NOTE

Effect of Leuprolide on Growth of Rat Prostatic Tumor (R 3327) and Weight of Male Accessory Sex Organs

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Abstract

Leuprolide, a synthetic LHRH analog, inhibited growth of the Dunning R 3327 androgen-sensitive rat prostatic tumor and induced weight loss in male accessory sex organs. The relationship between the mode of administration and efficiency of the treatment was examined. Maintenance of the drug level in vivo seemed to be one of the important factors in the suppression of tumor growth, while a decrease in the weight of the accessory sex organs was mainly dependent on the dose administered. No treatment with leuprolide surpassed the effect caused by castration. Cytosolic androgen receptor and acid phosphatase activity in the tumor tissues were not changed significantly after treatment with leuprolide.

Synthetic LHRH (luteinizing hormone-releasing hormone) analog shows a potent agonistic effect at the beginning of the treatment but a strong antagonistic effect, which decreases in the serum concentration of LH, FSH and testosterone, is developed thereafter (Schally et al., 1980). The antagonistic effect of the LHRH analog has been put to medical use, and pharmacological castration with this type of drug has been applied to treatment of prostatic cancer (Trachtenberg, 1983, Walker et al., 1983, Smith et al., 1985, Murphy et al., 1987), endometriosis (Shaw et al., 1983), and precocious puberty (Comite et al., 1981) and to contraception (Bergquist et al., 1979). The half life of the LHRH analog in vivo is relatively short (Yamazaki and Okada, 1980), and this has been a disadvantage in medical application. The Dunning R 3327 rat prostatic tumor (R 3327) is a transplantable androgen-sensitive tumor, which originated from the prostate of the Copenhagen rat (Smolev et al., 1977, Isaacs et al., 1978), and it was reported that growth of the R 3327 was inhibited by treatment of tumor-bearing rats with [D-Trp\(^6\)]LHRH (Redding and Schally, 1981, Schally et al., 1983). In this respect, we examined the inhibiting effect on growth of the R 3327 of leuprolide (D-Leu\(^6\)-[des-Gly\(^10\)-NH\(_2\)]-LHRH ethylamide acetate, Fujino et al., 1974), a synthetic LHRH analog with potent biological activity, to evaluate its usefulness in controlling prostatic cancer.

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Materials and Methods

Animals

Rats of the Copenhagen strain were obtained from the National Cancer Institute (Bethesda, Md, USA), and have been constantly inbred. Male rats of this strain, 10 weeks old and with a body weight of approximately 200 g, were used as host animals of the R 3327.

Castration was performed via the scrotal route under ether anesthesia. Leuprolide was obtained from Takeda Chemical Ind. (Osaka, Japan) as an aqueous solution for daily injections and also as a slow-release formulation injected once per month for continuous administration (Shimamoto, 1987).

Tumor

The R 3327 was obtained from the Papanicolaou Cancer Research Institute (Miami, Fl, USA). Two months after transplantation into male rats, the tumor became detectable as a subcutaneous nodule, then growth proceeded exponentially. The tumors transplanted into female rats appeared later than male rats and remained as a small nodule for a long time, suggesting androgen-sensitive growth of the R 3327. Tumors used in this study were of the 43th-44th generations.

Measurement of tumor volume

After the tumor became palpable as a subcutaneous nodule, the three dimensions of the mass were measured with calipers and tumor volume was calculated according to the formula \( L \times W \times H \times 0.5236 \) (Janik et al., 1975).

Tissue preparation and determination of cytosolic androgen receptor

Tumor tissues were used immediately after removing or after keeping at \(-80^\circ C\) for an appropriate period. The tissues were minced, then homogenized in 5 volumes of 10 mM Tris-HCl buffer (pH 7.4) containing 1 mM EDTA, 1 mM \( \beta \)-mercaptoethanol, 10 mM Na₂MoO₄, 0.1 mM phenylmethylsulfonylfluoride and 10% (w/v) glycerol. The homogenate was passed through nylon cloth and centrifuged at 105,000 \( \times g \) for 60 min, and the resultant supernatant was served for measurement of cytosolic androgen receptor using \(^3\)H-R 1881 (Akimoto et al., 1985). The dissociation constant (Kd) and maximum binding sites (Bmax) were obtained by the method of Scatchard (1949).

Other analytical methods

Serum testosterone was determined by radioimmunoassay (Hosaka et al., 1978). Activity of acid phosphatase in tumor tissue homogenate was measured colorimetrically (Bialy and Pincus, 1967). Protein was determined by the biuret method (Gornall et al., 1949).

Results

Effect of leuprolide or castration on tumor growth and weights of tumor, male accessory sex organs and testis

Approximately 2 months after tumor transplantation, the tumor appeared as a subcutaneous nodule and then accelerated growth was observed (Fig. 1). Castration caused obvious inhibition of growth. Administration of leuprolide suppressed growth, and the rate of suppression was dose-dependent following daily single injections. Injection twice a day with leuprolide inhibited growth more effectively than injection once a day with a larger amount of the drug. The slow-release type of leuprolide showed the strongest suppressive effect on growth but the rate of inhibition did not surpass the level caused by castration.

Animals were killed two months after starting the treatment, and weights of the tumor, male accessory sex organs and testis were compared with those of the untreated control (Fig. 2). Suppression of tumor weight was almost the same as inhibition of tumor growth. However, a daily single injection of a large dose of leuprolide effectively influenced weights of the accessory sex organs when compared with effect of the same treatment on the tumor. The weight of testis decreased following injection of the drug in a dose-dependent manner.
Effect of leuprolide or castration on serum testosterone, cytosolic androgen receptor and acid phosphatase

Two months after the start of the treatment, animals were sacrificed and serum testosterone was measured (Table 1). Less than 0.1 ng/ml of testosterone was observed in the leuprolide-treated and castrated animals, except some rats injected with a daily dose of 333 µg/kg as a single injection, confirming that treatment of animals with leuprolide decreased the concentration of serum testosterone to almost the level of castrated animals at any dose used in the present experiments.

At the same time, no significant differences were observed in Kd's and Bmax's

Table 1. Testosterone in sera from untreated, leuprolide-injected and castrated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Testosterone ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (7)</td>
<td>0.93 ± 0.12c</td>
</tr>
<tr>
<td>2 (7)</td>
<td>0.13 ± 0 (3d), n.d.a) (4)</td>
</tr>
<tr>
<td>3 (5)</td>
<td>n.d.</td>
</tr>
<tr>
<td>4 (7)</td>
<td>n.d.</td>
</tr>
<tr>
<td>5 (9)</td>
<td>n.d.</td>
</tr>
<tr>
<td>6 (6)</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

a) Groups are the same as described in the legend to Fig. 1.
b) Numbers in parentheses are the number of rats used for determinations. Animals in Groups 2 and 3 were sacrificed at 24 hr and in Group 4 at 12 hr after the last injection.
c) M ± S.E.
d) Average for three rats.
e) Not detected; less than 0.1 ng/ml.

Table 2. Androgen receptors in cytosols of tumors from untreated, leuprolide-injected and castrated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Kd (\times 10^{-9} \text{M} )</th>
<th>Bmax fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (5)b</td>
<td>0.2 ± 0.04</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>2 (5)</td>
<td>0.1 ± 0.03</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>3 (5)</td>
<td>0.1 ± 0.02</td>
<td>48 ± 9</td>
</tr>
<tr>
<td>4 (4)</td>
<td>0.1 ± 0.04</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>5 (4)</td>
<td>0.1 ± 0.01</td>
<td>57 ± 10</td>
</tr>
<tr>
<td>6 (4)</td>
<td>0.1 ± 0.04</td>
<td>50 ± 8</td>
</tr>
</tbody>
</table>

Data are shown as M ± S.E.
a) Groups are the same as described in the legend to Fig. 1.
b) Numbers in parentheses are the number of determinations.
of the cytosolic androgen receptor and acid phosphatase activity in the tumor tissues obtained from untreated, leuprolide-treated and castrated animals (Table 2 and 3).

Discussion

It was known that castration of the host animals bearing the R 3327 caused a temporal suppression of tumor growth (Smolev et al., 1977). Prolonged treatment with [D-Trp^6]LHRH also inhibited growth of the R 3327 probably due to deprivation of the serum androgens (Redding and Schally, 1981, Redding et al., 1984). The suppressive effect of leuprolide on growth of the R 3327 was also confirmed in the present study. The effect of leuprolide was less profound than that of castration. This may be attributable to the initial agonistic action of leuprolide on tumor growth, but...
it is not certain since the agonistic action of leuprolide lasts only for 2–3 days (Rivier et al., 1979). It was reported that concomitant application of antiandrogen potentiated the effect of LHRH analog on the treatment of prostatic cancer (Labrie et al., 1983, 1985), but there is a conflicting report: simultaneous treatment with antiandrogen did not enhance the effect of LHRH analog on growth of the R 3327 (Redding and Schally, 1985). From these results it seems evident that treatment with LHRH analog is not sufficient to deprive the androgens of circulation.

For inhibition of tumor growth it was revealed that continuous releasing of a small dose of leuprolide was more effective than the daily administration of a larger amount in a single injection. Redding et al. (1984) reported strong inhibition of growth of the R 3327 with a long acting type of [D-Trp⁶]LHRH. These results suggest that continuous supply of LHRH analog influences tumor growth more profoundly than pulsatile injections. In the accessory sex organs, however, it was observed in the present study that the influence of leuprolide was correlated with the dose rather than the mode of administration. Since the cell cycle may be shorter in tumors than in normal tissues such as the male accessory sex organs (Lala, 1971), maintenance of the level of leuprolide by frequent or continuous administrations seems to be more important in suppressing tumor growth.

Hierowski et al. (1983) observed binding sites resembling LHRH receptors in the R 3327, while such binding sites were not observed in the normal prostate. The physiological role of these binding sites in the R 3327 is not clear at present, and the direct effect of LHRH analog on the tumor, if any, is not growth-promoting, since treatment with LHRH analog does not induce proliferation in the R 3327.

The presence of the androgen receptors in the R 3327 was reported (Lea and French, 1981, Chung et al., 1981, Minagawa et al., 1983), and the injection of leuprolide or castration did not alter the receptors in the cytosol of the tumor tissues either qualitatively or quantitatively. The activity of acid phosphatase in the tumor tissues was not modified by these treatments administered to the host animals. These results show that tumors from LHRH analog-treated or castrated rats retain their original biological activities although growth rate is markedly suppressed.

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References


