Antitumor and Antimitotic Properties of cis-Dichloro(dipyridine)platinum(II)¹

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SUMMARY

Cis-dichloro(dipyridine)platinum(II), an aryl congener of the totally inorganic antitumor compound cis-dichlorodiammineplatinum(II), was evaluated for pharmacological similarities to the inorganic complex. The parameters assessed were its effects on bacterial cytokinesis, on development of the Ehrlich ascites carcinoma in vivo, on nucleic acid and protein synthesis in tumor cells in vitro, and on phytohemagglutinin-induced mitogenesis of human lymphocytes. The aryl derivative possessed actions similar to those of the inorganic species but was somewhat less potent. The merits of the organic derivative as a tool for studies of absorption, binding, distribution, and excretion by virtue of its amenability to tritium labeling were discussed.

INTRODUCTION

Since the serendipitous observation by Rosenberg et al. (9) that the bacterial cytokinetostasis that occurred in cultures of *Escherichia coli* through which an alternating electric current was passed was due to electrolytic products from the "inert" platinum electrode, a number of reports have described pharmacological and chemotherapeutic properties of various organic and inorganic platinum compounds. Those most active as inhibitors of cell division in *E. coli* and of development of several transplantable rodent tumors are cis isomers of Pt(II) and Pt(IV) existing as neutral complexes (7, 8, 10, 11). With various treatment regimens, Rosenberg's group obtained over 83% increased survival times of mice bearing the L1210 leukemia (10) and recently reported complete regression of advanced Sarcoma 180 by the 2 totally inorganic complexes, cis-[Pt(NH₃)₂Cl₂]₀ and cis-[Pt(NH₃)₂Cl₄]₀ (7). The Ehrlich ascites carcinoma is also quite susceptible to the action of cis-[Pt(NH₃)₂Cl₂]₀, and biochemical studies have indicated that a persistent and selective inhibition of DNA synthesis in *vivo* may be related to its chemotherapeutic efficacy (3).

The cis substituents of the 4 most active compounds reported thus far are the diammine (2[NH₃]) or monoethylenediamine (H₂NCH₂CH₂NH₂) derivatives of the square planar Pt(II) or the octahedral Pt(IV). The NH₃ groups of the totally inorganic species or the NH₂ groups of the 5-membered organometallic ring established in the case of the ethylenediamine derivative appear to be critical for biological activity, since substitution of NO₂ for NH₃ of the inorganic species yields a compound devoid of inhibitory action on bacterial cytokinesis (8). The current study was consequently undertaken to determine whether an aryl organic platinum derivative, cis-PPC² exerts pharmacological actions similar to those of the more active platinum compounds reported previously. In addition to studies with *E. coli* and the Ehrlich ascites carcinoma, the actions of this organic derivative on mitogenesis of human lymphocytes in culture were assessed, since experiments currently underway in this laboratory indicate that cis-[Pt(NH₃)₂Cl₂]₀ is a potent inhibitor of PHA-induced blastogenic transformation.

MATERIALS AND METHODS

cis-PPC was prepared by the method of Kauffman (4). Elemental analyses (Galbraith Laboratories, Inc., Knoxville, Tenn.) showed, in percentages (theoretical values are in parentheses): Pt, 46.08 (45.99); C, 28.12 (28.31); H, 2.37 (2.38); N, 6.74 (6.60); and Cl, 16.55 (16.72). Solutions of cis-PPC were prepared immediately prior to use by dissolution in 1 part dimethyl sulfoxide and 9 parts 0.9% NaCl solution.

*E. coli* strain B (ATCC No. 11303) was grown at 37°C in a synthetic liquid medium described by Davis and Mingioli (1). Cells for microscopy were stained for 30 sec with gentian violet and photographed with a Leitz Orthoplan device.

The carcinostatic activity of cis-PPC was assessed against the Ehrlich ascites tumor in BALB/c mice (Flow Laboratories, Inc., Rockville, Md.). Ascitic fluid from donor mice was diluted with 0.9% NaCl solution, and each recipient was given approximately 5 X 10⁶ cells i.p. Treatment was begun 24 hr later with either single or multiple dose regimens. Controls were given an appropriate volume of 10% dimethyl sulfoxide in 0.9% NaCl solution. The few mice that survived over 60 days were considered to have survived only 60 days in the calculations of percentage of increase of survival.

The methods used to measure the incorporation of thymidine-methyl-³H, uridine-⁵³H, and L-leucine-¹⁴C into the acid-insoluble fraction of tumor cells in *vivo* have been described (3). Isotopically labeled compounds were from New England Nuclear, Boston, Mass., and MEM was from Microbiological Associates, Bethesda, Md.

Antimitotic activity of cis-PPC was measured with the use of ²The abbreviations used are: cis-PPC, cis-dichloro(dipyridine)-platinum(II); PHA, phytohemagglutinin; MEM, Eagle's minimum essential medium with Hanks' balanced salt solution, 2.0 mM with respect to L-glutamine.
of peripheral lymphocytes from presumably normal human subjects. The cells were isolated as described by Ohno and Hersh (5); erythrocytes were sedimentated with dextran (M.W. 204,000). Mitogenesis was initiated by the addition of 0.005 ml of PHA-P (Difco Laboratories, Inc., Detroit, Mich.) per ml of culture. The medium used was MEM supplemented with autotchthonous serum (20%), NaHCO₃ (1.7 mM), penicillin G (100 units/ml), and streptomycin (100 ìg/ml). The total volume of each culture was 2.0 ml, and the cell density varied from 3 × 10⁶ to 5 × 10⁸ cells/ml. Incubation was at 37° in 5% CO₂-95% air without agitation. At 46 hr, thymidine-methyl-³H, 1.0 µCi/ml, was added to each culture. Two hr later, the cells were washed with cold 0.9% NaCl solution, and nucleic acid was precipitated with cold 5% trichloroacetic acid. The acid-insoluble fraction was then processed for liquid scintillation counting as described for the tumor cells (3).

RESULTS

E. coli B was sensitive to the action of cis-PPC, and virtually total inhibition of growth was achieved at an inhibitor concentration of 8 × 10⁻⁵ M in the synthetic medium. Photomicrographs showed that cells grown in medium containing 5 × 10⁻⁵ M cis-PPC were extremely elongated (Fig. 1), quite analogous to the appearance of cells grown in the presence of the active inorganic platinum compounds (2, 6, 8).

The effects of cis-PPC on the survival times of mice bearing the Ehrlich ascites tumor are shown in Table 1. In each case, the extension of survival was of a high order of statistical significance. Of the regimens assessed, the greatest degrees of

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of mice</th>
<th>Dose/injection (mg/kg)</th>
<th>Day(s) treated</th>
<th>Mean survival time (days ± S.D.)</th>
<th>% increase of mean survival time</th>
<th>p</th>
<th>No. of toxic deaths</th>
<th>No. of 50-day survivors</th>
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<td>A</td>
<td>10</td>
<td>0</td>
<td>1–6</td>
<td>15.2 ± 2.8</td>
<td>22</td>
<td>&lt;0.005</td>
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<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>1–6</td>
<td>18.6 ± 1.8</td>
<td>50</td>
<td>&lt;0.05</td>
<td>0</td>
<td>1</td>
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<td></td>
<td>10</td>
<td>10</td>
<td>1, 4, 7, 10</td>
<td>22.8 ± 13.5</td>
<td>80</td>
<td>&lt;0.005</td>
<td>0</td>
<td>1</td>
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<tr>
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<td>10</td>
<td>10</td>
<td>1–5</td>
<td>27.4 ± 11.9</td>
<td>80</td>
<td>&lt;0.005</td>
<td>0</td>
<td>1</td>
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<td>10</td>
<td>15</td>
<td>1–4</td>
<td>29.7 ± 8.8</td>
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<td>&lt;0.0005</td>
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<td>10</td>
<td>25</td>
<td>1</td>
<td>37.5 ± 12.7</td>
<td>147</td>
<td>&lt;0.0005</td>
<td>0</td>
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<td>8</td>
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<td>1</td>
<td>14.6 ± 3.9</td>
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<td>&lt;0.0005</td>
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<td>40</td>
<td>1</td>
<td>33.4 ± 3.4</td>
<td>129</td>
<td>&lt;0.0005</td>
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<td>&lt;0.0025</td>
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<td>39.0 ± 6.7</td>
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* The tumor was given on Day 0.

The effects of cis-PPC on the in vitro synthesis of DNA, RNA, and protein synthesis are shown in Chart 1. Since preliminary experiments revealed that the onset of action was time dependent, requiring a number of hours to be manifest fully, these 3 parameters were monitored over a 100-fold range of cis-PPC concentration after 3 hr of exposure of the cells to the inhibitor. Very little selectivity against DNA synthesis was noted, and the concentration that conferred 50% inhibition of each process was about $2 \times 10^{-5}$ to $5 \times 10^{-5}$ M. By reducing the concentration of cis-PPC to $10^{-7}$ M and varying the period of exposure of the cells to the inhibitor prior to the brief pulse-labeling period, a moderate degree of selectivity against DNA synthesis was observed; after incubation of the cells for 6 hr with the inhibitor at this concentration, the rate of synthesis of DNA was reduced to less than 50% of the control value, while the rate of RNA synthesis was virtually unaltered.

Blastogenesis of human lymphocytes promoted by PHA was quite sensitive to the action of cis-PPC, and the concentration that conferred 50% inhibition, as assessed by incorporation of thymidine into an acid-insoluble form, was almost an order of magnitude lower than that found when the tumor cells were used in a protein-free medium (Chart 2).

**DISCUSSION**

Of the pharmacological properties of cis-PPC surveyed in this work, no remarkable differences were found between the actions of this neutral organic platinum species and the totally inorganic cis-[Pt (NH$_3$)$_2$Cl$_2$] $^9$. The only dissimilarity appears to be in regard to potency. Certainly, this decreased potency of the organic derivative is no great attribute for a compound of the so-called precious metal group. However, animal toxicity was reduced considerably by substituting 2 pyridine groups for the 2 ammine groups. For example, BALB/c mice are consistently killed by a single i.p. injection of cis-[Pt (NH$_3$)$_2$Cl$_2$] $^9$ at about 17 to 20 mg/kg (unpublished data) or by 8 daily injections of 4 mg/kg (3), while a single injection of cis-PPC at 100 mg/kg yielded only 30% mortality. Unequivocally, considerably more evaluation is essential to determine whether the reduced toxicity of cis-PPC, in spite of its reduced potency otherwise, confers any distinct therapeutic advantage over the inorganic compound.

A decided advantage of the pyridine-substituted derivative resides in its amenability to tritium labeling, which facilitates studies of absorption, distribution, binding, and excretion, and the 10 potential labeling sites on the dipyridine congener would permit a greater specific activity of the ensuing compound than could be obtained with cis-dichloroethylenediamineplatinum(II), which contains only 4 hydrogen atoms unlikely to exchange with aqueous solvent. We have now synthesized cis-PPC using pyridine-$^3$H and have obtained a product with a specific activity of 3.2 mCi/mmol; binding and distribution studies are underway and will be communicated subsequently.

**REFERENCES**


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