Advances in Brief

Transplacental Carcinogenicity of Cisplatin: Initiation of Skin Tumors and Induction of Other Preneoplastic and Neoplastic Lesions in SENCAR Mice

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Abstract

Cis-Dichlorodiammineplatinum (cis-DPP), an anticancer agent sometimes used in pregnant women for the treatment of malignant ovarian and uterine tumors, was tested for transplacental carcinogenic and/or tumor-initiating effects in SENCAR mice. Pregnant mice were given a single i.p. injection of either cis-DPP (7.5 mg/kg body weight) in 2.5% NaCl or the same weight-adjusted volume of NaCl (5 ml/kg body weight) on day 17 of gestation. Offspring were delivered and raised by their natural mothers until weaning at 3 weeks of age. Starting at week 4, offspring in experimental groups received topical applications of 2 µg 12-O-tetradecanoylphorbol-13-acetate (TPA) in acetone twice a week for 20 weeks while those in control groups received only acetone (0.2 ml/application) for the same duration. The experiment was terminated at 25 weeks of age. A high incidence (18 of 37; 48.7%) of papillomas was observed in offspring exposed transplacentally to cis-DPP and postnatally to TPA, while only 10% (4 of 40) of offspring exposed to TPA alone developed such tumors (P < 0.0002). Although no skin tumors were observed without TPA promotion, transplacental administration of cis-DPP resulted in development of thymic lymphomas, lung tumors, and proliferative kidney lesions in offspring. These results provide the first evidence that cis-DPP can initiate and/or induce preneoplastic and neoplastic lesions in multiple tissues transplacentally.

Introduction

cis-DPP, a broad-spectrum antineoplastic drug, has been widely used by itself and in combination for the treatment of various types of cancers including ovarian and testicular carcinomas; carcinomas of the head, neck, urinary bladder, and prostate; and pediatric osteogenic sarcomas. The drug, however, has been shown to cause a variety of mutagenic effects in mammalian cells in culture (1, 2) and is carcinogenic in both mice and rats (3, 4). In a standard initiation-promotion model, cis-DPP initiated skin tumors in CD-1 mice (5) and enhanced conversion of skin tumors from benign to malignant phenotype in SENCAR mice (6).

In pregnant mice, cis-DPP has been shown to cross the placenta and enter the embryonal/fetal compartment (7). Cisplatin-DNA adduct formation has been documented in maternal and fetal tissues of patas (Erythrocebus patas) monkey dams exposed to cis-DPP during pregnancy (8). cis-DPP is highly embryolethal in rats and mice (9, 10). However, no reports have been published on possible transplacental carcinogenic effects of cis-DPP in any experimental species. Since cis-DPP or cis-DPP-based chemotherapy is used during pregnancy in human patients for the treatment of malignant ovarian and uterine tumors (11–13), and cis-DPP can reach the infant through the mother’s milk (14), the potential transplacental and translactational effects of maternal cancer chemotherapy remain a major concern to clinicians caring for the infants of these patients. It is estimated that about 3500 cases of cancer (0.8% of all cancer cases in women) occur in pregnant women annually in the United States (15). We, therefore, investigated the transplacental carcinogenic and/or tumor initiating effects of cis-DPP in different rodent species. In the present report, we describe our results on the tumor-initiating effects of cis-DPP in mouse skin after a single transplacental exposure to this drug. This regimen of cis-DPP was also found to induce preneoplastic and neoplastic lesions in other tissues of offspring exposed during the final week of gestation.

Materials and Methods

Male and female SENCAR mice, 6–8 weeks old, were obtained from the Animal Production Area of the NCI-Frederick Cancer Research and Development Center. They were caged together overnight and females were checked the next morning for vaginal plugs. The day on which a vaginal plug was observed was counted as day 1 of gestation. The females were palpated two weeks later to confirm pregnancy.

cis-DPP (Sigma Chemical Company, St. Louis, MO) was dissolved in 2.5% sterile aqueous sodium chloride solution. Fig. 1 shows the experimental design. Pregnant mice received either a single i.p. injection of cis-DPP (7.5 mg/kg body weight; group 1) or the same volume (5 ml/kg body weight; group 2) of 2.5% NaCl on day 17 of gestation. Pregnant mice were allowed to deliver normally and the number of live offspring per litter was recorded. After sexing at 3 weeks of age, offspring from each litter treated with cis-DPP (group 1) or vehicle (group 2) were distributed randomly into two subgroups (groups 1A and 2A, respectively) of approximately equal number of males and females each. Starting at 4 weeks of age, offspring of groups 1B and 2B received topical applications of 2 µg TPA (Sigma) in acetone, twice a week for 20 weeks (0.2 ml/application), while those of groups 1A and 2A received only acetone (0.2 ml/application) for the same duration. Skin tumors were counted and tabulated weekly for the duration of the experiment. Animals were sacrificed at 25 weeks of age.

At necropsy, all skin tumors, tumors of other organs and any abnormal lesions were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 µm, and routinely stained with hematoxylin and eosin for histological evaluations.

Results and Discussion

Although cis-DPP has been shown to initiate skin tumors in adult mice (3, 5), the present report showed for the first time its effectiveness in initiating such tumors in fetal mice. The incidence, multiplicity, and average size of skin tumors in offspring of mice at 25 weeks of age are summarized in Table 1. No skin tumors were observed in offspring exposed to transplacental cis-DPP alone (group 1A) or vehicle alone (group 2A). On the other hand, many offspring (48.7%; Fig. 2) in group 1B (transplacental cis-DPP/postnatal TPA) developed skin tumors during the experimental period. A low incidence (4 of 40; 10%) of offspring in group 2B that were exposed transplacentally to vehicle and treated postnatally with TPA developed a single skin tumor each (Fig. 2; Table 1). Multiple tumors occurred in offspring of...
group 1B; these tumors were also larger in size than in those of group 2B. As shown in Fig. 2, skin tumors also occurred earlier in offspring of m-DDP transplacental tumor-initiating activity of m-DDP in SENCAR mice. Of sacrifice (25 weeks of age), many offspring in groups 1A (7 of 20; 35%) and IB (9 of 37; 24%) developed preneoplastic or dysplastic foci of renal cortical tubular epithelium (Table 2). The epithelium lining dysplastic foci exhibited varying degrees of cellular atypia and more layers of irregular epithelium; in a few cases, atypical cells occupied most or all of a single lumen. No sex difference in the incidence of such lesions was observed in any group. Nephropathy, such as focal degenerative and regenerative tubular changes, dilated or cystic tubules, and glomerular degeneration or necrosis, was commonly seen in offspring exposed to transplacental cis-DDP with or without postnatal exposure to TPA. Moderate to severe hyperplasia of pelvic transitional cell epithelium was observed in three mice receiving cis-DDP transplacently (groups 1A and B) while no such lesions were observed in groups 2A and 2B.

Nephrotoxic effects of cis-DDP have been studied extensively in both experimental animals (16) and humans (17, 18). In fact, a significant impairment in renal function, characterized by elevated blood urea nitrogen and plasma creatinine concentrations and reduction in glomerular functions, remain a major limiting factor in the use of this drug in humans. In rats, cis-DDP administration caused renal cyst formation 1–6 months after a single i.p. injection (19). More recently, Bulger and Dobyan (20) reported the development of hyperplastic lesions of the proximal tubular epithelium and of the epithelium of the renal papilla in rat kidneys following a single dose of cisplatin. Similar proliferative lesions of altered proximal tubular epithelial cells and pelvic transitional epithelial cells were found in offspring of mice exposed transplacently to cis-DDP in this study.

![Fig. 1. Experimental design for the determination of transplacental carcinogenic and/or tumor-initiating activity of cis-DDP in SENCAR mice.](image)

![Fig. 2. Incidence of skin papillomas in cis-DDP-initiated or uninitiated offspring exposed postnatally to TPA.](image)

### Table 1 Skin papillomas initiated transplacently by cis-DDP and promoted postnatally by TPA in Sencar mice

<table>
<thead>
<tr>
<th>Treatment (group)</th>
<th>Incidence (%)</th>
<th>No. of papillomas/mouse with papillomas</th>
<th>Mean no. of papillomas/mouse with papillomas (mean ± SD)</th>
<th>Av. diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-DDP/TPA (1B)</td>
<td>48.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1–5</td>
<td>1.8 ± 1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>6.3</td>
</tr>
<tr>
<td>Vehicle/TPA (2B)</td>
<td>10.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1</td>
<td>1.0 ± 0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

<sup>a</sup> cis-DDP/TPA, 37 mice (17 male and 20 female); vehicle/TPA, 40 mice (20 male and 20 female).

<sup>b</sup> P < 0.00024 compared to vehicle/TPA group by Fisher's exact test.

<sup>*</sup> P = 0.085 compared to vehicle/TPA group by Mann-Whitney U test.

### Table 2 Induction of preneoplastic and neoplastic lesions in internal organs of mouse offspring exposed prenatally to cis-DDP and postnatally to TPA

<table>
<thead>
<tr>
<th>Treatment groups (no.)</th>
<th>Total no. of offspring</th>
<th>Lymphomas</th>
<th>Lung tumors</th>
<th>Incidence</th>
<th>Multiplicity (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-DDP/acetone (1A)</td>
<td>20</td>
<td>4 (20)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3 (15)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 (35)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>2.5 ± 1.3</td>
</tr>
<tr>
<td>cis-DDP/TPA (1B)</td>
<td>37</td>
<td>5 (14)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2 (5)</td>
<td>9 (24)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.6 ± 1.3</td>
</tr>
<tr>
<td>Vehicle/acetone (2A)</td>
<td>40</td>
<td>0</td>
<td>1 (3)</td>
<td>0</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Vehicle/TPA (2B)</td>
<td>40</td>
<td>0</td>
<td>2 (5)</td>
<td>0</td>
<td>1.5 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01 compared to vehicle/TPA group by Fisher's exact test.

<sup>b</sup> Numbers in parentheses, percentage.

<sup>c</sup> P < 0.05 groups 1A + B combined (5 of 57) versus groups 2A + B combined (1 of 80).

<sup>*</sup> P < 0.02 compared to vehicle/TPA group by Fisher's exact test.
In summary, we have found that cis-DDP, an important antineoplastic agent, is an effective transplacental initiator of skin tumors in mice. Prenatal administration of cis-DDP also induced preneoplastic and neoplastic lesions in pulmonary and renal epithelium and thymic lymphoid tissue. Our results suggest that infants of cancer patients who had received or are receiving cis-DDP-based chemotherapy during pregnancy may be at increased risk for the development of preneoplastic and/or neoplastic lesions in later life and should be followed clinically to monitor any such adverse effect of exposure to this drug.

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References

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