Antimetastatic Activity of the New Platinum Analog \{\text{Pt}(\text{cis-dach}) (DPPE)\cdot2\text{NO}_3\}\ in a Metastatic Model of Human Bladder Cancer

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Abstract. Surgical orthotopic implantation (SOI) of histologically intact human RT-4 bladder tumor tissue in nude mice resulted in local growth, invasion, regional extension and metastases as well as distant metastases to other organ sites and lymph nodes, thus mimicking the bladder cancer patient. This metastatic bladder tumor animal model was treated with two doses of new platinum analog \{\text{Pt}(\text{cis-dach})(\text{DPPE})\cdot2\text{NO}_3\}\ for the evaluation of antimetastatic efficacy compared to two doses of cisplatin. Unlike the untreated control group or the group treated with the low dose of cisplatin, there were no metastases in either the high or low-dose platinum-analog-treated groups and the high-dose cisplatin-treated group. The results obtained with this patient-like nude-mouse model of bladder cancer indicate that the new platinum analog appears to be a valuable lead compound with antimetastatic efficacy and clinical potential.

Cis-diamminodichloroplatinum (cisplatin) is a first line chemotherapeutic agent for the treatment of testicular cancer, bladder cancer, and other cancers. However, the efficacy of cisplatin is limited by several dose-limiting toxicities, including nephrotoxicity, nausea, neurotoxicity, ototoxicity and myelosuppression (1,2).

Consequently, there is a need to obtain platinum analogs that have less toxicity and have more favorable therapeutic indices. To accomplish this goal, we have synthesized a new platinum analog containing DACH as a carrier ligand and 1,2-bis(diphenyl phosphino)ethane (DPPE) as a leaving group (1,2). Previously, we have shown that this new platinum analog has much less nephrotoxicity than cisplatin (1) and similar or more potent anticancer efficacy than cisplatin (2).

In particular, we now wish to evaluate the antimetastatic efficacy of the platinum analog. The surgical orthotopic implant (SOI) models that our laboratories have developed have been shown to mimic metastatic clinical human cancer (11). In this study we utilized SOI to transplant the human RT-4 bladder tumor xenograft to nude mice (3). This SOI model of human bladder cancer results in extensive local growth and invasion as well as regional and distant metastasis.

In the present study we report the antimetastatic efficacy of a new platinum analog on the SOI RT-4 bladder tumor model.

Materials and Methods

Mice. Four-week-old outbred nu/nu mice of both sexes were used for tumor implantation. All animals were maintained in a sterile environment; cage, bedding, food, and water were all autoclaved. All animals were maintained on a daily 12-hour light/12-hour dark cycle. Bethaprime pediatric suspension (containing sulfamezoxazole and trimethoprim) was added to the drinking water. Four-to-six-week outbred nu/nu mice of both sexes were used for subcutaneous injection and for the surgical orthotopic implantation (SOI) experiments.

Surgical orthotopic implantation. RT-4 human bladder tumor cells were obtained from the ATCC (Rockville, MD, USA) and grown into subcutaneous tumor tissue in 4-week-old outbred female nude mice. Two months later, the subcutaneous growth tumors were excised and cut into 2 mm³ pieces to be used for surgical orthotopic implantation (SOI) previously described (3-5). Briefly, the nude mice were anesthetized by isoflurane inhalation and the lower abdomen was sterilized with iodine and alcohol swabs. A small midline incision was made and the urinary bladder was exposed. The surgical adhesive 2-cyanacrylate acid ester was applied on one side of the 2 mm³ tumor.
xenograft tissue and 5 pieces of tumor were glued on the serosa of the urinary bladder. The abdominal incision was closed with 7-0 silk surgical sutures in one layer. The animals were then kept in a sterile environment.

Drug treatment. Four weeks after SOI, mice were randomized into control and treated groups and cisplatinum and the new platinum analog (Pt(cis-dach)(DPPE) - 2NO3) (1) were administering. Both agents were dissolved in 0.2 ml physiological saline solution and administered intraperitoneally (ip) as bolus. 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 analog, which were equivalent to the LD50 and 2 x LD50 respectively, for cisplatinum, and 30% and 60% of the LD50 respectively, for the platinum analog. The drugs were given ip on day 1, 2, and 7. Mice were observed every day and sacrificed on the 7th day after the last injection. At the time of sacrifice, the tumors on the bladder wall were then removed from each mouse and weighed. All major organs were grossly examined for metastasis. Each organ and lymph node were then fixed in 10% formol, dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic evaluation of metastases.

Results and Discussion

The mice were sacrificed one week after the last treatment. By this time, the orthotopically implanted fragments of RT-4 tumor tissue had fused and become a well growing tissue on the bladder. Histopathologic findings revealed a typical well-differentiated transitional-cell carcinoma (Figure 1A,B). Figure 1C and 1D show the primary bladder tumor and abdominal lymph node metastatic lesion in a mouse from the low-dose cisplatinum (4.5 mg/kg) treated group. These tumors were also well-differentiated transitional cell carcinoma.

However, as shown in Table 1 and Figure 2, the animals treated with the low- and high-dose DPPE cisplatinum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of metastases/No. of animals</th>
<th>Site of metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2/5</td>
<td>Abdominal wall</td>
</tr>
<tr>
<td>Cisplatinum (9 mg/kg)</td>
<td>0/5</td>
<td>None detected</td>
</tr>
<tr>
<td>Cisplatinum (4.5 mg/kg)</td>
<td>2/5</td>
<td>Abdominal wall</td>
</tr>
<tr>
<td>DPPE* (50 mg/kg)</td>
<td>0/5</td>
<td>Abdominal LN**</td>
</tr>
<tr>
<td>DPPE (25 mg/kg)</td>
<td>0/5</td>
<td>None detected</td>
</tr>
</tbody>
</table>

* DPPE: (Pt(Cis-dach)(diphenylphosphinoethane) - 2NO3)
** LN: lymph node
In this study, we utilized an orthotopically implanted bladder tumor model to evaluate the anticancer efficacy of DPPE. There were no metastatic lesions in the DPPE-treated groups including the low dose treated group. Based on these results, this new platinum analog is a promising developing anticancer chemo-therapeutic agent with anticancer activity with clinical potential.

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