Verapamil Enhancement of Antitumor Effect of \textit{cis}-Diamminedichloroplatinum(II) in Nude Mouse-grown Human Neuroblastoma\textsuperscript{1}

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ABSTRACT

The antitumor effect of combined use of \textit{cis}-diamminedichloroplatinum(II) (CDDP) and verapamil, a calcium influx blocker, was examined in neuroblastoma transplanted to BALB/c athymic mice. The response of the tumor to CDDP was related to the dose administered. Regression of the tumor was observed when CDDP was administered at 4.2 mg/kg/injection. The retardation of tumor growth was observed in the group to which CDDP was administered at 2.1 mg/kg/injection. When verapamil was administered with CDDP, regression of the tumor was observed in the group treated with CDDP at 2.1 mg/kg/injection, and the retardation of tumor growth was observed in the group treated with CDDP at 1.4 mg/kg/injection. These results indicate that verapamil enhances the antitumor effect of CDDP against transplanted neuroblastoma in BALB/c athymic mice.

INTRODUCTION

Neuroblastoma is the most common solid tumor of infancy and childhood. The incidence of this malignant disease is thought to be approximately 1 per 20,000 infants in Japan. Despite recent multidisciplinary treatment including surgery, radiotherapy, and chemotherapy, the survival rate for advanced neuroblastoma has remained unchanged.

In a number of recent publications, various therapeutic modalities used in treating advanced neuroblastoma have been discussed (1, 2). The results of these studies show that some chemotherapeutic agents including CDDP\textsuperscript{3} have beneficial effects and that these agents used in various combinations lead to a complete tumor response of several months duration. From these clinical results it is thought that neuroblastoma cells are sensitive to a chemotherapeutic agent initially but become progressively resistant after some periods.

Calcium influx blockers including verapamil were reported to enhance the cytotoxicity of vincristine or Adriamycin in tumor cells (3-13), and the resistance of tumor cells to these chemotherapeutic agents was circumvented through this enhancement of cytotoxicity. We thought that the cytotoxicity of CDDP would also be enhanced by verapamil and that the combined use of these drugs would lead to a better outcome in treating advanced neuroblastoma. We explored the antitumor effect of combined use of CDDP and verapamil in human neuroblastoma transplanted to BALB/c athymic mice.

MATERIALS AND METHODS

Nude mouse-grown poorly differentiated ganglioneuroblastoma, a primary explant derived from an 11-month-old female was used. Xeno-grafts of this tumor transplanted in nude mice exhibited significant sensitivity to CDDP, cyclophosphamide, and vincristine and were resistant to anthracyclines. A small piece of tumor measuring approximately 3 mm\textsuperscript{3} was inoculated s.c. into the anterior aspect of the lateral thoracic wall of 5- to 7-wk-old male BALB/c nu/nu athymic mice. Treatment was initiated when tumors had reached a measuring size of 100 to 300 mm\textsuperscript{3}. Tumor-bearing mice were randomized to groups of 3 to 4 animals each. Experimental groups include (a) control, (b) groups treated with verapamil, (c) groups treated with CDDP, and (d) groups treated with CDDP and verapamil. CDDP was administered i.p. on Days 0, 4, and 8. Verapamil was injected s.c. on Days 0 and 4. All animals were weighed, and tumor volumes were measured every second day with calipers using this formula.

\[
\text{Length} \times \text{width}^2 \times 0.5
\]

The effects of treatment were evaluated on Day 12, and the animals were autopsied. Results of the experiments were evaluated according to a protocol of Battelle Columbus Laboratories (14). Treatment-related histopathological changes in the autopsied specimen were evaluated by comparing random sections of each group. Statistical analysis was done using Student's t test.

RESULTS

The tumor showed marked sensitivity to CDDP treatment. Table 1 summarizes the evaluation of tumor response to various treatments. Regression of the tumor was observed when CDDP was administered 3 times every 4 days at 4.2 mg/kg/injection. The retardation of tumor growth was observed in the group to which CDDP was administered at 2.1 mg/kg/injection. Although there was a statistically significant difference in the relative tumor weight between the control group and the CDDP group at 1.4 mg/kg, no effect of treatment expressed as retardation of tumor growth was observed in the CDDP group at 1.4 mg/kg (Fig. 1).

When verapamil was administered at 12.5 mg/kg/injection, regression of the tumor was observed not only in the group treated with CDDP at 4.2 mg/kg/injection but also in the group treated with CDDP at 2.1 mg/kg/injection. CDDP treatment administered at 2.1 mg/kg/injection with verapamil at 12.5 mg/kg/injection resulted in tumor response comparable to that observed in the group treated with CDDP at 4.2 mg/kg/injection without verapamil (Fig. 2).

Verapamil administered at 25 mg/kg/injection with CDDP has a more dramatic effect on tumor response. Tumor regression was observed in the group to which CDDP was administered at 2.1 mg/kg/injection. Although CDDP administered at 1.4 mg/kg/injection with verapamil at 12.5 mg/kg/injection had no effect on tumor growth, there was the retardation of tumor growth in the group to which CDDP was administered at 1.4 mg/kg/injection with verapamil at 25 mg/kg/injection. Combined use of CDDP (4.2 mg/kg) and verapamil (25 mg/kg) was toxic as all mice in this group were dead 4 days after the initiation of treatment (Fig. 3).

During the experimental period marked weight loss was observed in the group to which CDDP (4.2 mg/kg) and vera...
Table 1 Effect of verapamil on antitumor activity of CDDP

| Drug and dosage | RW*  | \(T_{\text{ew}}/C_{\text{ew}}\) (%)
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Control</td>
<td>4.83 ± 0.50*</td>
<td>100</td>
</tr>
<tr>
<td>CDDP (4.2 mg/kg)</td>
<td>0.08 ± 0.04*</td>
<td>1.7*</td>
</tr>
<tr>
<td>CDDP (2.1 mg/kg)</td>
<td>1.79 ± 0.21*</td>
<td>37.1*</td>
</tr>
<tr>
<td>CDDP (1.4 mg/kg)</td>
<td>3.16 ± 0.52*</td>
<td>65.4</td>
</tr>
<tr>
<td>Control (12.5 mg/kg)</td>
<td>5.71 ± 1.24</td>
<td>100</td>
</tr>
<tr>
<td>Verapamil (12.5 mg/kg) + verapamil (12.5 mg/kg)</td>
<td>6.91 ± 0.73</td>
<td>121.0</td>
</tr>
<tr>
<td>CDDP (4.2 mg/kg) + verapamil (12.5 mg/kg)</td>
<td>0.07 ± 0.04*</td>
<td>1.2*</td>
</tr>
<tr>
<td>CDDP (2.1 mg/kg) + verapamil (12.5 mg/kg)</td>
<td>0.56 ± 0.32*</td>
<td>9.8*</td>
</tr>
<tr>
<td>CDDP (1.4 mg/kg) + verapamil (12.5 mg/kg)</td>
<td>3.51 ± 0.41*</td>
<td>61.5</td>
</tr>
<tr>
<td>Control (25 mg/kg)</td>
<td>4.25 ± 1.28</td>
<td>100</td>
</tr>
<tr>
<td>Verapamil (25 mg/kg) + verapamil (25 mg/kg)</td>
<td>4.53 ± 1.32</td>
<td>106.6</td>
</tr>
<tr>
<td>CDDP (4.2 mg/kg) + verapamil (25 mg/kg)</td>
<td>0.11 ± 0.07*</td>
<td>2.6*</td>
</tr>
<tr>
<td>CDDP (2.1 mg/kg) + verapamil (25 mg/kg)</td>
<td>1.21 ± 0.27*</td>
<td>28.5*</td>
</tr>
<tr>
<td>CDDP (1.4 mg/kg) + verapamil (25 mg/kg)</td>
<td>1.21 ± 0.27*</td>
<td>28.5*</td>
</tr>
</tbody>
</table>

* RW, relative tumor weight; \(T_{\text{ew}}/C_{\text{ew}}\), comparison of relative tumor weight between treated and control groups.

\(\beta\) Mean ± SD.

\(\gamma\) Statistically significant \((P < 0.05)\) by Student's t test as compared with that of the control group.

\(\delta\) Regression of tumor was observed.

\(\epsilon\) Retardation of tumor growth was observed.

**VERAPAMIL ENHANCEMENT OF ANTITUMOR EFFECT OF CDDP**

**DISCUSSION**

It was reported that verapamil, a calcium influx blocker, enhanced the cytotoxicity of vincristine and that the resistance to vincristine was overcome in vitro and in vivo (3–6, 11). It was also reported that the cytotoxicity of Adriamycin was enhanced and that the resistance to Adriamycin was overcome by verapamil (5–13). Other calcium influx blockers, diltiazem, nicardipine, nifedipine, niludipine, and nimodipine, were also reported to enhance the cytotoxicity of chemotherapeutic
VERAPAMIL ENHANCEMENT OF ANTITUMOR EFFECT OF CDDP

Fig. 4. Changes in animal body weight during the experimental period. Each point represents the mean of animal body weights. A: O, control; O, CDDP (4.2 mg/kg); V, CDDP (2.1 mg/kg); C, CDDP (1.4 mg/kg). B: O, control; •, verapamil (12.5 mg/kg); •, CDDP (4.2 mg/kg) + verapamil (12.5 mg/kg); W, CDDP (2.1 mg/kg) + verapamil (12.5 mg/kg). C: O, control; •, verapamil (25 mg/kg); •, CDDP (4.2 mg/kg) + verapamil (25 mg/kg); W, CDDP (2.1 mg/kg) + verapamil (25 mg/kg); A, CDDP (1.4 mg/kg) + verapamil (25 mg/kg).

Fig. 5. No tumor cells remained or nonviable cells remained in tumors of the group to which CDDP (2.1 mg/kg) and verapamil (25 mg/kg) were administered. H & E, x 220.

agents (15–17). The precise mechanism by which verapamil enhances chemotherapeutic efficacy in drug-sensitive and drug-resistant tumor cells was investigated. Tsuruo et al. speculated that calcium influx blockers including verapamil increased the accumulation of chemotherapeutic agents in tumor cells by inhibiting the efflux from tumor cells and led to enhanced cytotoxicity of the drugs (3–6, 15). Although it is possible that the calcium environment plays a role in the active efflux of chemotherapeutic agents (18), it is reported that no relationship between calcium fluxes and anthracycline transport was found (7, 19). On the basis of these results, the advantages of the clinical use of calcium influx blockers in cancer chemotherapy were proposed. These include enhancement of the cytotoxicity of chemotherapeutic agents, circumvention of drug resistance, improvement in clinical results at a given drug concentration, and reduction in the amount of drug administered and subsequent risk of toxicity.

The combined use of chemotherapeutic agents and calcium influx blockers has not yet been investigated in childhood solid tumors, especially neuroblastoma. Our coworker investigated the enhancement of the antitumor effect of vincristine by verapamil in neuroblastoma in vivo (20). The combined use of these agents had a significant antitumor effect against transplanted neuroblastoma in the athymic mice. However, its effect was limited to the retardation of tumor growth. In the present study, we found that verapamil also enhanced the antitumor effect of CDDP in neuroblastoma transplanted to BALB/c athymic mice. However, the combined use of CDDP (4.2 mg/kg) and verapamil (25 mg/kg) was toxic, and all mice in this group died. The effect of verapamil on the systemic circulation is a possible explanation for this toxicity. But it is possible that verapamil also enhances the toxic effect of CDDP to nontumorous cells. If this undesirable effect of the combined use of CDDP and verapamil is overcome, the clinical use of verapamil will be a beneficial method in treating advanced neuroblastoma.

ACKNOWLEDGMENTS

CDDP was obtained from Nippon Kayaku Co., Ltd., Tokyo, Japan, and verapamil was obtained from Eisai Co., Ltd., Tokyo, Japan.

REFERENCES

VERAPAMIL ENHANCEMENT OF ANTITUMOR EFFECT OF CDDP


Erratum

The article "Verapamil Enhancement of Antitumor Effect of cis-Diamminedichloroplatinum(II) in Nude Mouse-grown Human Neuroblastoma" by Hitoshi Ikeda et al., which appeared on pp. 231–234 of the January 1, 1987, issue, contains errors in Figures 1–4. The location of the arrows which indicate the administration of CDDP and verapamil is incorrect. Revised figures showing the correct placement of the arrows follow.
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