Enhancement of Naturally Occurring Human Spontaneous Monocyte-mediated Cytotoxicity by cis-Diamminedichloroplatinum(II)

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ABSTRACT

The effect of the antineoplastic agent cis-diamminedichloroplatinum(II) (cis-DDP) on spontaneous cytotoxicity of human mononuclear cells in vitro was assessed. cis-DDP enhanced spontaneous monocyte-mediated cytotoxicity 300% compared to control values. Further, this spontaneous monocyte killing developed earlier (3 to 4 days) than did that of untreated cells (5 to 7 days). Since monocyte cytotoxicity is under the control of lymphocyte suppressor cells, the effect of cis-DDP on separated populations of lymphocytes and monocytes was examined. Unlike X-irradiation which enhanced monocyte cytotoxicity through the indirect pathway of inactivation of lymphocyte suppressors, cis-DDP directly stimulated the monocyte population. Direct stimulation of killer monocytes may be an important mechanism of the antitumor effect of cis-DDP.

INTRODUCTION

cis-DDP² (cis-platin) is a potent chemotherapeutic agent for the treatment of a variety of human tumors, including testicular, ovarian, and head and neck carcinoma (7). The mechanism by which cis-DDP kills tumor cells in culture is thought to derive from direct damage to the DNA template (21). Although this mechanism may also be responsible for the antitumor effect of the drug in whole animals, other mechanisms are possible.

Since immune responses are likely to be important in the host’s defense against malignant disease, enhancement of these responses could also result in tumor regression. It has been suggested that natural cytotoxicity or natural killing may be an important mechanism of “nonimmune” defense against tumor invasion. This natural cytotoxicity is triggered prior to the more specific antibody-dependent responses which take time to generate. In vitro models which measure natural killing function correlate well with in vivo tumor resistance in mice (10). An analogous in vitro system has been developed in our laboratory. Using RBC targets, our model studies the generation of spontaneous monocyte-mediated cellular cytotoxicity in human cells. Under the conditions of this assay, monocytes become spontaneously cytotoxic after 6 to 7 days of in vitro culture. This phenomenon is independent of exogenous stimulus by antigen, antibody, or lectin and is due to the inactivation of potent lymphocyte suppressor cells (18, 19). With this assay, it becomes possible to study not only the regulation of monocyte-mediated cytotoxicity but also the effect of various drugs on monocyte killing.

cis-DDP, like other antineoplastic agents which damage DNA, has been considered immunosuppressive (1-3, 8, 12-17, 29). However, in several animal models, an immunostimulatory effect has also been described (26). Because we believed that the effect of cis-DDP on the immune response needed further investigation, the following studies were conducted to determine the effect that cis-DDP had on one aspect of human monocyte function, spontaneous cytotoxicity.

MATERIALS AND METHODS

Cytotoxicity Assay. Spontaneous monocyte-mediated cytotoxicity was assayed by methods described previously (18). Briefly, peripheral blood from normal human donors was fractionated on Ficoll-Hypaque gradients, and the MNL-rich fraction was washed twice and resuspended to contain 3.5 x 10⁶ MNL/ml in Medium NCTC 109 (Microbiological Associates, Bethesda, Md.) with added penicillin (50 units/ml), streptomycin (50 μg/ml), glucose (4 mm), and 2% fetal calf serum (Microbiological Associates). Aliquots were then incubated with c/s-DDP or a media control for 15 min at 37°. After incubation, the cells were washed once in balanced salt solution and resuspended in supplemented Medium NCTC 109 at 3.5 x 10⁶ MNL/ml. Two-ml aliquots were added to wells of 15-mm plastic Linbro cluster plates (Bellco Glass Co., Vineland, N. J.) and incubated at 37° in a humidified 5% CO₂ incubator for a specified time period, usually 5 to 7 days. At the end of the culture period, the cells were vigorously suspended with a Pasteur pipet, washed once, and adjusted to 3.0 x 10⁶ viable cells/ml in supplemented Roswell Park Memorial Institute Tissue Culture Medium 1640 (Microbiological Associates). The cultured cells were then assayed for cytotoxicity by quantitating their ability to lyse ⁵¹Cr-labeled chicken RBC targets as described previously (18). Cytotoxicity was expressed as percentage of total ⁵¹Cr released.

Separation of MNL into Monocyte and Lymphocyte Populations. Percoll (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) is a medium based on a colloidal suspension of silica particles coated with a polyvinylpyrrolidone in water. The Percoll particles are heterogenous in size and create a density gradient by sedimenting at different rates in a gravitational field (23). Upon centrifugation, cell populations layered on top of a preformed Percoll gradient will be separated on the basis of their relative densities into 2 distinct bands. The upper band is enriched for monocytes (75 to 90%), while the lower band is predominantly composed of lymphocytes (80 to 95%). MNL (70 x 10⁶) were layered onto preformed Percoll gradients in 15-ml polycarbonate tubes and spun in swing-out buckets in a refrigerated centrifuge at 10000 x g for 20 min, with the brake off. After separation, portions of each population were treated with 10μM cis-DDP or a media control for 15 min at 37°. The cells were washed once in balanced salt solution and then

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² The abbreviations used are: c/s-DDP, cis-diamminedichloroplatinum(II); MNL, mononuclear leukocyte(s).

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Effect of cis-DOP on spontaneous monocyte cytotoxicity

Drugs. cis-DDP (NSC 119875) was obtained from the Drug Synthesis and Chemistry Branch of the Division of Cancer Treatment, National Cancer Institute, dissolved in Roswell Park Memorial Institute Tissue Culture Medium 1640 with added glutamine, penicillin, and streptomycin, and used within 60 to 90 min.

Viability. Viability of all cultures was assessed in trypan blue. There was no difference in viability between the control and the treated populations.

RESULTS

Effect of cis-DDP on Spontaneous Monocyte Cytotoxicity. Treating normal MNL with 10 μM cis-DDP for 15 min enhanced spontaneous cytotoxicity (Table 1) (*p < 0.01). Chart 1 shows that significant enhancement was seen at concentrations as low as 10^{-9} M cis-DDP (*p < 0.05). With doses less than 10^{-9} M, cis-DDP did not significantly enhance spontaneous cytotoxicity.

Previous experiments have shown that normal MNL become spontaneously cytotoxic after 4 to 7 days in culture (18). Chart 2 shows that treating normal MNL with cis-DDP accelerated the generation of spontaneous cytotoxicity. Not only was cytotoxicity enhanced in the cis-DDP-treated MNL but their ability to kill began 24 to 48 hr earlier than that of the control cells.

To determine whether cis-DDP had any direct effect on the target cell or could immediately activate the effector cells, 10 μM cis-DDP was added directly to targets and cultured untreated effector cells for the duration of the 18-hr cytotoxicity assay. This had no effect on the expression of spontaneous cytotoxicity.

Selective Effect of cis-DDP on Spontaneous Cytotoxicity. To determine whether enhancement of spontaneous cytotoxicity by cis-DDP was due to a selective effect on one cell population, MNL were separated into monocyte and lymphocyte populations prior to treatment. Each population was treated separately with 10 μM cis-DDP or a media control, washed once, reconstituted, and assayed for cytotoxicity on Day 5. Table 2 shows that treating the lymphocyte populations with cis-DDP did not increase cytotoxicity. However, treating the monocytes with cis-DDP increased cytotoxicity.

Selective Effect of Irradiation on Spontaneous Cytotoxicity. Because intracellular DNA is thought to be the target of both cis-DDP (30) and X-irradiation and previous data suggested that X-irradiation enhanced monocyte killing by inactivating suppressor lymphocytes (20), we sought to determine what effect selective X-irradiation had on spontaneous cytotoxicity as a control for the monocyte activation induced by cis-DDP. Once again, MNL were separated into monocyte and lymphocyte populations on Percoll gradients. Portions of each population received 330 R X-irradiation. The cell populations were reconstituted and assayed for cytotoxicity on Day 5. Table 3 shows that irradiating the lymphocyte population alone increased cytotoxicity; however, irradiating the monocyte population produced no significant increase in cytotoxicity.

Table 1

<table>
<thead>
<tr>
<th>Donor</th>
<th>% of 51Cr released</th>
<th>% of enhancement by cis-DDP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>cis-DDP</td>
</tr>
<tr>
<td>L. Z.</td>
<td>19</td>
<td>73</td>
</tr>
<tr>
<td>L. Z.</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>D. P.</td>
<td>23</td>
<td>89</td>
</tr>
<tr>
<td>D. P.</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>D. P.</td>
<td>20</td>
<td>57</td>
</tr>
<tr>
<td>D. M.</td>
<td>12</td>
<td>27</td>
</tr>
<tr>
<td>D. M.</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td>S. M.</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>S. M.</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

* MNL from normal donors were incubated with 10 μM cis-DDP for 15 min at 37°, washed, and subsequently assayed for cytotoxicity on Day 5.
* Mean ± S.E.
cis-DDP-enhanced Monocyte Killing

Table 2
Selective effect of cis-DDP on human monocytes

<table>
<thead>
<tr>
<th>Cell combination</th>
<th>Cells treated with cis-DDP</th>
<th>% of 51Cr released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes + lymphocytes</td>
<td>None</td>
<td>6 ± 1b</td>
</tr>
<tr>
<td>Monocytes + lymphocytes</td>
<td>Lymphocytes</td>
<td>8 ± 0</td>
</tr>
<tr>
<td>Monocytes + lymphocytes</td>
<td>Monocytes</td>
<td>33 ± 4</td>
</tr>
</tbody>
</table>

Monocytes and lymphocytes were separated on Percoll gradients. Each cell population was treated with 10 μM cis-DDP or a media control for 15 min, washed, combined, and subsequently assayed for cytotoxicity on Day 5.

Table 3
Selective effect of irradiation on human monocytes

<table>
<thead>
<tr>
<th>Cell combination</th>
<th>Cells treated with 330 R X-irradiation</th>
<th>% of 51Cr released</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes + lymphocytes</td>
<td>None</td>
<td>8 ± 2b</td>
</tr>
<tr>
<td>Monocytes + lymphocytes</td>
<td>Lymphocytes</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>Monocytes + lymphocytes</td>
<td>Monocytes</td>
<td>10 ± 2</td>
</tr>
</tbody>
</table>

Monocytes and lymphocytes were separated on Percoll gradients. Portions of each cell population received 330 R irradiation. The cells were combined and assayed for cytotoxicity on Day 5.

Discussion

Other investigators have proposed that cis-DDP exerts its antineoplastic effect by damaging DNA and inhibiting cell division (30). However, the hypothesis of tumor regression by direct cytotoxicity does not explain the following experimental findings: (a) tumors which ultimately regress have been reported to continue growing 3 to 4 days after cis-DDP treatment (26); (b) animals cured of large tumors by cis-DDP retained prolonged immunity against reinoculation with the original tumor (25); (c) cis-DDP did not selectively accumulate in tumor tissue (11, 28); and (d) a large percentage of cis-DDP is excreted within 2 hr of administration, and thus the amount of cis-DDP retained in the body is low compared to that used in in vitro studies (11). Furthermore, within 3 hr of administration, about 90% of the cis-DDP administered is protein bound. Evidence suggests that only free cis-DDP is active, and therefore the amount of active drug in the circulation approaches 100 to 280 ng/ml 2 hr after an i.v. bolus (6, 22). This is lower than the 10 to 100 μM concentrations that were used in other in vitro studies. Thus, although inhibiting cell division may be an important mechanism of antitumor activity, it does not necessarily explain the selective tumoricidal action of cis-DDP in vivo.

Ineffective immune responses in tumor-bearing hosts are well described (9). Therefore, the effects of cancer chemotherapy on the immune system of the individual undergoing such treatment may be important. Macrophages are immune effector cells that can phagocytize and destroy antigenic material. Their accumulation at sites of inflammation and their ability to kill foreign antigens are important to host defense. Host suppressor cells have been hypothesized to interfere with antitumor immune responses, thereby allowing unimpeded tumor growth (4). Natural killer cells may be inhibited by such suppressors (27). Thus, tumor regression could be promoted by elimination of suppressor cells or by stimulation of killer cells by chemotherapeutic agents.

cis-DDP enhanced monocyte-mediated cytotoxicity (Table 1) and stimulated an earlier response (Chart 2). This could not be explained by the enhancement of target antigenicity, inasmuch as cis-DDP added directly to the RBC targets did not increase monocyte killing. Such enhanced killing could result from inactivation of the suppressor lymphocyte population, which is thought to control the killer monocyte (19), as was shown in the case of X-irradiation (Table 3). In the case of cis-DDP, however, a direct stimulatory effect on the monocyte population was found (Table 2). Enhanced spontaneous cytotoxicity could explain much of the puzzling data presented above. This enhanced cytotoxicity develops with time and thus could explain the initial growth of tumor following cis-DDP administration prior to eventual tumor regression. Because enhanced cytotoxicity by cis-DDP occurred at low concentrations (10^-5 M), this may be an important factor in the treated host even after extensive urinary excretion and protein binding has decreased the amount of the active platinum species. Furthermore, the enhancement of naturally occurring immune function requires no tumor uptake of drug to cause tumor regression.

The ability of cis-DDP to enhance immune function in the tumor-bearing host has been described. Six days after cis-DDP treatment, lymphocytes and macrophages have been observed in the tumor mass of mice with Sarcoma 180 but not in the tumors of untreated mice (28). Other histological studies have shown phagocytizing macrophages in all areas of treated tumor (24). Hydrocortisone (an immunosuppressive agent) decreased cis-DDP cures in tumor-bearing animals, whereas zymosan (an immunostimulant) increased the cure rate (5). Thus, cis-DDP has behaved as an immunostimulant in vivo, and this immunostimulation appears to result in an antitumor effect. The current work supports this concept, and we would therefore propose that direct activation of killer monocytes may be an additional mechanism of the antitumor activity of cis-DDP.

References


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