Antitumor Action of Two Rhodium and Ruthenium Complexes in Comparison with cis-Diamminedichloroplatinum(II)¹

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SUMMARY

Two organometallic complexes, acetylacetonate-1,5-cyclooctadienerhodium(I) ([RhacacCOD]°) and cis-dichlorotetrakisdimethylsulfoxide ruthenium(II) ([cis-Ru(II)(DMSO)₄Cl₂]°), have been examined for antitumor activity. RhacacCOD had an activity comparable with that of cis-diammine dichloroplatinum(II) (cis-PDD) against Ehrlich ascites carcinoma, yielding 100% cures at two dosages, whereas the comparative activity of [cis-Ru(II)(DMSO)₄Cl₂]° was slightly lower. Only cis-PDD and [cis-Ru(II)(DMSO)₄Cl₂]° had any significant activity against L1210 leukemia.

The minimal structural requirements for antitumor activity to be the most thoroughly investigated for antitumor activity in various experimental models (6), no previous systematic examination appears to have been done. The aim of this paper, therefore, is to report the effects of a new rhodium complex, [RhacacCOD]°, on Ehrlich ascites and L1210 leukemia growth and on the incorporation of labeled precursors into macromolecules of the tumor cells. RhacacCOD is structurally related to a series of rhodium derivatives for which antitumor properties have already been investigated and reported by some of the present authors (12). These complexes, like cis-PDD, have a cis-, square, planar configuration (Chart 1).

[RhacacCOD]°, however, differs from the previously tested complexes by having 1 bidentate oxygen donor-leaving group instead of nitrogen- or chlorine-leaving groups. [cis-Ru(II)(DMSO)₄Cl₂]° was also included in this study since, unlike rhodium derivatives, it has effects on bacteria similar to those caused by cis-PDD, such as induction of prophage and filamentous growth and greater toxicity for DNA damage repair-deficient strains of Escherichia coli (16). A histological examination of intestinal mucosa, spleen, and kidney damage caused by the compounds tested, in comparison with cis-PDD, was also performed to evaluate any possible qualitative or quantitative differences. This may be of particular relevance in the case of renal toxicity, which appears to be the major limiting factor for the therapeutic efficacy of cis-PDD (13, 14, 21).

INTRODUCTION

Among the organometallic complexes, cis-PDD² appears to be the most thoroughly investigated for antitumor activity. Since the earlier reports on its effects against Sarcoma 180 and leukemia L1210 in mice (19), it has been shown to have activity against a large spectrum of rodent tumors (22). Encouraging results have also been obtained in preliminary clinical trials (13), and data concerning its mode of action, pharmacology, and toxicology have been reported (14, 21).

The minimal structural requirements for antitumor activity by platinum complexes have been largely determined (14, 21), and elegant structure-activity relationship studies have provided new derivatives with remarkably high therapeutic indices in the experimental system used (3, 7).

Although many other transition metal complexes have been examined for antitumor activity in various experimental models (6), no previous systematic examination appears to have been done. The aim of this paper, therefore, is to report the effects of a new rhodium complex, [RhacacCOD]°, on Ehrlich ascites and L1210 leukemia growth and on the incorporation of labeled precursors into macromolecules of the tumor cells. RhacacCOD is structurally related to a series of rhodium derivatives for which antitumor properties have already been investigated and reported by some of the present authors (12). These complexes, like cis-PDD, have a cis-, square, planar configuration (Chart 1).

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MATERIALS AND METHODS

Synthesis. The rhodium and ruthenium complexes were prepared following known methods (2, 9, 15). cis-PDD was kindly provided by Professor B. Rosenberg, Department of Biophysics, Michigan State University.

Animal Treatment. cis-PDD and [cis-Ru(II)(DMSO)₄Cl₂]° were dissolved in water. [RhacacCOD]° was suspended in 1% carboxymethyl cellulose in water (w/v) by sonic disruption for 10 sec with an MSE 150-watt Ultrasonic Disintegrator at maximum power setting. Freshly prepared drug solutions were immediately injected i.p. into mice, using 0.1 to 0.2 ml per 10 g of animal weight.

Evaluation of Antitumor Activity. This was performed according to the United States National Cancer Institute protocols for Ehrlich ascites carcinoma (4) and L1210 leukemia (10).

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Antitumor Action of Organometallic Complexes

\[
\text{CIS-DICHLORODIAMINOPlatinum (II)}
\]

Antitumor Activity. cis-PDD proved to be quite effective against Ehrlich ascites carcinoma, yielding 100% cures at 2 sublethal dosages. \([\text{cis-Ru(II)(DMSO)}_4\text{Cl}_2]\)° had a pronounced activity, yielding low T/C ratios at 3 of the dosages and cures at the highest dosage, in 1 of 2 duplicate experiments (Table 1).

\([\text{RhacacCOD}]\)° showed an activity comparable with that of cis-PDD yielding 100% cures in duplicate experiments at 2 sublethal dosages. At the same time, a smaller reduction in body weight was observed. Of the compounds tested, only cis-PDD and \([\text{cis-Ru(II)(DMSO)}_4\text{Cl}_2]\)° displayed any significant activity against L1210 leukemia, yielding T/C ratios greater than 1.25 (15).

Histological Effects. The data reported in Tables 2 and 3 show that \([\text{cis-Ru(II)(DMSO)}_4\text{Cl}_2]\)° caused a pattern of histological damage qualitatively similar to that of cis-PDD but that its effects were slightly less pronounced on the intestinal mucosa and spleen at both dosages used. The effects of \([\text{RhacacCOD}]\)° are noteworthy, since at the lower dosages still capable of curing animals bearing Ehrlich ascites carcinoma cells, histological damage, compared with cis-PDD, was less pronounced in the renal structure and markedly less severe in the intestinal mucosa and spleen.

Incorporation of Labeled Precursors into Macromolecules. \([\text{RhacacCOD}]\)° caused a marked dose-dependent depression of labeled uridine incorporation into acid-insoluble material (Chart 2). Thymidine and, especially, leucine incorporation, however, were less affected. At the lowest dosage, devoid of antitumor effects, only uridine incorporation was reduced, by about 55%.

The effects caused by \([\text{cis-Ru(II)(DMSO)}_4\text{Cl}_2]\)° consisted, surprisingly, of a similar depression in DNA, RNA, and protein synthesis at the higher dosages studied. No significant alteration, however, was observed at the lower dosages still possessing antitumor activity (Chart 2).

DISCUSSION

The data reported thus far show that \([\text{RhacacCOD}]\)° and, to a lesser extent, \([\text{cis-Ru(II)(DMSO)}_4\text{Cl}_2]\)° have activity against Ehrlich ascites carcinoma comparable with that of cis-PDD. Antileukemic activity was displayed only by the ruthenium complex, where it was barely significant and slightly smaller than that of cis-PDD. Host toxicity as indicated by the reduction in body weight was less for the rhodium than for the platinum or ruthenium derivatives.
### Table 1

**In vivo effects of the organometallic complexes tested against Ehrlich ascites and L1210 leukemia**

The treatment was performed daily for 4 days, starting on Day 1 after tumor transplantation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>No. of cures*</th>
<th>% Body weight variation*</th>
<th>Toxic deaths</th>
<th>L1210 leukemia (T/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cis</em>-PDD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1/6</td>
<td>-23.7</td>
<td>5/6</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6/6</td>
<td>-16.8</td>
<td>0/6</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>8/8</td>
<td>-12.1</td>
<td>0/8</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6/6</td>
<td>-9.3</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8/8</td>
<td>-6.8</td>
<td>0/8</td>
<td></td>
</tr>
<tr>
<td><em>[cis-Ru(II)(DMSO)Cl2]</em></td>
<td>800</td>
<td>2/2</td>
<td>20.4</td>
<td>0/6</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td>565</td>
<td></td>
<td>11.9</td>
<td>0/6</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>6/6</td>
<td>-20.4</td>
<td>0/6</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.072</td>
<td>-10.3</td>
<td>0/6</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.054</td>
<td>0.5</td>
<td>0/6</td>
<td></td>
</tr>
<tr>
<td><em>[RhacacCOD]</em></td>
<td>142</td>
<td>5/5</td>
<td>-7.2</td>
<td>0/5</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td></td>
<td>4.3</td>
<td>0/5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>5/5</td>
<td>-3.2</td>
<td>0/5</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>5/5</td>
<td>-5.1</td>
<td>0/5</td>
<td>1.08</td>
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<td></td>
<td>12.5</td>
<td>0.13</td>
<td>-5.6</td>
<td>0/6</td>
<td></td>
</tr>
</tbody>
</table>

* Groups of mice ranged in number from 5 to 8.
* Three mice/group.
* Animals without tumor cells and ascitic fluid at sacrifice.
* Compared to the controls at the end of treatment.

### Table 2

**Renal histopathological alterations caused by the organometallic compounds tested**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>Thickening of glomerular epithelium and periglomerular fibrosis</th>
<th>Enlargement of descending loop of Henle</th>
<th>Degeneration of the epithelium of the proximal convoluted tubules</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cis</em>-PDD</td>
<td></td>
<td>++ + + + Extensive with focal necrosis</td>
<td>+++</td>
<td>+ + + Extensive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>++ Extensive</td>
<td>+++</td>
<td>+ + Focal</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>++ Extensive</td>
<td>+++</td>
<td>+ + Focal</td>
</tr>
<tr>
<td><em>[cis-Ru(II)(DMSO)Cl2]</em></td>
<td>400</td>
<td>++ Focal</td>
<td>+ + Focal</td>
<td>+ + Focal</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>++ Focal hemorrhagic extravasation</td>
<td>+ + Focal</td>
<td>+ + Focal</td>
</tr>
<tr>
<td><em>[RhacacCOD]</em></td>
<td>50</td>
<td>++ + + Extensive</td>
<td>+ + Focal</td>
<td>+ + Focal</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>+ Focal</td>
<td>+ + Focal</td>
<td>+ + Focal</td>
</tr>
</tbody>
</table>

* -, absent; +, light; ++, moderate; +++, severe.

The histological damage on all the tissues examined caused by the complexes appeared less pronounced for the rhodium complex, which showed remarkably little damage to the intestinal mucosa and spleen. These findings might be related to a selective toxicity for tumor tissues. The mechanism of selectivity could be the unstable oxidation state of rhodium(I) complexes, since they easily undergo oxidation to the corresponding octahedral rhodium(III) forms, which give substitution reactions at a much lower rate. The reduced redox potential of tumor tissue (5) might cause a lower rate of oxidation of the complex, thus leading to a selective inactivation of the drug in normal tissues.

In the study of precursors' incorporation, it is interesting to compare the results obtained with those reported in our study of 1,5-cyclooctadienepiperidinechlororhodium(I) (12). This complex appears to depress leucine incorporation more markedly than that of thymidine and uridine, particularly at the lowest dosage used. One could speculate that this different behavior may be attributed to their different chemical reactivities. Indeed, the lability of the leaving group(s) is greater for 1,5-cyclooctadienepiperidinechlororhodium(I) than for [RhacacCOD]*, and this difference could account for a greater ratio between nuclear and cytoplasmic concentrations of the unchanged drug in the case of the less labile complex. In turn, this could lead to interac-
Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>Small intestine degeneration of mucous membrane</th>
<th>Spleen Parenchymatous hemorrhage</th>
<th>Spleen Structural derangement</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-PDD</td>
<td>2</td>
<td>Extensive necrosis</td>
<td>++++</td>
<td>Extensive</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>[cis-Ru(II)(DMSO)4Cl]°</td>
<td>400</td>
<td>++++</td>
<td>+ Extensive</td>
<td>+ Focal</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>++++</td>
<td>+ Extensive</td>
<td>+ Focal</td>
</tr>
<tr>
<td>[RhacacCOD]°</td>
<td>50</td>
<td>Blunting of the intestinal villi</td>
<td>+ Focal</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td></td>
<td>+ Focal</td>
<td>--</td>
</tr>
</tbody>
</table>

* --, absent; +, light; ++, moderate; ++++, severe.

* Metaplasia.

A

Chart 2. Effects of the organometallic complexes tested on the incorporation of labeled precursors into macromolecules of tumor cells. Ehrlich ascites carcinoma-bearing mice were treated i.p. with [RhacacCOD]° (A) or [cis-Ru(II)(DMSO)4Cl]° (B) at the doses indicated. Four hr after sacrifice, the in vitro incorporation of labeled precursors into acid-insoluble material of tumor cells was determined as described in "Materials and Methods." Each point is the mean (± S.E.) of 4 determinations, expressed as a percentage of the controls.

Concerning the reported correlation between in vivo antitumor activity and effects on bacteria, such as induction of prophage and filamentous growth in E. coli found for platinum complexes (20), it is of interest to note that only ruthenium falls within this pattern.

Finally, these results and considerations indicate a need for a further examination of the structure-activity relationships and the mode of action of these classes of rhodium complexes.
and ruthenium derivatives. In particular, the study of the effects of ligands on the chemical reactivity, oxidizability, and physicochemical parameters such as water solubility and partition coefficients could lead to the development of agents with increased therapeutic efficacy. Part of such work is in already progress.

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REFERENCES

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