Clinical and Pharmacological Studies with 
cis-Diamminedichloroplatinum(II)1

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SUMMARY

cis-Diamminedichloroplatinum(II) is the first of a group of platinum coordination complexes with antineoplastic activity to be studied in humans. This Phase I investigation characterizes the toxicity and pharmacological disposition of the drug in 10 patients. Plasma levels of cis-diamminedichloroplatinum(II) decayed in a biphasic mode, with an initial half-life of 25 to 49 min and a secondary phase ranging from 58 to 73 hr. Protein binding exceeded 90% of radioactivity in this phase. Intracellular leukocyte levels approximated 6 to 11% of coincident plasma samples. Urinary excretion was incomplete, with only 27 to 45% of radioactivity excreted in the first 5 days. The initial fractions of radioactivity were largely unchanged drug, although this changed with time. The incorporation of thymidine-3H into DNA was inhibited in leukemic leukocytes only after prolonged exposure in vitro to cis-diamminedichloroplatinum(II). Acute lymphocytic cells appeared more sensitive than myelocytic cells in vitro, although the only objective antineoplastic response noted was a transient decrease in blast count in a patient with acute myelocytic leukemia. Renal impairment was the dose-limiting toxicity in the single-dose escalation scheme used. Rises in serum creatinine occurred in three of six patients who received doses of 1.95 mg or more per kg, and progressive renal failure contributed to the death of one patient.

INTRODUCTION

In 1965, Rosenberg (17) first inadvertently demonstrated that platinum compounds inhibit cell division in *Escherichia coli*. Since then, various studies have defined the chemistry of a number of derivatives and demonstrated antineoplastic activity in many animal tumor-screening systems. cis-Pt-II2 is the agent that has been studied most and the one that appears to have the greatest antineoplastic activity. It is more sensitive than myelocytic cells in vitro, although the only objective antineoplastic response noted was a transient decrease in blast count in a patient with acute myelocytic leukemia. Renal impairment was the dose-limiting toxicity in the single-dose escalation scheme used. Rises in serum creatinine occurred in three of six patients who received doses of 1.95 mg or more per kg, and progressive renal failure contributed to the death of one patient.

MATERIALS AND METHODS

Patient Selection and Evaluation. Ten patients with advanced neoplastic disease considered resistant to conventional treatment were studied. Two patients with acute leukemia had had multiple courses of combination drug therapy. The 2 subjects with carcinoma of the cervix had previously been unresponsive to bleomycin, and 4 of 5 patients with gastrointestinal cancer had been treated with both 5-fluorouracil and 1,3-bis(2-chloroethyl-1-nitroso)-

Patients were hospitalized for study and treatment in the Clinical Pharmacology Research Unit of the Yale-New Haven Medical Center, and written informed consent was obtained. Pretreatment assessments of the extent of disease were made by physical examination, X-ray, peripheral blood and bone marrow findings, and multiple studies of liver and renal function. Peripheral blood counts and bone marrow aspirates were normal in patients without leukemia. Serum creatinine values were all within normal limits (< 1.2 mg/100 ml). Patients were carefully observed for symptoms and signs of drug toxicity as well as antineoplastic effects. Serial hematological, hepatic, and renal function studies as well as audiograms were performed as indicated. Serial 24-hr urine collections were obtained prior to, during, and immediately after administration of the drug.

1 Supported in part by Grants CA 5138, CA 08341, and Ca 12317 from the USPHS, as well as by Grant ET-14G from the American Cancer Society.

2 The abbreviations used are: cis-Pt-II, cis-diamminedichloroplatinum(II); HIA, heavy ion accelerator; TCA, trichloroacetic acid.

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Clinical and Pharmacological Studies with cis-Pt-II

Chemicals. cis-Pt-II was provided by Dr. Steven Carter of the Cancer Therapy Evaluation Branch of the National Cancer Institute. Thymidine-methyl-3H was purchased from New England Nuclear (Boston, Mass.), elemental platinum was from the Atomergic Chemetal Co. (Long Island, N. Y.), and enriched 193Pt was purchased from Oak Ridge National Laboratory (Oak Ridge, Tenn.). Elemental osmium was obtained from Alfa Inorganics, Inc. (Beverly, Mass.).

Preparations of Labeled cis-Pt-II. The cis-Pt-II compound was labeled with radioactive 193mPt, which has a half-life of 4.4 days and decays by an isomeric transition to 193Pt. The 193mPt can be counted by liquid scintillation counting, with the conversion electrons or with a sodium iodide scintillation counter, and with the use of 67keV X-rays generated by K capture.

Radioactive 193mPt was prepared by 2 methods. (a) Osmium metal was bombarded with α particles at the Yale HIA. The HIA bombardment uses the reaction, 192Os(α, 3n) 195Pt. The natural abundance of 192Os is 41.0%. High specific activity of 193mPt is produced, since the radioactive platinum can be prepared in a carrier-free state. After chemical separation of the 193mPt from the osmium target, the radioactive 193mPt was combined with about 10 μg of platinum metal dissolved in aqua regia in preparation for the cis-Pt-II synthesis. (b) Enriched (11.52%) 192Pt was subjected to neutron irradiation (General Electric Co., Beverley, Mass.).

The synthesis of labeled cis-Pt-II was carried out by conversion of the platinum metal to K2PtCl4 and then reduction of this compound to K2PtCl4 with hydrazine (12). The purity and authenticity of the material was established by chromatography on Whatman MM 3 paper, with the use of acetone:water (9:1, v/v) and filament elongation of E. coli. (17). The labeled cis-Pt-II was dissolved in 0.9% NaCl solution and filtered through a 0.45 μm Millipore filter before being combined with unlabeled drug for patient studies.

Administration of Drug. Patients received a single i.v. dose of drug at 4- to 6-week intervals. The starting dose selected was 0.15 mg/kg, and in successive patients the initial dosages of drug were escalated according to a Fibonacci scheme. Drug was administered as a rapid i.v. push over a 1- to 5-min period. Care was taken to ensure adequate hydration, and i.v. fluids were used when necessary to prevent dehydration due to nausea and vomiting.

One patient (R. R.) received a 2nd dose of drug 16 days after the initial dose because of progression of leukemia after initial evidence of response.

Studies of the Metabolism of cis Pt-II. Patients received 0.066 to 3.15 mg of cis-Pt-II/kg, containing 193mPt at levels of 0.0033 to 1.178 x 10^8 cpm (determined in a Picker Spectroscaler γ counter with a 2-inch crystal). The drug was administered through a Volu-trole. Serial blood and urine samples were obtained, and plasma was separated from whole blood by centrifugation. We counted all samples in a γ counter and applied corrections both for decay and for any variations due to the geometry of differing sample size (as much as 5 ml was counted for later blood samples), by counting a variety of standards. All counting was carried out on duplicate samples to within a S.E. of ±2.5%. The variation between duplicate samples was 6% or less.

Clinical Effects. Objective evidence of antineoplastic effects could be documented in only 1 patient (Table 1). This subject (R. R.) who had acute granulocytic leukemia, experiences a fall in peripheral blast count from 25,000 to 3,000 after receiving the largest single dose used in this study, 3.15 mg/kg. Because this response was short, a 2nd smaller dose of drug (1.20 mg/kg) was given 16 days after the initial dose. In the week that followed, he developed progressive azotemia and died with florid leukemia and
Table 1  
Drug dosage and effects of cis-diaminedichloroplatinum

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Disease</th>
<th>Drug dosage (mg/kg)</th>
<th>Gastro-intestinal</th>
<th>Hematological</th>
<th>Auditory</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. P.</td>
<td>63</td>
<td>M</td>
<td>Carcinoma, rectum</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. I.</td>
<td>38</td>
<td>M</td>
<td>Acute lymphocytic leukemia</td>
<td>0.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. C.</td>
<td>59</td>
<td>M</td>
<td>Carcinoma, tonsil</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J. G.</td>
<td>52</td>
<td>F</td>
<td>Carcinoma, stomach</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.20</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. B.</td>
<td>62</td>
<td>F</td>
<td>Carcinoma, cervix</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
<td>Tinnitus</td>
</tr>
<tr>
<td>L. E.</td>
<td>35</td>
<td>F</td>
<td>Carcinoma, cervix</td>
<td>0.75</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. B.</td>
<td>66</td>
<td>M</td>
<td>Carcinoma, colon</td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R. R.</td>
<td>32</td>
<td>M</td>
<td>Acute myelomonocytic leukemia</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. N.</td>
<td>67</td>
<td>M</td>
<td>Carcinoma, colon</td>
<td>3.15</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. C.</td>
<td>31</td>
<td>F</td>
<td>Carcinoma, stomach</td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
<td>Tinnitus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nausea and vomiting occurred in all patients who received doses of more than 0.75 mg/kg. These symptoms usually began 1 to 2 hr after drug administration and seemed somewhat more severe at the higher dose levels, with persistence of gastrointestinal symptoms for as long as 1 to 2 days. Hematological toxicity was mild; 1 patient developed a transient fall in platelet count to 90,000/cu mm and another experienced a leukocyte fall to 2,600/cu mm. No alterations in liver function could be attributed to the drug rather than to the progression of metastatic disease. Two patients developed tinnitus without hearing loss; this persisted for several hr in one patient and for 1 week in another. Additional courses of drug did not reproduce the symptom. One patient (R. R.) developed a 30-db high-frequency hearing loss after his initial dose (3.15 mg/kg).

**Metabolism of cis-Pt-II.** After administration of doses of cis-Pt-II of 0.066 to 3.15 mg/kg containing $^{193m}$Pt, the plasma curves for radioactivity shown in Chart 1 were obtained. Data were expressed as cis-Pt-II, although this was not necessarily the only chemical species present. These curves were clearly biphasic with a relatively rapid initial clearance ($t_{1/2}$, 25.5 to 49.0 min) followed by a phase of extremely slow loss from the circulation ($t_{1/2}$, 58.5 to 73.0 hr). Levels of radioactivity were not sufficient to enable us to undertake a full-scale chromatographic study with this rapidly decaying isotope. Gel filtration of such samples indicated that in all patients, 64.8 to 97.3% of the plasma radioactivity was associated with protein. In general, there was a time-dependent increase in the percentage bound $^{193m}$Pt, and once the plasma level reached the slow secondary phase, such binding exceeded 90%. For 2 subjects (L. E. and R. R.), this finding was studied in somewhat more detail. It was found that raising the NaCl concentration to 2 M caused no dissociation. When a double-reciprocal plot of the binding (in $\mu$g/ml plasma) versus the concentration of $^{193m}$Pt (expressed as $\mu$g of cis-Pt-II/ml) was

![Chart 1. Curves for plasma clearance of $^{193m}$Pt after administration of cis-Pt-II. Radioactivity is expressed in terms of $\mu$g equivalents of cis-Pt-II per ml.](chart1.png)
drawn, straight lines were obtained from which apparent
K_m values of 12.5 and 20.0 µg/ml were derived (Chart 2). Binding between unlabeled plasma protein and cis-Pt-II-
^{193m}Pt also occurred in vitro, and values thus obtained
were completely superimposable on the plots of the in vivo data.

As might be expected for a drug exhibiting a high degree
of association with plasma protein, levels of radioactivity in
the white cells were in the range of 6 to 11% of the coinci-
dent plasma concentrations. Since levels of radioactivity
in whole blood lay in the range of 64 to 80% of those of the
plasma, it is evident that penetration of cis-Pt-II into red
cells is also limited.

The urinary excretion of \(^{193m}Pt\) after administration of
labeled cis-Pt-II followed the pattern (Chart 3) of rapid
initial output, followed by a very slow and incomplete
phase. Fifteen to 27% of the radioactivity was excreted in the
1st 6 hr. By 24 hr, only 18 to 34% was excreted and,
after 5 days, a total of only 27 to 45% was recovered. Chromatography was applied to the early urines only. In 1 case
(A. B.), 67% of the \(^{193m}Pt\) excreted in the 1st 6 hr was in the
form of cis-Pt-II, while in another patient (R. R.) given
a higher dose (3.15 mg/kg), only 27.8% of the label in the
same urine fraction was present as unchanged drug. Patient
L. E. showed a somewhat greater proportion (62.6%) of
unchanged drug after the larger dose (0.75 mg/kg) than
after the small dose (0.066 mg/kg), when only 44.4% was
excreted as cis-Pt-II in 6 hr. The very short half-life of
\(^{193m}Pt\) has prevented any thorough investigation of the
nature of the metabolites, of which the major component
did not migrate in the acetone: H_2O solvent. Another minor
component had an R_f value of 0.20 on Whatman No. 3MM
paper.

**Effects of cis-Pt-II in Vitro on DNA Synthesis in Leu-
kemic Leukocytes.** Incorporation of thymidine-\(^3H\) into
DNA was used as a measure of DNA synthesis in isolated
white cells. We used various times of preincubation with
cis-Pt-II before the addition of thymidine to determine the
most suitable conditions. Inhibiting effects were small and
irregular for periods of less than 5 hr, and at least 18 hr
were needed to give consistent significant effects. This beh-
avior has been described previously (7) in human amnionic
AV_3 cells. Table 2 shows the data for the percentage
inhibition of thymidine-\(^3H\) incorporation into DNA for a range
of human peripheral leukemic leukocytes. It is evident that
the cells of acute lymphoblastic leukemia were the most
sensitive to cis-Pt-II, while acute myelogenous leukemia cells
showed marked variation in response. In Patient D. I., cells
were isolated at various times over a 48-hr period following
injection of cis-Pt-II (0.3 mg/kg). At no time was the in
vitro incorporation of thymidine-\(^3H\) by these cells de-
pressed below baseline activities with this low dose.

![Chart 3. Urinary excretion of \(^{193m}Pt\) after administration of labeled
 cis-Pt-II.](chart3.png)

**Table 2**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>0.1 µg/ml</th>
<th>0.5 µg/ml</th>
<th>2.0 µg/ml</th>
<th>5.0 µg/ml</th>
<th>10.0 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>100.4</td>
<td>49.2</td>
<td>43.0</td>
<td>12.1</td>
<td>4.7</td>
</tr>
<tr>
<td>ALL</td>
<td>85.7</td>
<td>68.5</td>
<td>46.4</td>
<td>33.2</td>
<td>26.4</td>
</tr>
<tr>
<td>ALL (D. I.)</td>
<td>83.9</td>
<td>77.3</td>
<td>59.0</td>
<td></td>
<td>35.9</td>
</tr>
<tr>
<td>AML</td>
<td>115.4</td>
<td>101.0</td>
<td>136.7</td>
<td>99.0</td>
<td></td>
</tr>
<tr>
<td>AML (R. R.)</td>
<td>96.0</td>
<td>100.9</td>
<td>107.7</td>
<td>118.8</td>
<td>85.4</td>
</tr>
<tr>
<td>AML</td>
<td>93.9</td>
<td>97.2</td>
<td>83.7</td>
<td>54.0</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>94.4</td>
<td>75.4</td>
<td>66.5</td>
<td>43.1</td>
<td>30.3</td>
</tr>
<tr>
<td>PCL</td>
<td>104.6</td>
<td>81.0</td>
<td>81.3</td>
<td>100.1</td>
<td>67.5</td>
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<tr>
<td>CLL</td>
<td>93.2</td>
<td>100.0</td>
<td>98.7</td>
<td>99.8</td>
<td>89.3</td>
</tr>
</tbody>
</table>

*The abbreviations used are: ALL, acute lymphocytic leukemia;
AML, acute myelomonocytic leukemia; PCL, plasma cell leukemia;
CLL, chronic lymphocytic leukemia.*

*Radioactivity in crude cpm/sample placed in counting vials was:
ALL, 444 to 1481; AML, 241 to 529; PCL, 317; CLL 87.*
DISCUSSION

This limited study has helped define the single-dose limitations of cis-Pt-H in humans. Single doses of more than 1.95 mg/kg are likely to produce renal impairment and may result in severe morbidity or, as in 1 patient in this study, may contribute to death from acute tubular necrosis. Similar findings have been reported with the use of such doses in other Phase I studies (8, 9, 18, 20), and further exploration of higher doses appeared unwarranted. Lippman et al. (14), using a daily dosage schedule, noted significant renal damage in 9 of 16 patients who received total doses of more than 2 mg/kg. Thus, human tolerance for this compound is quite similar to that described for dogs and monkeys (5).

The persistence of radioactivity in the blood is certainly a function of the high degree of protein binding that occurs. Interaction of cis-Pt-II with DNA has been explored in depth by spectrophotometric methods (10). In those experiments, chloride and phosphate anions were markedly inhibitory to the interaction, which involved at least 5 reacting species (10, 15). In contrast, binding to plasma protein in our studies did not show significant sensitivity to NaCl over a concentration range of 0.005 to 2.0 M, in terms of either dissociation of preformed complex or of inhibition of the association in vitro. Thus, the interaction with plasma protein must differ in nature from that with DNA, with which a ligand bond between platinum and the base azonitrogen may occur (10). However, the reaction with plasma protein apparently follows saturable Michaelis-Menten kinetics and thus is presumably reversible. Hence, the complex could serve as a pool for the supply of free cis-Pt-II, provided of course that chemical change to some other entity is not a prerequisite for binding. We have not attempted to determine which plasma protein selectively binds cis-Pt-II. However, experiments with the related agent cis-dichloro(dipyridine)platinum(II) (11) indicated that it does not bind to bovine serum albumin. The prolonged retention and slow urinary elimination of cis-Pt-II and its breakdown products, seen in this and other studies (9), provide confirmation of the existence of a pool of bound material and pose a possible clinical danger. Drug treatments scheduled at too-close intervals may lead to accumulation of a toxic body load of platinum. Furthermore, preclinical studies of tumor inhibition (13, 16) suggest that single large doses given at weekly intervals are more effective than daily treatment. Thus, both clinical pharmacological data and animal tumor studies indicate the desirability of widely spaced treatments.

The studies carried out in vitro with leukemic leukocytes do not add very significantly to knowledge of the mode of action of this agent. Inhibition of DNA synthesis required prolonged exposure, both in these and other studies (7). In one subject (D. I.), cells isolated during treatment showed no impairment of DNA synthesis despite being sensitive to this agent, added in vitro. The low dose given (0.3 mg/kg) may have failed to achieve or maintain comparable adequate plasma levels of drug in vivo. Alternatively, this could suggest that inhibition of DNA synthesis is not the key mechanism of action of the agent. cis-Pt-II is able to effect some sort of alkylation of DNA (10), and there is evidence, both at the biochemical level (11) and from studies of tumor cross-resistance (1), that there is a considerable resemblance to the mode of action of nitrogen mustard derivatives. Thus, although there appears to be some selectivity for inhibition of DNA synthesis, other areas of metabolism are affected at least with the dipyridine derivative (6), and the crucial events leading to cell death may not be expressed through DNA synthesis. Certainly, examination of mouse bone marrow after treatment with cis-Pt-II indicated that inhibition of mitosis commenced within 1 hr and persisted for up to 72 hr (22). Such an inhibition cannot be secondary to changes in DNA synthesis or to cell death, since it precedes both of these. It is evident that much more work will be needed to elucidate the mechanism of action of cis-Pt-II.

REFERENCES

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