Platinum and Palladium Complexes Containing Ethylenediamine Derivatives as Carrier Ligands and Their Antitumor Activity

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We prepared new antitumor active platinum complexes containing N,N’-bis(2-chloroethyl)ethylenediamine (C1EN) as an alkylating carrier ligand in order to obtain increased antitumor effects. Some of the platinum complexes synthesized showed enhanced antitumor activity. However, palladium(II) complexes containing C1EN were inactive against L1210 and P388.

Keywords platinum complex; antitumor activity; alkylating agent; palladium(II) complex

Efforts to develop new antitumor-active Pt complexes with greater potency and reduced toxicity as compared with cis-diaminedichloroplatinum(II) (cis-DPP) have yielded Carboplatin (cis-diamine (1,1-cyclobutanedicarboxylato)platinum(II)), which is now clinically used in the U.S.A., Europe, and Japan. Such Pt(II) complexes contain ammonia as a carrier ligand in common and tumor cells can develop cross-resistance to them. Thus, the third-generation Pt complexes are needed to overcome the problem of acquired resistance. Oxaliplatin (1R,2R-cyclobutanediamine(oxalato)platinum(II)) is currently under clinical trials in Europe and Japan a,b and was reported to overcome no cross-resistance in some cis-DPP resistant tumors. 3 A number of other antitumor-active Pt complexes have been reported, whose carrier ligands are amines or diamines without specific physiological activities. So we attempted to synthesize Pt and Pd complexes containing an alkylating group in a carrier ligand in order to increase the activity. We synthesized an ethylenediamine derivative containing a chloroethyl moiety as an alkylating functional group, i.e., N,N’-bis(2-chloroethyl)ethylenediamine (C1EN), and in the process of its synthesis another carrier ligand without the alkylating group, i.e., N,N’- bis(hydroxyethyl)ethylenediamine (HOEn), was also obtained. Its Pt and Pd complexes were also prepared for comparative purposes.

The alkylating group was effective in Pt(IV) complexes, since [PtCl4(C1EN)] exhibited moderate activity against murine leukemia L1210, while [PtCl4(HOEn)] showed only marginal activity.

Experimental

HOEn and C1EN were prepared according to the method reported by Ishidate et al. 8,9

Syntheses of Pt and Pd Complexes Dichloro[N,N’-bis(2-hydroxyethyl or 2-chloroethyl)ethylenediamine]platinum(II) [PtCl4(HOEn)] or [PtCl4(C1EN)]; K2PtCl4 (415 mg, 1 mmol) and HOEn (148 mg, 1 mmol) or C1EN: 2HCl (258 mg, 1 mmol) were dissolved in 10 mL of H2O and the pH of the solution was adjusted to 7 with dilute HCl or NaOH solution. The solution was stirred at room temperature for 24 h and yellow precipitates that deposited were collected by filtration and washed with small amounts of H2O and then with ethanol. The obtained precipitates were dried in vacuo at 80°C for 4 h. Yields of [PtCl4(HOEn)] and [PtCl4(C1EN)] were 85 and 80%, respectively.

The nitrato Pt complexes were prepared by reacting the corresponding dichloro Pt complexes with 2 eq of AgNO3 in H2O. The resulting nitrato Pt(II) complexes were reacted with an equivalent amount of oxalic acid in H2O to give oxalato Pt(II) complexes.

Tetrachloro[N,N’-bis(2-hydroxyethyl or 2-chloroethyl)ethylenediamine]platinum(IV) [PtCl4(HOEn)] or [PtCl4(C1EN)]; [PtCl4(HOEn) or C1EN] (2 g) was suspended in 45 mL of H2O, through which Cl2 gas was bubbled for 90 min. The undissolved yellow precipitates were filtered off and the filtrate was concentrated to one-third of the original volume under reduced pressure. The yellow precipitates were collected by filtration and washed with small amounts of cold water and then with ethanol.

Pd(II) complexes were synthesized according to the same procedure as used for the Pt(II) complexes, from Na2PdCl4 as a starting material. The results of elemental analyses of these Pt and Pd complexes are shown in Table I.

Evaluation of Antitumor Activity Murine leukemia L1210 cells(106) were transplanted intraperitoneally into CDF1 mice on day 0. Treatment was given intraperitoneally three times on days 1, 5, and 9, according to the National Cancer Institute (Bethesda, Md.) protocol. The mean survival times (d) of the treated (T) and control (C) groups (6 mice/group) were calculated, and the antitumor activity was expressed as T/C%. Values of T/C% exceeding 125 were taken as indicating effectiveness.

Cell Survival Two hundred thousand cells were seeded in a test tube (2 mL of RPMI 1640 medium). After 48 h of incubation, the cells were centrifuged at 3000 rpm for 5 min and resuspended in fresh medium, then the cells were incubated for another 48 h. The medium was again replaced with fresh RPMI 1640 containing or not containing a test drug, and the cells exposed to the drug for 2 h. The medium was then changed

Table I. Results of Elemental Analyses of Platinum and Palladium Complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Found (%)</th>
<th>Calcd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>PtCl4(HOEn)</td>
<td>17.28</td>
<td>3.98</td>
</tr>
<tr>
<td>PtCl4(C1EN)</td>
<td>15.85</td>
<td>3.03</td>
</tr>
<tr>
<td>Pt(NO3)3(HOEn)-4H2O</td>
<td>13.55</td>
<td>4.23</td>
</tr>
<tr>
<td>Pt(NO3)3(C1EN)-H2O</td>
<td>14.18</td>
<td>3.00</td>
</tr>
<tr>
<td>Pt(ox)(HOEn)-H2O/2H2O</td>
<td>21.58</td>
<td>3.74</td>
</tr>
<tr>
<td>Pt(Hox)(NO3)2(C1EN)</td>
<td>16.33</td>
<td>3.15</td>
</tr>
<tr>
<td>PtCl4(HOEn)</td>
<td>14.69</td>
<td>3.41</td>
</tr>
<tr>
<td>PtCl4(C1EN)-H2O</td>
<td>13.71</td>
<td>2.72</td>
</tr>
<tr>
<td>PdCl4(HOEn)</td>
<td>21.93</td>
<td>5.06</td>
</tr>
<tr>
<td>PdCl4(C1EN)</td>
<td>19.64</td>
<td>3.90</td>
</tr>
<tr>
<td>Pd(ox)(HOEn)-2H2O</td>
<td>27.54</td>
<td>4.71</td>
</tr>
<tr>
<td>Pd(ox)(C1EN)</td>
<td>24.93</td>
<td>3.86</td>
</tr>
</tbody>
</table>

ox and Hox indicate divalent and monovalent oxalate ions, respectively.

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**Results and Discussion**

Since nitrogen atoms have a strong tendency to bind to Pt and Pd ions, amines or diamines have been favored as carrier ligands in contrast to more labile leaving groups. Usually Pt(II) and Pd(II) complexes take square planar structures and Pt(IV) complexes octahedral ones. Therefore, the obtained Pt(II) and Pd(II) complexes are expected to coordinate to the central metal ions through secondary amine nitrogen atoms and their putative structures are illustrated in Fig. 1. Elemental analysis of the oxalato Pt(II) complex of CIEn indicated that nitrate ions were included, and its IR spectrum also exhibited a strong peak at 1280 cm⁻¹ due to νN⁻O⁻. Therefore oxalate is considered to coordinate to Pt(II) as a unidentate ligand.

**Antitumor Activity**

The newly prepared Pt and Pd(II) complexes were tested against murine leukemia L1210 at doses of 100, 50 and 25 mg/kg and the results are shown in Table II.

Although CIEn and HOEn did not show any antitumor activity against L1210, [PtCl₂(CIEn)] showed T/C% values of 131 and 125 at doses of 50 and 25 mg/kg, respectively, while [PtCl₂(HOEn)] exhibited a T/C% value of 125 at the highest dose of 100 mg/kg. Clearly, the CIEn ligand enhanced antitumor activity in dichloro Pt(II) complexes. More significant effects were observed for Pt(IV) complexes, that is, [PtCl₄(CIEn)] exhibited T/C% values of 130 and 140 at 50 and 25 mg/kg, while the corresponding [PtCl₄(HOEn)] did not show any activity, so that the difference is considered to be attributable to that of carrier ligands.

The Pd(II) complexes of HOEn and CIEn did not show antitumor activity at any dose, possibly due to their rapid substitution reaction with biological components.

**Cell Survival**

Murine leukemia L1210 was chosen for initial screening because of its high sensitivity to Pt complexes. However, it is not necessarily sensitive to other metal complexes, so we tested the Pd(II) complexes against murine leukemia P388 in vitro. The results are collected in Table III. Although the IC₅₀ values of the Pt complexes are less than a few μM, being compatible with activity in vivo, those of the Pd(II) complexes are more than 100 μM, indicating that they are ineffective against P388.

In the case of CIEn, the nitrogen atoms function as both coordination sites and binding sites of the alkylating group, and this may have an unfavorable influence on the antitumor activity. If the coordination sites and alkylating sites can be separated, a more profound enhancement of antitumor activity might be obtained. For example, we have prepared a ligand with an alkylating group, which acts as a leaving group. The ligand is [N,N-bis(2-chloroethyl)carbamoyl]aminomalonic acid, whose Pt(II) complex with 1R,2R-cyclohexanediimine exhibited excellent antitumor activity, giving T/C% value of 263 with 3 cured mice out of 6 at the optimal dose of 6.25 mg/kg. Encouraged by this finding we are now designing a carrier ligand, which contains the alkylating group and coordination sites at different positions in the molecule.

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**References**