Carcinogenicity of Antitumor cis-Platinum(II) Coordination Complexes in the Mouse and Rat

Wilbur R. Leopold, Elizabeth C. Miller,2 and James A. Miller

ABSTRACT

Several cis-platinum coordination complexes were tested for their carcinogenic and mutagenic potencies in view of the known electrophilic activities of antitumor platinum complexes toward cellular nucleophiles and the mutagenicities of some of these complexes. cis-Dichlorodiammineplatinum(II) (total dose, 32.5 mg/kg) administered i.p. over 10 to 19 weeks increased the lung adenoma multiplicity in A/Jax mice from the control level of 0.5 to 0.8 adenoma/mouse to 10 to 16 adenomas/mouse at 8 months. Injection i.p. of cis-dichlorobis(cyclopentylamine)platinum(II) (total dose, 189 mg/kg) caused an average of 2.7 adenomas/mouse. Repeated i.p. doses of cis-dichlorodiammineplatinum(II) (total dose, 25.9 mg/kg) and concurrent and subsequent topical applications of croton oil induced skin papillomas in female CD-1 mice. At 41 weeks, 50% of the mice had papillomas with an average of 3.2 papillomas/mouse. Mice treated only with the platinum complex or with croton oil developed no papillomas. Sarcomas developed in 35 and 25%, respectively, of male Fischer rats that received multiple s.c. injections of cis-dichlorobis(cyclopentylamine)platinum(II) and cis-dichlorobis(pyrrrolidine)platinum(II) (total doses, 18 and 15 mg/rat, respectively).

cis-Dichlorodiammineplatinum(II), cis-dichlorobis(cyclopentylamine)platinum(II), and cis-dichlorobis(pyrrrolidine)platinum(II) were each directly mutagenic for Salmonella typhimurium TA100.

Treatment of patients with platinum antitumor complexes may impose a risk of induction of second tumors in long-term survivors.

INTRODUCTION

There is considerable interest in the use of complexes of platinum and related metals in the treatment of a variety of human neoplasms. The best studied of this class of antitumor agents is DDP, which was shown by Rosenberg et al. to be cytostatic for Escherichia coli (29) and to be active against Sarcoma 180 and L1210 leukemia in mice (30). The antineoplastic activity of DDP has been confirmed and extended (5, 6, 13, 16, 28, 33). Many platinum complexes have been tested for antitumor activity (5, 6, 19), and several are in clinical trials (10, 17, 31). DDP has shown significant antitumor activity both alone and in combination with other antineoplastic agents against several human cancers (31).

Strong mutagenic potential of DDP has been demonstrated for E. coli (2) and for Salmonella typhimurium TA100 (15, 22). Furthermore, the in vitro reactivity of DDP toward cellular nucleophiles and, especially, toward DNA is well documented (11, 18, 20, 23, 27). DDP also causes induction of prophages in bacteria (26), inhibition of DNA synthesis (8), and increases in the capacity of denatured DNA to renature (11). In these biological activities, DDP is similar to the bifunctional alkylating agents; the labile chlorine atoms of DDP make it structurally somewhat similar to the nitrogen and sulfur mustards.

It now appears that most chemical carcinogens are electrophilic per se or are metabolized to reactive electrophilic forms (21). In view of the mutagenic activity and electrophilic reactivity toward cellular nucleophiles of the platinum antitumor coordination complexes, the carcinogenic activities of some of these complexes were examined as a further test of the correlation between electrophilic reactivity and activity as an ultimate carcinogen. The structures of the platinum complexes studied are shown in Chart 1.

MATERIALS AND METHODS

UV spectra were taken in either water or DMSO solution on a Beckman DB spectrophotometer with a Sargent SRL recorder. IR spectra of compounds in KBr pellets were obtained on a Beckman IR-10 instrument.

DDP was purchased from Polysciences, Inc. (Warrington, Pa.) or ICN-K & K Laboratories, Inc., (Plainview, N. Y.) and purified by solution in dimethylformamide (Aldrich Chemical Co., Milwaukee, Wis.), filtration to remove insoluble impurities and reprecipitation by addition of 2 volumes of acetone and 1 volume of ethyl ether. Purity was assessed by IR and/or UV absorption spectroscopy and by thin-layer chromatography on cellulose plates (Brinkmann Instruments, Inc., Westbury, N. Y.) with either 1-butanol:acetic acid:water (12:3:5) (System A) or acetone:water (9:1) (System B). In all cases, the purified DDP gave a single spot with Rf's of 0.28 and 0.60 in Systems A and B, respectively, when sprayed with 5% SnCl5 in 0.1 N HCl (7). The IR spectrum (KBr) is: 3280, s, broad; 3220, sh; 2100, w; 1630, m; 1540, m; 1315, sh; 1300, s; 800, s; 310, s. The UV spectrum is: Amax: 360 nm (ε = 25), 299 nm (ε = 114), 277 nm (ε = 98).

DDC and DPP were synthesized from K2PtCl6, (ICN-K & K Laboratories, Inc.) by the method of Connors et al. (6). Stoichiometric amounts of K2PtCl6 and either cyclopentylamine or pyrrrolidine (Aldrich) in aqueous solution were

1 This work was supported by Grants CA-07175, CA-22484, and CA-09135 from the National Cancer Institute, USPHS.
2 To whom requests for reprints should be addressed.
3 The following abbreviations are used: DDP, cis-dichlorodiammineplatinum(II); DMSO, dimethyl sulfoxide; s, strong, sh, shoulder; w, weak; m, medium; DGP, cis-dichlorobis(cyclopentylamine)platinum(II); DPP, cis-dichlorobis(pyrrrolidine)platinum(II).
stirred at room temperature for 3 hr. The yellow precipitates were collected, washed successively with water, methanol, acetone, and ether, and dried under reduced pressure. Structural identities and purities were determined by IR and visible spectroscopy, thin-layer chromatography, and elemental analysis (Huffman Laboratories, Inc., Wheatridge, Colo.).

Chart 1. cis-Square planar complexes of platinum(II) used in this study.

<table>
<thead>
<tr>
<th>COMPLEX</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDP</td>
<td>NH₃</td>
</tr>
<tr>
<td>DCP</td>
<td>NH₂</td>
</tr>
<tr>
<td>DPP</td>
<td>H₂N</td>
</tr>
<tr>
<td>DMP</td>
<td>(CH₃)₂</td>
</tr>
</tbody>
</table>

Lung adenomas were induced in 8-week-old female A/Jax mice (The Jackson Laboratory, Bar Harbor, Maine) by weekly i.p. injections of either DDP or DCP in either 0.85% NaCl solution (6.5 ml/kg) or trioctanoin suspension (5.0 ml/kg). These experiments were terminated 8 months after the first injection, and the lungs of all animals were excised and fixed in neutral buffered formalin for the enumeration of lung adenomas (>1 mm in diameter).

For the induction of skin tumors, groups of 40 eight-week-old female CD-1 mice (Charles River Laboratories, Wilmington, Mass.) received weekly i.p. injections for 16 weeks. Each dose was 1.62 mg of DDP per 5 ml 0.85% NaCl solution per kg body weight. As a positive control, another group of mice received 2 weekly i.p. injections of ethyl carbamate (1 g per 5 ml 0.85% NaCl solution per kg body weight); negative controls received only the 0.85% NaCl solution. Some groups of mice received applications on the skin of 0.15 ml of 0.6% croton oil (Amend Drug and Chemical Co., New York, N.Y.) in redistilled acetone twice weekly (1 and 4 days after the injections) for the entire experimental period. The mice were shaved with an electric clipper prior to the first application of croton oil and thereafter as needed.

Sarcomas were induced in male Fischer rats (Charles River Breeding Laboratories), 160 to 170 g, by 6 weekly s.c. injections in the right hind leg with either 3.0 mg of DCP or 2.5 mg of DPP suspended in 0.1 ml of heat-sterilized trioctanoin (Sigma Chemical Co., St. Louis, Mo.). The compounds were suspended by a brief period of sonication. Suspensions were maintained by agitation with a vortexing machine.

All animals were subjected to a gross routine autopsy, which included examination of the skin, the s.c. tissue, and the organs of the abdominal and thoracic cavities. Representative samples of the lung adenomas and of the benign appearing skin tumors, all skin tumors with suspected malignancy, and all other tumors or suspected tumors were fixed in neutral buffered 10% formalin, sectioned at 5 to 6 μm, and stained with hematoxylin and eosin. The histopathological diagnoses were made by Dr. H. C. Pitot of the McArdle Laboratory for Cancer Research.

RESULTS

Toxicity of the Platinum Coordination Complexes. The water solubility of DDP facilitates its rapid removal from sites of injection; rodents given toxic doses develop renal necrosis (12, 16). Preliminary studies in our laboratory showed that weekly i.p. administration of 25% of the dose of DDP lethal to 50% of the mice (13 mg/kg) (6) for up to 10 weeks permitted survival of about 90% of the CD-1 mice. Higher doses or larger numbers of injections caused larger weight losses and resulted in more deaths. In agreement with the literature (6), the poorly water-soluble platinum complexes DCP and DPP were much less toxic on i.p. administration. The relative toxicities of these compounds are further documented by the average weight changes of the rats and mice during the periods of administration of the compounds (Tables 1 and 3).

Lung Adenomas in Female A/Jax Mice. DDP was quite active in the induction of lung adenomas in female A/Jax
mice (Table 1). In the first experiment, all of the mice that received weekly i.p. doses of 3.25 mg/kg body weight for 10 weeks or 1.62 mg/kg for 19 weeks developed lung adenomas by 8 months; the average multiplicities were 14.2 and 15.8 adenomas/mouse, respectively. Of the mice that received only injections of the 0.85% NaCl solution, 67% developed lung adenomas with an average of 0.8 adenoma/mouse. The injections for the mice in Group 1 were interrupted for 1 week at Week 7 owing to their poor condition at that time. These findings were extended in a second experiment. Of the mice given DDP, 94 to 100% developed lung adenomas. The average multiplicities were 14.2 and 15.8 adenomas/mouse for the group treated only with the vehicle. Administration of one-fourth of this level of DCP yielded more lung tumors than were observed in either control group (Groups 3 and 11), but the difference was not statistically significant. The 3 groups of mice that received ethyl carbamate (1 injection of 500 mg/kg) developed averages of 14.9, 18.9, and 29.4 adenomas/mouse. DDP induced 10 to 30 times and DCP 1 to 3 times as many lung adenomas/μmol of compound administered as did ethyl carbamate. However, direct comparisons in which the ethyl carbamate and the platinum complexes were injected on the same schedule have not been made.

**Skin Tumors in Female CD-1 Mice.** Administration of 16 weekly i.p. doses of DDP (total dose, 25.9 mg/kg) did not yield any gross skin tumors by the termination of the experiment at 52 weeks (Table 2). However, mice given the same dose of DCP and treated topically with 0.6% croton oil in acetone during the entire experimental period developed tumors in the croton oil-treated area. At 41 weeks, 50% of the 30 surviving mice had papillomas with an average multiplicity of 3.2 papillomas/mouse. Three mice developed epidermal carcinomas by the termination of the experiment at 52 weeks. For comparison, 61% of the mice given 2 weekly i.p. injections of ethyl carbamate (total dose, 2 g/kg) developed papillomas by 30 weeks with a multiplicity of 3.3 papillomas/mouse. Four of the mice in this group developed epidermoid carcinomas by the 52nd week. None of the mice that received only the croton oil treatments developed any skin tumors.

**Sarcomas in Male Fischer Rats.** Of 20 male rats given 6 weekly injections of 2.5 mg of DPP or 3.0 mg of DCP suspended in trioctanoin, 3 and 6 rats, respectively, developed sarcomas at the injection site by the termination of

### Table 1

**Induction of lung adenomas in female A/Jax mice by DDP and DCP**

Female A/Jax mice (8 weeks old) received weekly i.p. injections of DDP or DCP in 0.85% NaCl solution or in suspension in trioctanoin as indicated. At 8 months, the lungs of the surviving mice were fixed in neutral 10% formalin for enumeration of adenomas. Other conditions are described in "Materials and Methods." The average initial weight of mice in Experiment 1 was 20 g, and that for Experiment 2 was 18.5 g.

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Treatment (mg/kg/dose × no. of injections)</th>
<th>Total dose (μmol/kg)</th>
<th>Av. wt change at 10 wk (g)</th>
<th>No. of mice</th>
<th>% of mice with lung adenomas</th>
<th>Av. no. of adenomas/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DDP</td>
<td>3.25&lt;sub&gt;a&lt;/sub&gt; × 10</td>
<td>108</td>
<td>−4</td>
<td>10</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>DDP</td>
<td>1.62&lt;sub&gt;a&lt;/sub&gt; × 19</td>
<td>103</td>
<td>+2</td>
<td>10</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>0.85% NaCl solution only</td>
<td>6.5 ml/kg × 19</td>
<td>+4</td>
<td>6</td>
<td>6</td>
<td>67</td>
<td>0.8 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>Ethyl carbamate</td>
<td>500&lt;sup&gt;c&lt;/sup&gt; × 1</td>
<td>5600</td>
<td>+2</td>
<td>6</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>DDP</td>
<td>3.25&lt;sub&gt;d&lt;/sub&gt; × 10</td>
<td>108</td>
<td>+2</td>
<td>20</td>
<td>17</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>DDP</td>
<td>1.62&lt;sub&gt;d&lt;/sub&gt; × 10</td>
<td>54</td>
<td>+3</td>
<td>20</td>
<td>18</td>
<td>94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>DDP</td>
<td>3.25&lt;sub&gt;d&lt;/sub&gt; × 10</td>
<td>108</td>
<td>−1</td>
<td>20</td>
<td>17</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>DDP</td>
<td>3.25&lt;sub&gt;d&lt;/sub&gt; × 5</td>
<td>54</td>
<td>+1</td>
<td>20</td>
<td>18</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>9</td>
<td>DCP</td>
<td>18.9&lt;sub&gt;d&lt;/sub&gt; × 10</td>
<td>433</td>
<td>+3</td>
<td>20</td>
<td>19</td>
<td>95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>DCP</td>
<td>4.72&lt;sub&gt;d&lt;/sub&gt; × 10</td>
<td>108</td>
<td>+3</td>
<td>20</td>
<td>20</td>
<td>60</td>
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<tr>
<td>11</td>
<td>Trioctanoin</td>
<td>5 ml/kg × 10</td>
<td>+4</td>
<td>20</td>
<td>19</td>
<td>26</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>Ethyl carbamate</td>
<td>500&lt;sup&gt;d&lt;/sup&gt; × 1</td>
<td>5600</td>
<td>+5</td>
<td>15</td>
<td>15</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>Ethyl carbamate</td>
<td>500&lt;sup&gt;d&lt;/sup&gt; × 1</td>
<td>5600</td>
<td>+5</td>
<td>15</td>
<td>14</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dose given in 6.5 ml of 0.85% NaCl solution per kg body weight.

<sup>b</sup> Statistically different from controls (p < 0.005) by a χ² test.

<sup>c</sup> Mean ± S.D.

<sup>d</sup> Dose given in 5.0 ml of trioctanoin per kg body weight.
Groups of 40 female CD1 mice (8 weeks old) were given 16 weekly i.p. injections of DDP (1.62 mg per 5 ml 0.85% NaCl solution per kg body weight), or 5 ml 0.85% NaCl solution per kg body weight, or 2 weekly i.p. injections of ethyl carbamate (1 g/5 ml 0.85% NaCl solution per kg body weight). Groups that received croton oil were treated topically on the shaved back with 0.15 ml of 0.6% croton oil in acetone twice weekly for the duration of the experiment (52 weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15th wk</th>
<th>30th wk</th>
<th>41st wk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of mice</td>
<td>No. with papillomas/mouse</td>
<td>Av. papillomas/mouse</td>
</tr>
<tr>
<td>DDP + croton oil</td>
<td>39</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>DDP only</td>
<td>38</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ethyl carbamate + croton oil</td>
<td>37</td>
<td>13</td>
<td>2.6</td>
</tr>
<tr>
<td>0.85% NaCl solution + croton oil</td>
<td>40</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.85% NaCl solution only</td>
<td>40</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\(\text{a})\) One thymic lymphoma, 1 pulmonary adenoma.
\(\text{b})\) In external ear; carcinomas in the other groups occurred in the treated area.
\(\text{c})\) Two thymic lymphomas, 1 pulmonary adenoma, 3 mammary adenocarcinomas, 1 fibroliposarcoma (s.c.).
\(\text{d})\) Three thymic lymphomas, 16 pulmonary adenomas, 1 mammary adenocarcinoma, 1 fibromyosarcoma (s.c.).

Table 3

The induction of sarcomas in male Fischer rats by DCP and DPP

Groups of 20 male Fischer rats, with an average initial weight of 169 g, received 6 weekly s.c. injections in the right hind leg of DCP or DPP suspended in 0.1 ml of triocanoin. The experiment was terminated at 82 weeks. For other details, see "Materials and Methods."

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Av. change from initial wt (g)</th>
<th>No. of rats with sarcomas at the injection site by (wk)</th>
<th>No. of rats with sarcomas in the abdominal cavity(\text{a})</th>
<th>No. of rats with sarcomas in the abdominal cavity with other tumors (\text{b})</th>
<th>No. of rats with other tumors (\text{c})</th>
<th>No. of survivors at 82 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>mg/injection</td>
<td>6 wk</td>
<td>52 wk</td>
<td>48</td>
<td>60</td>
<td>82</td>
</tr>
<tr>
<td>DCP</td>
<td>3.0</td>
<td>+90</td>
<td>+244</td>
<td>1</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>DPP</td>
<td>2.5</td>
<td>+92</td>
<td>+256</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Triocanoin</td>
<td>0.1 ml</td>
<td>+103</td>
<td>+250</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\(\text{a})\) These rats did not have sarcomas at the injection site.
\(\text{b})\) One with an adenocarcinoma of the small intestine, 8 with interstitial cell tumors of the testes.
\(\text{c})\) One with a Grade I epidermoid carcinoma of Zymbal’s gland, 5 with interstitial cell tumors of the testes.
\(\text{d})\) Five with interstitial cell tumors of the testes.

The data presented above extend the general qualitative correlation between carcinogenicity, electrophilic reactivity, and mutagenic activity (21) to another class of chemicals, the platinum coordination complexes. Thus, in this study significant carcinogenicity of one or more of the 3
platinum complexes was observed in mouse lung and skin and in rat s.c. tissue. These 3 complexes were also mutagenic without activation in S. typhimurium TA100. Likewise, DDP and other platinum complexes are electrophilic and react with cellular nucleophiles (4, 32). Further qualitative and quantitative comparisons of the biological and chemical activities of these and other platinum complexes may have considerable theoretical and practical value.

The localization of tumor formation in the lungs, skin, and s.c. tissue in the present experiments was probably a consequence of the test systems; these are sites at which chemical carcinogens usually induce tumors with the protocols used. The high concentrations of platinum derivatives in the liver and kidney (14, 34) of rats and mice given these compounds also make these organs candidate sites for tumor formation. In view of our finding that skin tumor formation by DDP required treatment with croton oil, a potent promoting agent for the formation of these tumors in mice (3), promotion may also be required for tumor formation by the platinum(II) complexes in other tissues.

The platinum complexes examined in this study exhibit a wide range of toxicity and antitumor activity. The order of acute toxicity for mice of these complexes is DDP > DMP > DPP > DCP (5, 6). DDP is the most effective as an antitumor agent, but DCP and DPP are also active (6). DMP was not active against Sarcoma 180 (5).

DDP is moderately water soluble, while DPP and DCP are only poorly soluble in water. Thus, DDP would be expected to be widely distributed in the body fluids after administration, and this presumption is consistent with its acute renal toxicity and the development of tumors at sites (the lungs and skin) distant from the injection site. The much weaker carcinogenicity of DCP as compared to DDP for the mouse lung on i.p. administration may be related to its poorer solubility in water and consequent poorer delivery to peripheral tissues. DDP is a much stronger mutagen for S. typhimurium TA100 than were the other 3 platinum complexes. It is not evident whether these differences in biological activities reflect inherent differences in reactivity, transport, or rates of conversion to the aquo species (Refs. 4 and 32; Chart 3). Likewise, it is not clear why DMP, which is probably less reactive than are the corresponding aquo species (4, 32).

Binding of DDP and other reactive platinum complexes is likely to occur at nitrogen and sulfur atoms in proteins, especially the methionine sulfur and the imidazole nitrogens of histidine residues, and the nitrogen atoms of purine and pyrimidine bases in nucleic acids (32). Binding of DDP to guanosine, adenosine, and cytidine, but not appreciably to thymidine or uridine, has been reported (18). While both cis- and trans-DDP bind to N-7 of guanine residues, only the cis isomer can chelate the O4 and N-7 atoms of a single guanine residue (20). Since only the cis isomers of the platinum complexes have shown antitumor activity (5, 6), the ability of cis complexes to bind in a bidentate manner to sites on DNA may be important for their mutagenic, carcinogenic, and antitumor activities.

**ACKNOWLEDGMENTS**

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