Efficacy of New Platinum Analog DPPE in an Orthotopic Nude Mouse Model of Human Colon Cancer

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Abstract. A surgical orthotopic implantation (S.O.I.) model of the human colon cancer cell line Co-3 in nude mice was treated with two doses of the new platinum analogs \( \text{Pt(cis-dach)(DPPE)2NO}_3 \) and \( \text{Pt(trans-dach)(DPPE)2NO}_3 \). The analogs were evaluated for antimitastatic efficacy in comparison to two doses of cisplatin. Unlike the untreated control group, there were no mesenteric lymph node metastases in the groups treated with the high or low doses of both forms of new DPPE platinum analogs as well as cisplatin-treated group. However, much more body-weight loss occurred in the cisplatin-treated group than the DPPE-treated groups. The results obtained with S.O.I animal model of colon cancer demonstrated both cis- and trans-forms of DPPE had as strong an inhibitory effect on metastasis as that of cisplatin, but with much less toxicity. Thus the new platinum analogs appear to have promising clinical potential.

Cisplatinum induced nephrotoxicity is a major dose-limiting factor (1). There have been numerous attempts to systematically synthesized and characterize second and third-generation platinum compounds in the hope of identifying a platinum anticancer agent with less toxicity as well as a broad tumor range with improved efficacy against resistant tumors. Most of these platinum complexes have the 1,2-diaminocyclohexane (dach) carrier ligand, which decreases the nephrotoxicity of platinum (2). However, previous dach-platinum complexes appeared to be unsuitable for clinical development due to poor solubility and/or stability (3).

We have synthesized a new platinum analog containing DACH as a carrier ligand and 1,2-bis(diphenylphosphino)ethane (DPPE) as a leaving group. These nitrile salts improved water solubility and stability. Previously, we have shown that this new platinum analog has much less nephrotoxicity than cisplatinum (4) and similar or more potent anticancer efficacy than cisplatinum (5) with antimitastatic efficacy in a surgical orthotopic implantation (S.O.I) model of human bladder tumor (6).

The present study was designed to test ability of the new platinum analog DPPE administered intraperitoneally to inhibit the metastasis of a human colon carcinoma in comparison with cisplatinum following orthotopic implantation of the human colon carcinoma Co-3 on the colon of athymic nude mice.

In this study, we report the antimitastatic efficacy of the new platinum analogs cis-and trans-form DPPE on this Co-3 human colon cancer model.

Materials and Methods

Animals. Male and female CD-I athymic BALB/C nude mice between 4 and 5 weeks of age were used for this study. The animal were bred and maintained in a HEPA filtered environment with cages, food and bedding sterilized by autoclaving. The breeding pairs were obtained from Charles River Laboratories (Madison, WI, U.S.A.).

Study drugs. Cis- and trans-form of the synthetic platinum analog DPPE were supplied by Kyung Hee University, Seoul, Korea and stored at room temperature. Drug solutions were obtained by dissolving the compound in saline.

Human colon cancer cell lines. The human colon cancer cell lines Co-3 was used in this study. This cell line has been maintained in AntiCancer's animal facility by subcutaneous passage.

Tumor tissue fragments used for orthotopic implantation. Stock tumor tissues were from the last subcutaneous passage. Tumors were harvested when in log phase. Necrotic tissues were removed and fresh viable tissues were cut into 1mm3 pieces for subsequent implantation.

Orthotopic implantation. Surgical orthotopic implantation (S.O.I) was carried out as follows: The animals were anesthetized with isoflurane and the surgical area was sterilized using an iodine solution and alcohol. An incision approximately one cm long was made along the midline in the lower abdomen of the nude mouse using a pair of sterile scissors. The ascending colon was exposed. Six to eight pieces of tumor fragments of one mm3 were transplanted on the serosal surface of the ascending colon next to cecum with sterile 8-0 surgical sutures (nylon) after the serosa on the site has been stripped. The abdomen was closed.
with sterile 6-0 silk sutures. All surgical procedures were conducted under HEPA-filtered laminar flow hoods.

Study animals. All animals without tumor take were excluded from the study as well as animals with very small or larger tumors prior to the start of dosing. The orthotopically transplanted animals used for the study were selected to establish groups of similar mean tumor size as determined by caliper measurement.

Drug treatment. Administration of the test compounds and cisplatinum was begun when SO1 tumors had reached an approximate mean caliper-measured size of 55-65 mm³. All animals in all groups were dosed intraperitoneally based on a schedule of day 1, day 2 and day 7, for a total of 3 doses. Day 1 represents the day of first treatment. The doses of the drugs used were 4.5 mg/kg and 9 mg/kg for cisplatinum and 25 mg/kg and 50 mg/kg for the new platinum analogs, which were equivalent to the LD₅₀ and 2 x LD₅₀, respectively, for cisplatinum, and 30% and 60% of the LD₅₀, respectively, for the platinum analogs. All the test animals were sacrificed by CO₂ inhalation at day 14 after the first treatment.

Primary tumor size and weight. Primary tumor size and body weights for each animal were measured on a weekly basis. Primary tumor sizes were estimated by measuring the perpendicular minor dimension (W) and major dimension (L) using sliding calipers. Approximate tumor area was calculated by W x L. Body weight was measured by an electronic balance at time of tumor measurement. Upon necropsy and fixation of tissues in formalin, the weight of the primary tumors was obtained by weighing them on an electronic balance.

Histologic assessment of primary and metastatic tumors. Gross examination for metastasis was conducted at necropsy. Tissue samples of the primary tumor and those from relevant organ (lung, liver and mesenteric lymph node etc) were processed through standard procedures of H and E staining for subsequent microscopic examination.

Statistical methods used in efficacy evaluation. The primary tumor sizes measured on day 7 after the first treatment and tumor weight at necropsy were analyzed using ANOVA (with Turkey adjustment) with α=0.05 (two-sided). The metastic rate in each treatment group was compared with that control by the Fisher-exact test.

Table 1. Mean body weight at 7 days after initial treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body weight(g)</th>
<th>p value*</th>
<th>p value**</th>
<th>p value***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.63±.85</td>
<td></td>
<td>0.031</td>
<td>0.768</td>
</tr>
<tr>
<td>cis-DPPE 50mg/kg</td>
<td>22.80±2.26</td>
<td>0.884</td>
<td>0.009</td>
<td>0.281</td>
</tr>
<tr>
<td>cis-DPPE25mg/kg</td>
<td>22.16±2.85</td>
<td>0.966</td>
<td>0.011</td>
<td>0.383</td>
</tr>
<tr>
<td>trans-DPPE 50mg/kg</td>
<td>23.88±3.25</td>
<td>0.551</td>
<td>0.002</td>
<td>0.103</td>
</tr>
<tr>
<td>trans-DPPE 25mg/kg</td>
<td>22.42±1.74</td>
<td>0.930</td>
<td>0.008</td>
<td>0.311</td>
</tr>
<tr>
<td>Cisplatin 9.0mg/kg</td>
<td>14.60±.96</td>
<td>0.031</td>
<td>-</td>
<td>0.550</td>
</tr>
<tr>
<td>Cisplatin 4.5mg/kg</td>
<td>18.22±4.92</td>
<td>0.768</td>
<td>0.550</td>
<td>-</td>
</tr>
</tbody>
</table>

*All treated groups versus control
**Cisplatin 9.0mg/kg versus all other groups
***Cisplatin 4.5mg/kg versus all other groups

Results

Body weight. Unlike cisplatinum treatment, the body weight curve for the test compounds failed to show any apparent toxic effects from the two doses of new platinum analogs...
(Pt(cis-dach) (DPPE)-2NO3) or (Pt(trans-dach) (DPPE), 2NO3). The mean body weights at 7 days after initial treatment with the DPPE analogs did not have a statistically significant difference from the control group.

However, there was a statistically significant difference between the control group and the groups treated with the two doses of cisplatin (Table I). In the case of the cisplatinum treated animals, the body weights significantly decreased during the duration of the experiments (Figure 1).

Change of transplanted primary tumor volume. The size of the primary tumor was measured on a weekly using sliding calipers. Mean tumor area decreased in the platinum analog-treated groups compared with the control group. However, the new compounds did not show a statistically significant difference compared to the saline control group (Table II and Figure 2).

Primary tumor weight. Upon necropsy and fixation in formalin, the weights of the primary tumors were obtained by weighing them on an electronic balance. Primary tumor weight did not show a statistically significant difference between the test compound and saline control (Table III).

Micrometastasis. Five of 7 mice had mesenteric lymph node metastasis in the saline control groups (Figure 3). However, the animals treated with the low and high dose of both forms of DPPE platinum analogs had no metastases. The two doses of cisplatinum also eliminated metastases (Table IV).

Table II. Mean tumor area at 7 days after initial treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean tumor area (mm²)</th>
<th>p val</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>158.92 ± 38.80</td>
<td>-</td>
</tr>
<tr>
<td>cis-DPPE 50 mg/kg</td>
<td>115.69 ± 13.78</td>
<td>0.2</td>
</tr>
<tr>
<td>cis-DPPE 25 mg/kg</td>
<td>116.76 ± 32.79</td>
<td>0.11</td>
</tr>
<tr>
<td>trans-DPPE 50 mg/kg</td>
<td>127.58 ± 23.30</td>
<td>0.5</td>
</tr>
<tr>
<td>trans-DPPE 25 mg/kg</td>
<td>111.80 ± 36.48</td>
<td>0.11</td>
</tr>
<tr>
<td>Cisplatin 9.0 mg/kg</td>
<td>32.0 ± 18.56</td>
<td>0.00</td>
</tr>
<tr>
<td>Cisplatin 4.5 mg/kg</td>
<td>61.4 ± 15.82</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* All values were obtained by comparing the treated with the control.

Discussion

Since the antitumor activity of cisplatinum was first described by Rosenberg (7-9), it has become a first-line chemotherapeutic agent for many types of human cancers. Approximately 75% of patients with disseminated germ cell tumors are curable with cisplatinum-based combination chemotherapy (10). However, cisplatinum induced nephrotoxicity is a major dose limiting factor for other types of tumors treated with cisplatinum (1).

Our drug development program is aimed at produ
drugs capable of broadening the anticancer effect and decreasing the toxicity of platinum agents. To achieve these objectives, we have recently synthesized DPPE platinum analogs. We previously reported that DPPE has very low nephrotoxicity and significant anticancer efficacy in an in vitro study of histocultured human patient bladder tumors and cancer cell lines (5).

This new platinum analogs had antimetastatic efficacy in a surgical orthotopic implantation (SOI) model of human bladder cancer (6).

SOI models were used in the present studies since they were better reflect the clinical properties of human cancer including metastases (11-16) and have been shown to be valuable for the development of other new drugs and treatment strategies for cancer (12, 17, 18).

This study was designed to test the ability of the new DPPE platinum analogs administered intraperitoneally to inhibit the metastasis of a human colon carcinoma following
surgical orthotopic implantation of tumor tissues on the ascending colon of athymic nude mice.

The test compounds exerted a very strong anti-metastatic effect compared to the saline control with statistical significance. The control animals consisted of five mice demonstrating mesenteric lymph node metastasis while none of the animals treated with the test-compound (both forms) groups had metastatic lesions.

Based on these results, both forms of the test compounds (trans- and cis-) DPPE have strong antimetastatic efficacy with against the human colon cancer Co-3 orthotopically implanted in nude mice. Unlike cisplatinum, the new platinum compounds did not demonstrate toxicity in this nude mice model of human colon cancer as indicated by body weight loss. The new compounds thus have strong promise for further development.

Acknowledgements

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References


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