IMMUNOTOXIC EFFECT OF LOW DOSE CISPLATIN IN MICE

Yasuhide KOUCHI, Yasuhiro MAEDA, Akinobu OHUCHIDA and Motoyasu OHSAWA

Drug Safety Research Laboratory, Taiho Pharmaceutical Co., Ltd., 224-2, Ebisuno, Hiraishi, Kawauchi-cho, Tokushima 771-01, Japan

*Department of Environmental Toxicology, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01, Japan

(Received April 22, 1996 ; Accepted August 21, 1996)

ABSTRACT — The immunosuppressive effects of cisplatin at relatively low doses were investigated in CD-1 mice. Mice were injected intraperitoneally with 8, 40 and 200 μg/kg cisplatin for 10 days. A decrease in body and thymus weights was observed at 200 μg/kg. Though there were no dose-related effects on the IgM antibody response to sheep erythrocytes, a statistically significant reduction of the contact hypersensitivity response (CHR) was seen at 200 μg/kg. In vivo and in vitro effects of cisplatin on T- and B-lymphocyte function were assessed by proliferative response to concanavalin A and lipopolysaccharide, respectively. Cisplatin inhibited splenic T-lymphocyte function more than splenic B-lymphocyte function. These data indicate that a relatively low dose of cisplatin induce immunosuppressive effects in mice with a greater effect on T-lymphocytes than the B-lymphocytes.

KEY WORDS: Cisplatin, Immunotoxicity, Mice, Humoral immunity, Cell-mediated immunity

INTRODUCTION

Cisplatin, is one of the most widely used anticancer drugs, is effective against a wide variety of animal tumors and human cancers (Calabresi and Parks, 1985)

The drug exerts its antitumor effect is by through the binding of its highly reactive hydrated platinum complex to DNA, which inhibits nucleotide synthesis (Erickson et al., 1981; Roberts et al., 1988). Immunopotentiation with cisplatin has been reported. Murine macrophage monolayers treated with cisplatin showed significantly increased tumoricidal activity (Geetha and Sodhi, 1989). In addition, cisplatin enhanced natural killer cell function in rodents (Lichtenstein and Pende, 1986), and enhancement of host immunity may account for its antineoplastic effect.

Cisplatin also has a cytotoxic effect on immune cells when they are rapidly dividing. In cancer patients undergoing cisplatin treatment, a reduction in the number of T helper cells is seen with an increase in the number of T suppressor/cytotoxic (Onsrud et al., 1986). Suppression of the phytohemagglutinin (PHA) response has also been observed in humans (Kahn and Hill, 1971). Immunosuppression with this drug in humans seems to be transient.

A type of immunotoxic effect depends upon the anticancer agent used. For example, 5-FU suppresses a humoral immune response much stronger than cell-mediated immune response.
(Ohnuma et al., 1978). On the contrary, azathioprine appears to suppress T-cell mediated responses more than humoral responses in human and rodents (Spreafico and Aancelero, 1977). In rodents, cisplatin-induced immunosuppression was reported mostly at relatively high doses, and cisplatin suppressed both humoral and cell-mediated immune response (Khan and Hill, 1971; Thompson and Gale, 1971; Jilek et al., 1989).

Detecting the type of immune cells affected by cisplatin in the clinical dose range is important for understanding the drug's immunotoxic effects in human. Therefore, in this report, the immunotoxic effects of repeated injections of relatively low doses of cisplatin were investigated in mice.

MATERIALS AND METHODS

Animals

Seven-week old male CD-1 mice were purchased from Japan Charles River Inc. (Shiga, Japan), and acclimatized for 1 week. They were housed at 23±3°C at a relative humidity of 50±20% with a light cycle of 12hr (6:00-18:00). They were allowed food and water ad libitum.

Drugs

Cisplatin (Nippon Kayaku Co., Ltd.) was dissolved in saline, and cyclophosphamide (CPA, Shionogi seiyaku Co., Ltd.) was dissolved in distilled water. Solutions were sterilized by filtration before administration.

Administrations

Mice were randomized into five treatment groups of 15 mice each. Cisplatin was administered intraperitoneally at daily doses of 8, 40, or 200 μg/kg for 10 days. Saline was administered in the same manner to a vehicle control group. A positive control group received a single intraperitoneal injection of 200mg/kg CPA. A volume of 0.1ml per 10 g body weight was administered.

Body and organ weights

Mice treated with cisplatin or vehicle were weighed on day 0, 7, and 10 after the first administration. Spleen and thymus in all groups were weighed on day 10.

Spleen cell suspensions

Spleen single cell suspensions were prepared from spleens excised under sterile conditions. The cells were suspended in RPMI-1640 (Flow Lab., culture medium supplemented with 2mM L-glutamine, 500U penicillin, and 500 μg streptomycin per ml, and heat-inactivated 10% fetal bovine serum (GIBCO).

Plaque forming cell (PFC) response

The primary IgM responses to sheep red blood cells (SRBC) were quantitated as PFC responses by the method of Cunningham (1968). Five mice per treatment group were immunized with an i.v. injection of 5% SRBC suspension 3 days prior to the last drug treatment. One day after the last exposure to the test compound, the animals were sacrificed and the spleen cell suspensions were prepared and diluted 100-fold with culture medium. One-half milliliter of each diluted cell suspension, 0.05ml 50% SRBC suspension, and 0.05ml fresh guinea pig serum were mixed, and 0.1ml aliquots of the mixture were put into three cells of a Cunningham chamber. After incubation at 37°C for 1 hour, the number of PFC were counted. The spleen cells were counted and the number of PFC per 10⁶ spleen cells was calculated.

Mouse ear swelling assay

The contact hypersensitivity response (CHR) was assayed by quantitating the ear swelling response to a contact allergen using the method of Asherson and Ptak (1968). Briefly, on the last treatment day the mice were shaved on their dorsal surface and on the following day they were sensitized by a topical application of 25 μl 2% (w/v) 4-ethoxymethylene-2-phenyl oxazolone (OXAZ, Sigma) dissolved 4 : 1 in an acetone/olive oil mixture. Five days after sensitization, the mice were challenged with 10 μl 0.5% OXAZ by topical application to both the dorsal and ventral surfaces of the right ear. The acetone/olive oil vehicle was applied to the left ear as a vehicle control. CHR to OXAZ was quantitated as the difference in the thickness of the challenged ear before and after challenge. The thickness of the left ear was also determined.
before and after treatment by vehicle alone. Five mice per treatment group were used in this assay.

**Mitogen response assay**

In *in vivo* treatment of cisplatin, two days after the last treatment, spleen cell suspensions were prepared under sterile condition as described above and adjusted to a concentration of 2×10^6/ml culture medium. One hundred μl spleen cell suspension (2×10^5/0.1ml) was added to the wells of a flat-bottomed microtiter plate, and then the appropriate concentrations of mitogen (100 μl) was added. The plates were incubated at 37°C, 5% CO₂ in air in a humidified incubator for 3 days. Concanavalin A (Con A, Type IV, Sigma) and lipopolysaccharide (LPS, E. coli serotype 0128: B2, Sigma) were used as mitogens. Con A as a T cell mitogen was added at 2.0 and 4.0 μg/well, and LPS as a B cell mitogen was added at 20 and 40 μg/well.

The cell proliferation response was determined by an MTT colorimetric assay (Chung et al. 1990). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma) was dissolved in PBS at 5mg/ml and sterilized by filtration. After the cell suspension was incubated for 3 days, 20 μl MTT solution was added to each well of the culture plates, and the plates were reincubated at 37°C for 4 hours. The cultures were then centrifuged (2 min, 380g) and the supernatants were discarded by aspiration. Acidic isopropanol (150 μl 0.05N HCl in isopropanol) was then added to all wells and mixed in. Absorbance at 540nm of each well was read with a micro ELISA reader (Bio-Tek Instruments). Results were expressed as the mean of triplicate readings. Five mice per treatment group were used in this assay.

In *in vitro* treatment of cisplatin, the mitogen response assay was conducted as described above using spleen cells obtained from intact mice. Cisplatin was added to final concentrations of 10^-12~10^-5 M (3×10^-7~3×10^-9 μg/ml) to triplicate wells. For Con A, 4.0 μg/well was used, and for LPS, 40 μg/well was used.

**Statistical analysis**

Results were expressed as the mean±S.E. Statistical analysis was carried out by a standard two-way analysis of variance followed by Scheffe's F-test to establish significant differences. The default α-value of 0.05 was used as a cutoff for significance.

**RESULTS**

**Body and organ weights**

The only cisplatin-associated decrease in body weight gain was observed in the 200 μg/kg group on day 10, but it was not statistically significant (Fig. 1).

Organ weights are shown in Table 1. Though a statistically significant decrease in thymus weight was observed in the 200 μg/kg cisplatin group, there were no treatment-related effects on spleen weight. In the 200mg/kg CPA group, a statistically significant decrease in thymus weight and an increase in spleen weight were observed.

**Effect of cisplatin on IgM antibody production and spleen cell No.**

Results are shown in Table 2. The number of IgM antibody plaque forming cells in the control group was 1001 per 10^6 spleen cells. There were no significant changes in the number of IgM antibody plaque forming cells per 10^6 spleen cells and per spleen in the cisplatin-treated groups. A statistically significant reduction in
Table 1. Thymus and spleen weights in mice treated with cisplatin or cyclophosphamide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus (mg/10g B.W.)</th>
<th>Spleen (mg/10g B.W.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.82±2.9</td>
<td>43.4±7.2</td>
</tr>
<tr>
<td>40</td>
<td>8.76±1.3</td>
<td>45.4±8.0</td>
</tr>
<tr>
<td>200</td>
<td>4.20±1.4*</td>
<td>40.0±3.8</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>2.89±1.0*</td>
<td>69.6±13.1</td>
</tr>
<tr>
<td>None</td>
<td>8.04±2.2</td>
<td>41.5±4.9</td>
</tr>
</tbody>
</table>

*: Significantly different from control, P<0.05 (n=5)
Spleen and thymus were weighed one day after the last administration.
Mice were given a daily i.p. dose of cisplatin for 10 days or a single dose of cyclophosphamide as described in Table 1.

Table 2. Effects of cisplatin on IgM antibody response to sheep red blood cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PFC/10^6 spleen cells</th>
<th>PFC/spleen (×10^5)</th>
<th>Spleen cell no. (×10^8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>840±280</td>
<td>1114±442</td>
<td>1.32±0.27</td>
</tr>
<tr>
<td>40</td>
<td>1047±332</td>
<td>1738±725</td>
<td>1.66±0.43</td>
</tr>
<tr>
<td>200</td>
<td>900±496</td>
<td>1319±478</td>
<td>1.34±0.22</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>127±111*</td>
<td>218±32.8*</td>
<td>1.72±1.02</td>
</tr>
<tr>
<td>None</td>
<td>1001±324</td>
<td>1362±520</td>
<td>1.35±0.24</td>
</tr>
</tbody>
</table>

*: Significantly different from control, P<0.05 (n=5)
PFC: IgM antibody plaque forming cell
Mice were treated as in Table 1.

antibody production was observed in the CPA-treated positive control group. No significant change in spleen cell no. treated with cisplatin or CPA was observed.

**Effect of cisplatin on CHR**

Results are shown in Fig. 2. OXAZ-induced ear swelling was observed in the control groups 24 and 48 hours after the challenge. A reduction in ear swelling was observed in the cisplatin-treated groups, and a statistically significant reduction in CHR response was seen at 200 μg/kg.

**Effect of cisplatin on spleen cell proliferative response to Con A and LPS**

As shown in Fig. 3., though in vivo treatment of cisplatin caused no statistically significant changes in the cell proliferative response to LPS and Con A, the reduction in the cell proliferative

Vol. 21 No. 4
response to Con A was greater than that to LPS. As shown in Fig. 4, cisplatin markedly decreased the in vitro cell proliferative response to LPS and Con A dose-dependently at concentrations of $10^{-7}$–$10^{-3}$ M. Con A-induced cell proliferation was reduced by cisplatin even at $10^{-8}$ M.

Cell viability estimated by trypan blue dye exclusion was significantly decreased by cisplatin at concentrations of $10^{-7}$ M and higher (Data not shown).

DISCUSSION

The highest dose of cisplatin in this study corresponded to the clinical dose of 15–20 mg/m² for 5 consecutive days. In a 5-week subchronic toxicity study in rats (Tsubosaki et al., 1981), immunotoxic effects such as a decrease in the number of peripheral leukocytes and atrophy of thymus and spleen were observed at a daily dose of 216 µg/kg, but no obvious effects were seen in rats treated with 8 µg/kg. In this study, though a decrease in body and thymus weights was observed 200 µg/kg, there were no cisplatin-related effects were observed on spleen weight and spleen cell no. It was previously reported that thymic lymphocytes were more likely than lymphocytes of other lymphoid tissue to be vulnerable to the effects of cisplatin (Thompson and Gale, 1971).

In our study, there were no dose-related effects of cisplatin on the IgM antibody response to sheep erythrocytes. It has been shown that a single dose of 5–20 mg/kg cisplatin suppresses the antibody response in mice when given within 2 days before or after sensitization with SRBC (Berenbaum, 1971; Kahn and Hill, 1971; Okazaki et al., 1985). Dosage is also crucial in the immune response in mice, 12 mg/kg of cisplatin induced immunosuppression with leukopenia, but 2 mg/kg enhanced antibody production (Bagasro et al. 1985). We supposed that the doses of cisplatin used in the present experiments were not high enough to suppress antibody production. On the other hand, a significant decrease in thymus weight and antibody response were observed in the 200 mg/kg CPA group used as a positive control.
The effects of cisplatin on T- and B-lymphocyte function were assessed by polyclonal activation with the mitogens Con A and LPS, respectively. In vivo, cisplatin inhibited the T-lymphocyte response to Con A more than the B-lymphocyte response to LPS. In vitro, cisplatin significantly inhibited both T- and B-lymphocyte responses to mitogens, and the inhibition of the T-lymphocyte response was much stronger. Cisplatin at 10^{-8} M inhibited the T-lymphocyte response to Con A but induced no cytotoxic effect estimated by trypan blue dye exclusion (more than 90% were viable). At this concentration, cisplatin may inhibit the mitogenic response of T-lymphocytes not by cytotoxicity, but by impairing the cell function. Wierda and Pazdernik (1989) reported that the four platinum compounds, including cisplatin, were more toxic to T-lymphocyte function than to B-lymphocyte function in a mitogen assay. Cisplatin is also more inhibitory to the proliferation of human leukemic T cells than B cells (Ohnuma, 1978). As to T cell-mediated immunity, CHR was inhibited by cisplatin dose-dependently, and a statistical significant reduction in CHR was seen at the highest dose. Jilec et al. (1989) reported that 3.3 mg/kg cisplatin inhibited CHR only when it was applied on the day of sensitization. These data might indicate that proliferation of CD4 positive T cells in the draining lymph node after sensitization was inhibited by cisplatin.

In summary, this study has shown that cisplatin at relatively low doses suppressed the cell-mediated immune response, but not the humoral immune reponse in mice, and that T-lymphocytes were more sensitive than B-lymphocytes from the results of mitogen assay. This may be due to the different sensitivity of T- or B-lymphocytes to platinum compounds, and also to the doses used. During chemotherapy by cisplatin, both immunostimulatory and immunosuppressive effects may occur, and it depend on the immune status of the patients influenced by age, other therapeutic agents, nutrition, and also the frequency and dosage of cisplatin administration.

REFERENCES


agents on hemolytic plaque-forming cells. Syouwa Igakai Zassi, 45, 453-461.


