Human Prostate Cancer Xenograft as a Tool for Screening of Efficacious Antiandrogens

Yoshikazu Z. ITO, Sachiko HIROMACHI, Tamae FUTAWATARI, and Michiko ARAI
Department of Nursing, College of Health Sciences, Gunma University (Y.Z.I., S.H., M.A.), and Gunma Prefectural College of Health Sciences (T.F.), Maebashi, Japan

Received November 30, 1996

Key Words: Human prostate cancer, Antiandrogen, Prostate specific antigen, Prostatic acid phosphatase, Nude mice

Summary

The efficacies of the steroidal antiandrogens chlormadinone acetate (CMA), 17 α-acetoxy-6-chloro-2-oxa-4,6-pregnadiene-3,20-dione (TZP), and cyproterone acetate (CypA) for retardation of the growth of androgen-dependent human prostatic adenocarcinoma transplanted into nude mice (Honda tumor) were tested. CypA inhibited the growth of these tumors, while CMA and TZP did not affect the weight of cancer tissue at the doses examined. Serum levels of human prostatic acid phosphatase (PAP) in the tumor-bearing mice were very sensitive to the administration of antiandrogens, but the levels were not dependent on the changes in tumor weight. In contrast, human prostate-specific antigen (PSA) levels in the blood of the host mice were correlated with the weight of the tumors, and were independent of treatment with antiandrogens. Castration of the cancer-bearing mice resulted in reductions in all experimental parameters tested. The validity of PSA-secreting androgen-dependent human prostate cancer xenograft as a tool for screening of efficacious antiandrogens will be discussed.

Introduction

Almost 50 years ago, Huggins and Hodges first described the inhibitory effect of castration and estrogens on the growth of human prostate cancer (1). Although the therapeutic benefits of these hormonal manipulations are still controversial, these means remain the first choice for treatment of the disease, and are at least expected to ameliorate the symptoms related to progression of the illness (2). Estrogens, luteinizing hormone-releasing hormone (LH-RH) agonists, antiandrogens, and orchietomy are good candidates for such treatment.

Despite the assertion that the main cause of the death of patient suffering from this disease is the result of proliferation of hormone-resistant cancer cells
within tumors, it has been shown that total androgen blockade by combination of LH-RH agonists and orchietomy or antiandrogens prolonged the life of patients with Stage D cancer (3). Since lethal cardiac and pulmonary complications after estrogen treatment and psychological trauma induced by orchietomy cannot be tolerated by many patients, potent antiandrogens with least side effects, but which are as effective as orchietomy are required.

Analyses of the efficacy of antiandrogens have usually been conducted in rodents using the inhibitory effects on the weight of accessory sex glands, ventral prostate and seminal vesicles, of the animals as experimental parameters. The tests are usually performed prior to clinical trials (4). However, before the trials, it is necessary to determine the efficacy of antiandrogens using human tumor models, if possible, because species differences could affect the potency of the drugs. We report here the effects of various antiandrogens on the androgen-responsive human prostate cancer Honda transplanted into nude mice as xenografts.

Materials and Methods

Transplantation of the Honda tumor into nude mice was performed as described previously (5). Tumor fragments ca 1 mm in diameter were implanted into the flanks of nude mice using a trocar at the age of 8 weeks. Treatment with steroidal antiandrogens and castration were performed 4 weeks after tumor transplantation. Chlormadinone acetate (CMA, 50 mg/kg), 17α-acetoxy-6-chloro-2-oxa-4,6-pregnadiene-30,20-dione (TZP, 16 mg/kg) and cyproterone acetate (CypA, 16 mg/kg) were given orally to the mice with gum arabic for 28 days. In a separate experiment, the mice were treated with TZP (12.5 mg/kg) in the same manner as described above. The tumor-bearing mice were anesthetized with pentobarbital sodium, then tumors, ventral prostates, seminal vesicles, and testes were removed and weighed. Weight of the tumor-free carcasses of the mice was measured after removal of the gonads and accessory sex organs. Human prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) in the blood samples obtained from abdominal aorta were measured by radioimmunoaassay with Eiken kits (Eiken ICI). Assays were carried out according to the manufacturer's instructions. Statistical analysis of the data was performed with Student's t-test.

Results and Discussion

Table 1 shows a wide variety of differences in the experimental parameters: weight of the tumors and genitals of the host mice, PAP levels in the blood, and
Table 1. Effects of antiandrogens on Honda tumors, accessory glands of nude mice, and human prostatic acid phosphatase (PAP) levels in serum of tumor-bearing mice. Chlormadinone acetate (CMA, 50 mg/kg), 17α-acetoxy-6-chloro-2-oxa-4,6-pregnadiene-30,20-dione (TZP, 16 mg/kg), and cyproterone acetate (CypA, 16 mg/kg) were given orally with gum arabic for 28 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Honda Tumor(g)</th>
<th>Ventral Prostate(mg)</th>
<th>Seminal Vesicle(mg)</th>
<th>Testes(mg)</th>
<th>Tumor-free Carcass(g)</th>
<th>PAP (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n)</td>
<td>3.0±0.2 (6)</td>
<td>16.4±1.2 (6)</td>
<td>27.4±3.1 (6)</td>
<td>154.3±4.0 (6)</td>
<td>15.0±0.2 (6)</td>
<td>220.3±12.7 (6)</td>
</tr>
<tr>
<td>CMA (n)</td>
<td>2.8±0.3 (6)</td>
<td>12.3±1.6 (6)</td>
<td>17.8±1.6* (6)</td>
<td>132.8±8.8 (6)</td>
<td>16.5±0.2* (6)</td>
<td>94.4±17.7* (6)</td>
</tr>
<tr>
<td>TZP (n)</td>
<td>2.7±0.3 (6)</td>
<td>9.0±0.3* (6)</td>
<td>12.5±0.8* (6)</td>
<td>91.8±3.5* (6)</td>
<td>16.1±0.2* (6)</td>
<td>41.4±3.2* (6)</td>
</tr>
<tr>
<td>CypA (n)</td>
<td>2.0±0.1* (6)</td>
<td>14.3±1.1 (6)</td>
<td>22.1±3.4 (6)</td>
<td>153.6±8.9 (6)</td>
<td>16.3±0.5* (6)</td>
<td>47.2±10.6* (6)</td>
</tr>
<tr>
<td>Castration (n)</td>
<td>0.12±0.02* (8)</td>
<td>5.2±0.5* (8)</td>
<td>8.9±1.0* (8)</td>
<td>21.8±0.4* (8)</td>
<td>&lt;0.05 (8)</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from control.

body weight of the host mice after antiandrogen treatment. Antiandrogens and castration resulted in a overall reduction of PAP concentration and increase in tumor-free carcasses of the host mice regardless of the tumor weight. Only castration showed inhibitory effects on all of the experimental criteria examined. Among the steroidal antiandrogens tested in this experiment, CypA alone showed a significant inhibitory effect on tumor growth. However, this steroid did not reduce the weight of sex organs of the host mice. CypA is a biphasic antiandrogen which acts as an androgen in certain cases (6), suggesting that this agent may act differently on the tumor and accessory sex glands. TZP, a potent newly synthesized antiandrogen (7), decreased the weight of the ventral prostate and seminal vesicles but had no effect on the tumor growth. This agent reduced the weight of the testes of the host mice, suggesting that it has toxic effect against the gonads.

CMA showed insufficient antiandrogenic activity in rodents, reducing only the weight of seminal vesicles. This compound had no effect on the tumor weight at the dose used.

Interestingly, significant increases in body weight of the cancer-bearing mice after antiandrogenic treatment were observed. These changes paralleled the slight decreases in tumor weight, although the changes in tumor weight are statistically insignificant. The possible existence of inhibitory mechanisms of antiandrogens on the progression of cancer cachexia (8) cannot be excluded.

These results indicate that: 1) mechanisms of action on the target organs are quite different among different steroidal antiandrogens; 2) responses of human
prostatic cancer and reproductive organs of experimental animals are quite dissimilar, indicating differences in mechanisms of androgen-dependency; 3) oral administration may be inadequate to elicit the effects of antiandrogens; 4) synthetic antiandrogens are not as effective as castration against prostatic cancer; 5) PAP in the serum is very androgen-sensitive, and PAP levels do not depend on changes in the tumor weight; and 6) administration of antiandrogens to human prostatic cancer-bearing hosts may prevent the progression of cancer cachexia.

Table 2. Changes in concentrations of human prostatic acid phosphatase (PAP) and human prostate-specific antigen (PSA) in the serum of tumor-bearing mice after treatment with chlormadinone acetate (CMA, 50 mg/kg) or 17α-acetoxy-6-chloro-2-oxa-4,6-pregnadiene-30,20-dione (TZP, 12.5 mg/kg) for 28 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total dose (mg/kg)</th>
<th>Tumor Weight (g)</th>
<th>PSA</th>
<th>PAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(8)</td>
<td>3.88±0.46</td>
<td>283.5±57.5</td>
<td>436.7±75.4</td>
</tr>
<tr>
<td>CMA</td>
<td>(8) 50</td>
<td>4.37±0.74</td>
<td>162.7±32.3</td>
<td>178.7±55.1*</td>
</tr>
<tr>
<td>TZP</td>
<td>(8) 12.5</td>
<td>4.09±0.57</td>
<td>163.5±14.3</td>
<td>63.4±11.2*</td>
</tr>
<tr>
<td>Castration</td>
<td>(8)</td>
<td>0.03±0.005*</td>
<td>&lt;0.5*</td>
<td>0.10±0.01*</td>
</tr>
</tbody>
</table>

* Significantly different from control. † Limit of detection.

PAP and PSA are the most valuable tumor markers of the human prostate cancer (9, 10). Production of PAP has been proposed to be androgen-dependent related particularly with 5α-dihydrotestosterone (DHT), a hormonally active androgen, within the prostate cells (11). Significant decreases in PAP levels after treatment with antiandrogens is due to the inhibitory effects of these agents on the enzymatic conversion of testosterone to DHT within the tumor cells. Our results, however, showed that PAP levels in the serum merely indicate the hormonal status of the tumor cells, and do not represent the state of cancer growth.

PSA is produced in human prostate epithelia, regardless of malignant status, and secreted into the blood stream (12). The production of PSA has also been reported to be under the control of androgens (13). However, as shown in Table 2, PSA levels in the blood of tumor-bearing mice were independent of antiandrogen treatment. This indicates that PSA levels in the blood are dependent on the number of the cancer cells, and the production of the protein is not correlated with the blood level of DHT.
However, slight decreases in PSA levels in the antiandrogen-treated animals, although statistically insignificant, might be a consequence of the inhibitory process in the androgen-responsive elements of this substance (14).

The marked decreases in weight of the tumor and sex organs of the host, and in concentrations of PAP and PSA after castration indicate that the gonads are the sole source of androgens in nude mice. Castration of male nude mice thus induces total androgen blockade. Thus the oral administration of antiandrogens is far less efficient against prostate cancer than castration.

Taken together, the results presented here clearly indicate that fundamental methods to explore the efficacy of new drugs for the treatment of androgen-dependent human prostate cancer must include screening tests using human tumors that secrete PSA into the blood, and show hormone dependent-growth as a xenograft before entering commencement of clinical trials. The results of the present study additionally caution against clinical trials based on data obtained in animal experiments.

Honda tumor has been serially transplanted for more than 20 years over 105 passages (5). The tumor retains androgen-dependency and is morphologically poorly differentiated adenocarcinoma which secretes very high levels of PSA and PAP. Thus this tumor is an excellent candidate for such experiment. However, due to the heterogeneity of human prostate cancer, the establishment of many tumor xenografts models for the screening of curative antiandrogens is mandatory.

Acknowledgments

We are indebted to Mr. M. Mieda and his colleagues of Teikoku Hormone Manufacturing Co., Tokyo, Japan for their assistance.

References