The Successful Regression of Large Solid Sarcoma 180 Tumors by Platinum Compounds

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SUMMARY

The platinum compounds, cis-platinum (IV) diamminotetracloroide, and cis-platinum (II) diamminodichloride, have been reported to be active in inhibiting and regressing small solid Sarcoma 180 tumors and Leukemia L1210 tumors in mice. They were also active in successfully regressing large (Day 8) solid Sarcoma 180 tumors in 63 to 100% of the animals, with no apparent irreversible damage to the host, in a number of dose schedules.

INTRODUCTION

We have reported recently (5) that certain compounds of platinum are effective tumor inhibitors when tested in mice against the Sarcoma 180 tumor and the Leukemia L1210 tumor. These experiments showed that the compounds were capable of inhibiting the further growth of these tumors and regressing the transplants. More recent work in our laboratory has indicated that Sarcoma 180 can be inhibited with almost 100% success in certain dose schedules given the day after tumor implantation (B. Rosenberg and L. VanCamp, unpublished results). Additional studies conducted by 2 laboratories under contract to the Cancer Chemotherapy National Service Center, National Cancer Institute, have shown that cis-platinum (II) diamminodichloride approximately doubled the life-span of mice with Leukemia L1210 at doses of 8.0 mg/kg given as a single treatment and doses of 4.0 or 8.0 mg/kg given every 4th day (J. M. Venditti, personal communication). It therefore appears that these compounds are more effective than the preliminary tests indicated. The molecular structures of the 2 compounds that have shown the highest activity against tumors out of the 34 compounds we have tested so far are shown in Chart 1. These compounds are the cis-platinum (II) diamminodichloride and the cis-platinum (IV) diamminotetracloroide.

We report here some further results in our laboratory which indicate that both of these compounds, when injected into mice with large solid Sarcoma 180 tumors, can cause successful complete regression of these large tumors in 63 to 100% of the animals.

Chart 1. Structural diagrams of the 2 platinum compounds found to be active against Sarcoma 180 and Leukemia L1210 in mice: the octahedral complex, cis-Pt(IV)(NH3)2Cl4 and the square planar complex, cis-Pt(II)(NH3)2Cl2.

MATERIALS AND METHODS

Random-bred Swiss white mice, obtained from Spartan Research Laboratories (Williamston, Mich.) were implanted, according to Cancer Chemotherapy National Service Center protocols (1), with a Sarcoma 180 tumor which was originally obtained from Microbiological Associates, Bethesda, Md., at the request of Mr. S. Poiley of the Cancer Chemotherapy National Service Center staff. This original tumor line passed through 18 transplantations in ICR mice obtained from the Rawley Farms (Plymouth, Mich.), and then through 6 further transplantations in random-bred Swiss white mice before these tests.

The platinum compounds used in these tests were synthesized in our laboratory by Mr. Thomas Krigas according to procedures which have been previously published (3, 4). The crystalline platinum compounds were purified by repeated crystallization. The compounds were sent out for analytical tests to the Galbraith Laboratories, Inc. (Knoxville, Tenn.). The sum of the deviations from the predicted values was less than 0.5%. The chemicals were stored in the crystalline state in the dark until the day of use. Solutions of the appropriate strengths were prepared in 0.9% NaCl solution with distilled water and were injected within a short period of time after preparation to avoid unnecessary photochemical and hydrolytic decomposition of these compounds.

RESULTS

Preliminary Tests of Large Tumor Regression. The tests included 36 control animals. Of these, 12 were sacrificed on...
the day of injection, Day 8 (tumor implant on Day 0), and the tumors were excised and weighed to determine the average tumor size of the animals in this experiment. The resultant average tumor size and standard deviation for this experiment was 0.96 ± 0.44 g. Of the remaining 84 animals undergoing tests, we removed 12 animals with the smallest tumors from the group, thus biasing our tests in favor of larger initial tumors. Palpation tests indicated that no tumors weighed less than approximately 0.5 g. The remaining 72 mice were divided randomly into cages of 6 each for the various dose schedules. The doses used and the final results are given in Table 1 below. We chose dose schedules to include low doses on a daily basis as well as higher doses in single injections for both the cis-platinum (II) diamminodichloride and the cis-platinum (IV) diamminotetrachloride. The animals were weighed and any deaths were noted daily.

In testing the effects of chemicals on large, solid Sarcoma 180 tumors, we lose the ability to define precisely the cause of death as being due either to the presence of large tumors or to the effects of the platinum compounds. In a few cases, particularly of the daily low-dose injections, it was obvious that the platinum compounds were causing death. In all other cases, the cause of death, even after autopsy, was ambiguous.

Mice given single injections in the effective dose range began to lose weight after Day 8. This was expected from previous work. The cage weights diminished until Day 12, after which they started to rise again. In the control tumor animals, the weights began to drop slightly on Day 8 and usually continued to do so until death or spontaneous regression of the tumors. A typical plot of the average weight per mouse starting on Day 8, for 2 cages, 1 control and 1 treatment, are shown in Chart 2. This chart also indicates the days on which the tumors in the treated animals had completely disappeared and the days on which the control animals died.

In animals treated with effective doses (see Table 1), the size of the tumor remained static for a period of about 5 days after inoculation, while the control tumors continued to increase in size. Many of the tumors showed breakthroughs, with extensive necrotic tissue in the center of the tumors. In almost all of the treated animals that survived these tests, the tumors appear to have completely dropped

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose schedule (mg/kg)</th>
<th>No. of mice in test</th>
<th>Deaths</th>
<th>No. of cures</th>
<th>% cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Platinum(II)(NH₃)₂Cl₂</td>
<td>2.0 daily</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>0⁰⁺⁺</td>
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<tr>
<td>cis-Platinum(II)(NH₃)₂Cl₂</td>
<td>4.0, Day 8</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>17ᵇ</td>
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<tr>
<td>cis-Platinum(II)(NH₃)₂Cl₂</td>
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<td>6</td>
<td>2</td>
<td>4</td>
<td>67</td>
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<tr>
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<td>6.0, Day 8</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>cis-Platinum(II)(NH₃)₂Cl₂</td>
<td>6.0, Days 8 and 16</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>cis-Platinum(II)(NH₃)₂Cl₂</td>
<td>8.0, Day 8</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>4.0 daily</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>17ᵇ</td>
</tr>
<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>6.0, Day 8</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>17ᵇ</td>
</tr>
<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>6.0, Days 8 and 15</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>50</td>
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<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>8.0, Day 8</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>8.0, Days 8, 16, and 23</td>
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<td>1</td>
<td>5</td>
<td>83</td>
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<tr>
<td>cis-Platinum(IV)(NH₃)₂Cl₄</td>
<td>10.0, Day 8</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>17ᵇ</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>24</td>
<td>20</td>
<td>4</td>
<td>17ᵇ</td>
</tr>
</tbody>
</table>

*⁰⁺⁺ Deaths due to platinum compound.
ᵇ Expected spontaneous regression rate.
out, leaving an open wound in the tumored area. It is possible that, when the tumors became necrotic, the normal grooming activities of the animals removed all of this necrotic tissue. The skin then formed a flap and a scab, which slowly, over the next week or 2, healed to a modest scar, which then disappeared in the regrowth of hair over the area. This sequence occurred in spontaneous regression as well as in the treated animals. We have seen only 2 tumors disappear by internal resorption of the tissue. We shall therefore call the regressions caused either by the treatment or spontaneously which over a period of 5 to 6 months showed no new appearance of any tumor tissue “cures.”

Table 1 shows that single injections of 8.0 mg/kg of the cis-[Pt(NH3)2Cl2] have produced 6 out of 6 cures (100%). In addition, the 2 injections of 4.0 mg/kg of this compound, and 3 injections of 8.0 mg/kg each of the cis-[Pt(NH3)2Cl4] produced 5 out of 6 cures (83%). In these 2 cases the single death in each cage was a tumor death, occurring on Day 26 and Day 50, respectively. The controls had 4 spontaneous regressions of the tumor out of 24 animals (17%), which is slightly higher than previously reported rates of 8 to 10% (6) of spontaneous regression of this tumor, but is consistent with our other experiences with this tumor. It is obvious therefore that these 3 dose levels have produced cures at a significantly higher rate than spontaneous regression.

All animals in this sequence of tests that have been cured appear to be healthy, with sleek fur and weight gain. We can find no evidence of irreversible damage in these animals upon autopsy. In 2 cages we have noticed a small amount of hair loss about the nose or scalp 30 days after the treatment in some of the animals. This hair loss was reversible and after 2 weeks the hair returned and appeared quite normal.

From the results shown in Table 1, we can compare the data for the single injections of 4.0, 6.0, and 8.0 mg/kg of the cis-platinum (II) diamminodichloride as an indication of the dose-response curve. Our previous experience indicated that a single injection of 10 mg/kg produced about 20% deaths due to the platinum compound, and higher doses produced an increasingly larger number of deaths. It is obvious, therefore, that the dose-response curve for this compound for single injections is very narrow; however, the major point to be taken from this table is that at some dose level 100% cures can be effected.

Confirmatory Tests of Large Tumor Regression. To confirm the results given above with a large sampling of animals, we have repeated the test on a group of animals including 30 controls and 30 treated animals. Again, the same tumor and animal species were used, and the injection was given on Day 8 after tumor implantation. The animals were organized into 6 cages of 10 each. For this test we chose the optimum dose indicated in the previous test of 8.0 mg/kg single i.p. injections of the cis-platinum (II) diamminodichloride. The results of this test are shown in Table 2. In this case the spontaneous regression rate was 13%, which is slightly lower than the previous case but within the range of variability of previous spontaneous regression rates. Of the 30 animals in the treatment category, the platinum compound produced a cure rate of 19 out of the 30 animals (63%).

In these tests 9 of the treated animals did not show any tumor regression within a 2-week period after the injection. These animals were given a 2nd injection of the same compound at a dose of 4.0 mg/kg, and 3 of these 9 then showed tumor regression to the cure point and were included in the statistics given in Table 2.

Autopsies of some similarly treated animals 6 days after injection indicated that the tumors had lost the extensive blood supply the tumor usually recruits, that the tumors were extensively necrotic, and that, even in the healthier appearing tumor tissue, the mitotic indices were extremely low in comparison to untreated tumors.

DISCUSSION

It is obvious from the data given in Tables 1 and 2 that the platinum compounds, cis-platinum(II) diamminochloride and cis-platinum(IV) diamminotetrachloride, are both capable of completely regressing large solid Sarcoma 180 tumors in the Swiss white mice with about 63 to 100% success. The mice survived and appeared to be healthy. After a period of 6 months, the cured animals did not show any signs of a resurgence of the tumor. An extensive survey of the literature on the solid Sarcoma 180 tumor failed to turn up any other chemotherapeutic agent capable of successfully regressing such large tumors. These platinum compounds are therefore the first chemotherapeutic agents able to accomplish this regression. Clarke et al. (2) reported that treatment of Sarcoma 180 tumors with 6-mercaptopurine initiated 96 hr after tumor implantation did promote

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose schedule (mg/kg)</th>
<th>No. of mice in test</th>
<th>Deaths</th>
<th>No. of cures</th>
<th>% cures</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Platinum(II)(NH3)2Cl2</td>
<td>8.0, Day 8</td>
<td>21</td>
<td>5</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0, Day 8, and 4.0, Day 24</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>30</td>
<td>11</td>
<td>19</td>
<td>63</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>30</td>
<td>26</td>
<td>4</td>
<td>13</td>
</tr>
</tbody>
</table>
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approximately 50% eventual recovery. However, these tumor masses were an order of magnitude smaller than the initial tumor masses we have treated here. Also, they report that the tumors disappeared by resorption, rather than extrusion as we have observed.

These platinum compounds exhibit a very steep slope in the dose-response curve. Some data obtained by the Cancer Chemotherapy National Service Center on the percentage of increase in life-span of BDF1 mice with Leukemia L1210 (6) with the same dose schedule and chemical indicates roughly the same steep slope for the rising portion of the dose-response curve.

At the present time we are investigating the in vivo fate of the injected platinum compounds. We speculate, on the basis of the above results as one possible mechanism of action, that there must be a very large uptake rate of the tumor tissue for the platinum compound. It is also quite probable that the platinum compound remains in situ in the tumor with a long lifetime, while in the normal tissue of the animal it has a short lifetime. This is suggested by the recovery of the weight of the animal after 6 days, whereas tumors have disappeared sometimes 20 days or more after injection. We are now investigating the distribution and lifetime of the injected platinum in these animals with neutron activation techniques. Two other alternate hypotheses are (a) that the tumor tissue is more sensitive to the destructive action of the platinum compounds than is normal tissue [some studies presently underway of comparisons in in vitro tissue culture of normal and transformed cells' sensitivity to the cis-platinum(II) diamminodichloride indicate this (H. Harder, unpublished results)]; or (b) the antitumor activity is an indirect result of the stimulation of the immune mechanism of the host. This stimulation of the immune mechanism of the host has been suggested by one of the referees of this paper. We had already begun a series of tests on the cured animals to test if they would reject a reimplantation of the tumor. In the Swiss white mice, the tumor is completely rejected in 100% of the cured animals. The full details of these tests will be reported elsewhere.

ACKNOWLEDGMENTS

We thank Mr. Thomas Krigas and Miss Gail Valentine for their excellent assistance in the experimental work.

REFERENCES

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