Chemotherapy of Human Yolk Sac Tumor Heterotransplanted in Nude Mice

Masumi Sawada, Yoshiaki Matsui, and Yoshio Okudaira

ABSTRACT—The chemotherapeutic effects of cis-diamminedichloroplatinum+vinblastine+bleomycin (PVB) on 3 human yolk sac tumors (YST-1, YST-2, and YST-3) of the ovary, which were heterotransplanted into BALB/c nude mice, were compared with the effects of vincristine+actinomycin D+cyclophosphamide (VAC), the combination currently favored for treatment of yolk sac tumors. PVB and VAC therapies were performed for 3 weeks. Both PVB and VAC significantly reduced the tumor volume of all the treated tumors. The mean weights of tumors in animals treated with PVB or VAC were, in percent of the mean tumor weight in untreated animals: 1.3 and 1.8 for YST-1, 2.5 and 3.3 for YST-2, and 5.5 and 2.7 for YST-3, respectively. A strong correlation was noted between tumor volume and alpha-fetoprotein level in the sera of mice bearing YST-1 or TST-2 tumors.—JNCI 1983; 71:1221-1225.

Yolk sac tumor is a rare and highly malignant germ cell tumor of the ovary that occurs primarily in children and young adults (1-4). Until recently, the prognosis for patients with this tumor was very poor, but progress in combination chemotherapy has markedly improved patient survival (2). A VAC regimen introduced by Smith and Rutledge (5) is widely favored as first-line chemotherapy for germ cell tumors, including yolk sac tumor (2, 6, 7). VAC provides very good results when used as a surgical adjuvant in patients with totally resectable tumor, but patients in advanced stages who were treated with this regimen failed to achieve a complete remission (5, 6).

Since Einhorn and Donohue (8) reported good results with a PVB regimen in male patients with germ cell tumors, other investigators have used this regimen in the treatment of ovarian germ cell tumors (9, 10). However, whether the PVB regimen is more effective than VAC awaits further clinical investigation.

In 1977, we successfully transplanted tissues from a human yolk sac tumor into nude mice, and 3 human yolk sac tumors have been maintained by serial transplantation in nude mice in our laboratory (11, 12). With this human tumor-nude mouse system, we have been able to examine in detail the clinicopathology of human yolk sac tumor and to test the response of human tumors to various anticancer agents. In this paper, therapeutic responses of ovarian yolk sac tumors to PVB and VAC are discussed.

MATERIALS AND METHODS

Animals.—BALB/c female nude (nu/nu) mice 4–5 weeks old were obtained from Clea Japan Inc., Takatsuki, Osaka. They were kept under sterile conditions in autoclaved cages with filter bonnets in a laminar flow unit and were fed sterilized CL-2 pellets and distilled water. Mice were used for experiments when they weighed 20 g or more.

Tomors.—The human yolk sac tumors (YST-1, YST-2, and YST-3) used in this study were established by inoculation of fresh tumor tissues from patients into nude mice in our laboratory, as previously described (11, 12). The donor patients had not been treated with VAC or PVB before surgical removal of their tumors. For serial transplantation, the tumors were cut into small pieces (2–4 mm³) in ice-cold Eagle's minimum essential medium and transplanted sc onto the backs of nude mice by means of a trocar needle. At the time of the experiments described in this paper, the number of previous tumor tissue passages ranged from 10 to 20. The tumor take rate for all the tumor lines was 90–100%. The histologic appearance of serially transplanted tumors was similar to that of the original tumors: YST-1, reticular and microcystic; YST-2, solid with perivascular proliferation; and YST-3, an endodermal sinus pattern. All 3 tumor lines are able to produce AFP.

Drugs.—The chemotherapeutic protocol used in this study for PVB therapy was based on the work of Einhorn and Donohue (8), and that for VAC was based on the work of Smith and Rutledge (5). Both the protocols are comparable to those used in clinical situations. The PVB regimen was as follows: 2.3 mg cis-diamminedichloroplatinum from days 1 to 5, 1.0 mg vinblastine on day 1, and 2.5 mg bleomycin from days 1 to 5. The VAC regimen was 0.17 mg vincristine on day 1, 0.05 mg actinomycin D from days 1 to 5, and 25 mg cyclophosphamide from days 1 to 5. No drugs were given on days 6 and 7. PVB and VAC therapies were repeated for 3 weeks. The drugs were dissolved in 0.2 ml 0.9% NaCl solution and were injected ip into tumor-bearing nude mice 4-5 weeks after inoculation of fresh tumor tissues from patients into nude mice in our laboratory (11, 12). The donor patients had not been treated with VAC or PVB before surgical removal of their tumors. For serial transplantation, the tumors were cut into small pieces (2–4 mm³) in ice-cold Eagle's minimum essential medium and transplanted sc onto the backs of nude mice by means of a trocar needle. At the time of the experiments described in this paper, the number of previous tumor tissue passages ranged from 10 to 20. The tumor take rate for all the tumor lines was 90–100%. The histologic appearance of serially transplanted tumors was similar to that of the original tumors: YST-1, reticular and microcystic; YST-2, solid with perivascular proliferation; and YST-3, an endodermal sinus pattern. All 3 tumor lines are able to produce AFP.

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mice. Control mice were given ip the same volume of 0.9% NaCl.

Evaluation of chemotherapeutic effects.—When the tumors became palpable and were growing progressively, experimental mice were randomized into test groups of 5 mice each. The size of the growing transplant was measured with slide calipers twice a week, and the volume, in cubic millimeters, was calculated by means of the formula described by Houchens et al. (13): \( V = W^2 \times L \times 1/2 \), where \( W \) and \( L \) are the width and length in millimeters. Since individual growth of inoculated tumors showed slight variability, the treatment was initiated when the transplanted tumors had grown to 300–400 mm\(^3\). For comparison of different groups, the RV for each group was calculated from the formula \( RV = V/V_{n} \), where \( V \) is the mean tumor volume at any given time and \( V_{n} \) is the mean initial tumor volume when treatment was begun. T/C was calculated each time the tumors were measured. At the end of experiments, all treated and control tumors were resected and weighed, and the T/C in weight was calculated.

To ascertain whether there was a correlation between serum AFP levels and tumor volumes, we drew a blood sample (30 \( \mu l \)) at regular intervals from a tail vein of each mouse. The relative AFP value was calculated in a manner similar to that described for the RV. AFP determinations were done with an AFP radioimmunoassay kit (Eiken Immunochemical Laboratory, Tokyo) by Mr. K. Iwanaga in the Osaka Kessei Research Laboratories. AFP values in the sera of nontumorous nude mice were less than 5 ng/ml, the limit of detection of this assay. At the initiation of chemotherapy, the AFP levels in the tumor-bearing mice ranged from 200 to 600 ng/ml for YST-1, from 150 to 400 ng/ml for YST-2, and from 50 to 300 ng/ml for YST-3. To monitor the toxicity of the drugs, we weighed the mice once a week during the experimental period.

RESULTS

YST-1 Tumor

Tumor growth.—Treatment was initiated between 32 and 40 days post transplantation. As shown in text-figures 1A–C and 2A, both PVB and VAC produced significant decreases in the tumor volume. Text-figures 1A–C show the tumor growth curves for each mouse in the control and treated groups. The curves in text-figure 1B are parallel, showing that tumor response to PVB treatment followed the same pattern in all the treated mice. The tumor disappeared in 4 of 5 mice treated with PVB and in 2 of 4 mice treated with VAC. No statistical difference in the T/C values was observed between the PVB- and VAC-treated groups (table 1).

Changes in serum AFP values.—AFP values in sera of mice bearing YST-1 tumor paralleled the tumor volumes. The serum AFP of mice in the control groups increased with the increase of tumor volumes, whereas the serum AFP of mice treated with PVB or VAC decreased rapidly after initiation of the treatment (text-fig. 3A). The \( r \) between the tumor volumes and the serum AFP values was 0.71.

YST-2 Tumor

Tumor growth.—Treatment was initiated between 23 and 30 days post inoculation. As shown in text-figures 1D–F and 2B, both PVB and VAC suppressed the
initiation of treatment. Although the YST-2 tumor seemed to respond more quickly to PVB, no statistical difference in the T/C values was observed between the PVB- and VAC-treated groups, either for estimated volume or weight after resection (table 1).

Changes in serum AFP values.—Serum AFP levels in control mice paralleled tumor volumes. The AFP levels in sera of mice treated with PVB or VAC rose temporarily and then fell. Although the size of YST-2 tumors seemed to decrease more rapidly in response to PVB, the relative value of the serum AFP of mice was higher in the PVB-treated groups (text-fig. 3B). The r between tumor volumes and the serum AFP values was 0.68.

YST-3 Tumor

Tumor growth—Treatment was initiated between 27 and 34 days post transplantation. As shown in text-figures 1G–1 and 2C, both PVB and VAC caused regression of YST-3 tumors. The tumors were eliminated in 2 of 7 mice treated with PVB and in 2 of 5 mice treated with VAC. No statistical difference in T/C values was observed between the PVB- and VAC-treated groups (table 1).

Changes in serum AFP. Serum AFP levels in control mice paralleled the increase in the tumor volumes (r = 0.58; P < .001). However, increases in AFP levels were observed in the groups treated with PVB or VAC despite the decrease in tumor volumes (text-fig. 3C). No clear relationship was observed between tumor volumes and AFP levels in treated mice.

Drug Toxicity

A significant weight loss of 30–40% was noted in the mice treated with PVB, as compared with the weight loss in both the control groups and VAC-treated groups (table 1). The small but not statistically significant difference observed between the control mice and the VAC-treated mice reflects primarily the difference in the tumor weight in treated and untreated mice. No mice died due to drug toxicity.

Table 1.—Summary of the effect of PVB and VAC on human yolk sac tumors heterotransplanted in nude mice

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Treatment</th>
<th>No. of mice</th>
<th>Tumor weight, mga</th>
<th>Weight (P)</th>
<th>Volumeb (P)</th>
<th>Body weightc</th>
<th>AT, g</th>
<th>AT/BT, % change (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YST-1</td>
<td>NaCl (controls)</td>
<td>4</td>
<td>1,637±300</td>
<td></td>
<td></td>
<td></td>
<td>25.0±0.7</td>
<td>25.8±0.8 (+3)</td>
</tr>
<tr>
<td></td>
<td>PVB</td>
<td>5</td>
<td>26±19</td>
<td>1.3 (&lt;.001)</td>
<td>0.11 (&lt;.05)</td>
<td>24.4±0.5</td>
<td>23.7±0.5 (−3 &lt;.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAC</td>
<td>4</td>
<td>33±31</td>
<td>1.6 (&lt;.001)</td>
<td>0.17 (&lt;.05)</td>
<td>24.5±0.5</td>
<td>23.7±0.5 (−3 &lt;.1)</td>
<td></td>
</tr>
<tr>
<td>YST-2</td>
<td>NaCl (controls)</td>
<td>4</td>
<td>2,110±903</td>
<td></td>
<td></td>
<td></td>
<td>23.3±0.8</td>
<td>25.8±1.1 (+11)</td>
</tr>
<tr>
<td></td>
<td>PVB</td>
<td>5</td>
<td>52±6</td>
<td>2.5 (&lt;.01)</td>
<td>4.5 (&lt;.05)</td>
<td>22.8±1.2</td>
<td>17.0±1.0 (−29 &lt;.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAC</td>
<td>5</td>
<td>69±22</td>
<td>3.3 (&lt;.01)</td>
<td>6.0 (&lt;.05)</td>
<td>22.0±1.3</td>
<td>21.2±3.2 (−4 &lt;.1)</td>
<td></td>
</tr>
<tr>
<td>YST-3</td>
<td>NaCl (controls)</td>
<td>4</td>
<td>1,250±900</td>
<td></td>
<td></td>
<td></td>
<td>23.8±2.4</td>
<td>26.0±3.3 (+9)</td>
</tr>
<tr>
<td></td>
<td>PVB</td>
<td>7</td>
<td>69±62</td>
<td>5.5 (&lt;.05)</td>
<td>4.1 (&lt;.05)</td>
<td>25.0±1.1</td>
<td>16.2±1.5 (−35 &lt;.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VAC</td>
<td>5</td>
<td>34±25</td>
<td>2.7 (&lt;.05)</td>
<td>2.1 (&lt;.01)</td>
<td>24.7±1.5</td>
<td>24.8±1.6 (±0 &lt;.05)</td>
<td></td>
</tr>
</tbody>
</table>

*aValues are means ± SD.
*bBT=before treatment; AT=after treatment.
*cAnalysis was performed for control groups vs. treated groups, with the use of Student’s t-test.
*dValues were calculated from the final set of tumor volume measurements.
DISCUSSION

Yolk sac tumor of the ovary is a rare and highly malignant gonadal germ cell tumor. An in vivo experimental system had been earnestly desired for the purpose of examining the characteristics of this malignant tumor and for the testing of chemotherapeutic regimens. Since Rygaard and Povlsen (14) first reported heterotransplantation of malignant human tumors into nude mice, a number of related studies have been reported. However, transplantation of human yolk sac tumor has been reported by only a few investigators, including us (11, 12, 15, 16). The heterotransplanted yolk sac tumors YST-1, YST-2, and YST-3 are valuable because they preserve both histologic and functional characteristics of the original tumors. These transplantable tumors are solid and hypervascular. The human tumor–nude mouse system, therefore, is useful for the investigation of transplanted tumor response to various antitumor drugs and also in the search for a valid protocol for human yolk sac tumor chemotherapy.

The 3 heterotransplantable tumors had different origins. All tumors responded to both PVB and VAC, but the responses to chemotherapy were slightly different. Interestingly, the patient from whom the YST-2 tumor was derived responded well to PVB therapy and has remained disease-free for 45 months from the start of therapy. However, the patient from whom the YST-3 tumor was derived did not respond well to PVB therapy and died 12 months after the start of therapy. We could not perform PVB therapy on the patient from whom the YST-1 tumor was derived, because cis-diaminedichloroplatinum was not available. Whether PVB would have been effective in this patient is unknown. In the 2 patients for whom both clinical and experimental data are available, the responses of the original and transplanted tumors to PVB were similar.

In experimental chemotherapy of human tumors in nude mice, an important aspect is the determination of appropriate drug dosages. Such determinations are complicated by differences in the pharmacokinetic behavior of drugs in mice and humans and the insufficiency of toxicity data for nude mice. The dosages used in our study were based mainly on the clinical regimens and corresponded to about five times the doses (mg/kg) usually adopted in the treatment of patients with yolk sac tumors. In general, larger doses are required in mice than in humans (17-19), and larger doses may be required to treat tumors in nude mice than in conventional mice (13, 20). The doses we used in the present study were less than a lethal dose for nude mice and were below the 10% lethal dose (LD10) for conventional mice (21, 22). Although much more data would be required for a detailed comparison of the two types of therapy, or for determination of the optimal regimen for just one of the drugs for nude mice, the results obtained in this study suggest that PVB might be as effective as VAC against yolk sac tumors. We have treated 5 patients with yolk sac tumors with a PVB regimen based on the protocol of Einhorn and Donohue (8). Four patients have achieved a complete remission and are tumor-free 18–45 months after initiation of treatment.

Although severe weight loss (30–40%) was observed in mice treated with PVB, this toxicity does not necessarily preclude the use of PVB as a chemotherapy for yolk sac tumor. The conditions of treatment for nude mice and patients do not correspond exactly. As mentioned by Rozenwieg et al. (23) and other investigators (24, 25), intravenous hydration along with mannitol (with or without furosemide) decreases the nephrotoxicity of cis-diaminedichloroplatinum, without affecting its antitumor activity, and allows administration of higher doses.

The tumor volume, calculated by the formula described by Houchens et al. (13), showed good correlation with the tumor weight, as indicated by the following r: r = 0.86 for YST-1, r = 0.96 for YST-2, and r = 0.97 for YST-3. These results illustrate the usefulness of this
formula for evaluation of the efficacy of chemotherapy in xenotransplants that produce solid elipsoid tumors.

A major application of tumor marker assays has been the monitoring of clinical response of tumors to chemotherapy. A correlation between serum levels of tumor marker and tumor volume has been reported for human tumors heterotransplanted in nude mice, e.g., for AFP in the case of liver cell carcinoma (26) and for human chorionic gonadotropin in the case of choriocarcinoma (27). In the present study, a strong correlation was found between tumor volume and the AFP level in sera of mice bearing YST-1 or YST-2 tumors, but no clear correlation was observed with YST-3 tumors. Although AFP is considered to be the most useful marker for germ cell tumors (28-31), Talerman et al. (31) have cautioned that the presence or absence of elevated levels of a tumor marker indicates only that the tumor elements responsible for its production are present or absent. The discordant behavior of the increase in AFP and the tumor regression indicate that various neoplastic elements within YST-3 tumor may respond differently to chemotherapy. The intratumor element that produces AFP has not yet been identified.

This study illustrates the use of heterotransplantable yolk sac tumors of the ovary to screen antitumor drugs and for the biologic study of the tumor in relation to marker production. In the regimens tested, PVB was as effective as VAC against yolk sac tumor. We suggest that PVB might be considered for primary treatment of yolk sac tumor of the ovary.

REFERENCES