</td>
<td>0.84 ± 0.91</td>
</tr>
<tr>
<td>35</td>
<td>3.21 ± 3.04</td>
<td>1.03 ± 0.88</td>
<td>2.52 ± 3.01</td>
<td>1.05 ± 0.79</td>
<td>0.28 ± 0.36</td>
<td>2.02 ± 1.99</td>
</tr>
<tr>
<td>42</td>
<td>6.56 ± 4.51</td>
<td>1.99 ± 0.86b</td>
<td>5.14 ± 4.02</td>
<td>1.33 ± 0.87c</td>
<td>0.27 ± 0.18d</td>
<td>4.56 ± 3.13</td>
</tr>
<tr>
<td>49</td>
<td>7.07 ± 5.73</td>
<td>3.85 ± 2.79c</td>
<td>6.24 ± 4.97</td>
<td>2.68 ± 1.26d</td>
<td>0.58 ± 0.37e</td>
<td>6.88 ± 4.72</td>
</tr>
</tbody>
</table>

* Mean ± SD.

Table 2  Effects of cisplatin and calmodulin antagonists (W-7 or W-5) on lytic activity to SN target cells of spleen cells in nude mice with SN cells

<table>
<thead>
<tr>
<th>Spleen cells</th>
<th>% of cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palpable</td>
<td>50:1 (%)</td>
</tr>
<tr>
<td>Before inoculation</td>
<td>−3.7 ± 0.4</td>
</tr>
<tr>
<td>3 wk after inoculation, untreated group</td>
<td>10.9 ± 0.3</td>
</tr>
<tr>
<td>W-7 alone-treated group</td>
<td>12.1 ± 0.6</td>
</tr>
<tr>
<td>W-5 alone-treated group</td>
<td>11.2 ± 0.7</td>
</tr>
<tr>
<td>Cisplatin-treated group</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Cisplatin→W-7-treated group</td>
<td>16.2 ± 2.2</td>
</tr>
<tr>
<td>Cisplatin→W-5-treated group</td>
<td>18.1 ± 2.6</td>
</tr>
<tr>
<td>W-7→cisplatin-treated group</td>
<td>12.9 ± 1.0</td>
</tr>
<tr>
<td>W-5→cisplatin-treated group</td>
<td>13.2 ± 2.1</td>
</tr>
<tr>
<td>5 wk after inoculation, untreated group</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td>W-7 alone-treated group</td>
<td>6.1 ± 2.3</td>
</tr>
<tr>
<td>W-5 alone-treated group</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>Cisplatin-treated group</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Cisplatin→W-7-treated group</td>
<td>15.4 ± 1.3</td>
</tr>
<tr>
<td>Cisplatin→W-5-treated group</td>
<td>9.7 ± 2.6</td>
</tr>
<tr>
<td>W-7→cisplatin-treated group</td>
<td>6.8 ± 0.4</td>
</tr>
<tr>
<td>W-5→cisplatin-treated group</td>
<td>4.9 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± SD.

DISCUSSION

In the present study, we have demonstrated that a combination of cisplatin and calmodulin antagonists (W-7 and W-5) resulted in enhancement of its antitumor effects only when treatment with cisplatin was followed by treatments with calmodulin antagonists. We have already observed that in order to result in adjuvant effects of calmodulin antagonists to 5-fluorouracil on the inhibition of tumor cell proliferation in vitro, calmodulin antagonists should be administered after (not before) treatment with anticancer drugs (6). Calmodulin inhibitors have been reported to stabilize tumor cell membranes (9).
Recently, it has been reported that calmodulin antagonists caused a marked inhibition in recovery from bleomycin-induced potentially lethal damage of cells by preventing the repair of damaged DNA (10). Similarly, it is possible that W-7 disturbs the repair process of DNA damaged by cisplatin. Treatment with cisplatin followed by W-7 or W-5 brought about a significant prolongation in survival time. The concentrations of W-7 or W-5 and cisplatin used in the present study did not result in any adverse side effects as confirmed by measurement of body weight and hematocrit values (data not shown). Recently, we reported that preincubation of target cells with calmodulin antagonists (W-7 or W-5) resulted in restoration of decreased functional T-cells induced further under the microenvironment of the thymus and had as much adjuvant and restorative effects to cisplatin on tumor growth and the restorative effects of cisplatin-induced depressed immune function, W-5 had as much adjuvant and restorative effects as W-7. Recently, Onoda et al. (18) described the synergistic cytotoxicity of cisplatin combined with the calcium channel blocker, nifedipine. Therefore, it is possible that naphthalenesulfonamide potentiation of cisplatin activity is related more to calcium than to calmodulin. These observations provide a new strategy for enhancement of sensitivity of cancer cells to anticancer drugs and circumvention to their resistance.

REFERENCES


Table 3 Effects of W-7 or W-5 on lytic activity of spleen cells in intact nude mice to SN cells

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Untreated target cells</th>
<th>W-7-treated target cells</th>
<th>W-5-treated target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>-10 ± 1.2f</td>
<td>5.4 ± 1.0f</td>
<td>4.9 ± 1.2f</td>
</tr>
<tr>
<td>50:1*</td>
<td>-1.0 ± 2.1</td>
<td>7.8 ± 1.1</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>100:1</td>
<td>1.7 ± 1.3</td>
<td>3.6 ± 1.8</td>
<td>5.8 ± 2.6f</td>
</tr>
<tr>
<td>W-7-treated</td>
<td>50:1</td>
<td>2.1 ± 3.1</td>
<td>1.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>100:1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 continued

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Untreated target cells</th>
<th>W-7-treated target cells</th>
<th>W-5-treated target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>50:1*</td>
<td>1.7 ± 1.3</td>
<td>3.1 ± 1.0</td>
<td>5.2 ± 1.5</td>
</tr>
<tr>
<td>100:1</td>
<td>2.1 ± 3.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Effectortarget cell ratio.
# Mean ± SD.
% P < 0.01, compared to untreated target cells.
& P < 0.01, compared to untreated effector cells.
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