Enhancement of the antitumor efficacy of the antiprogestin, onapristone, by combination with the antiestrogen, ICI 164384

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Abstract. So far, no combination of endocrine treatments has been routinely used in the therapy of breast cancer. It was, therefore, our interest to determine whether the combination of the antiprogestin, onapristone (ON), and the pure antiestrogen, ICI 164384 (ICI), might provide a more effective therapy than either monotherapy in experimental mammary tumors containing both estrogen and progesterone receptors. In the MXT-mammary tumor of the mouse, ON (5 mg/kg) administered for 3 weeks exerted an ovariectomy-like antitumor effect (56% inhibition), whereas ICI (30 mg/kg) was weakly effective (28% inhibition). The combination of ON and ICI was, however, distinctly more effective than the monotherapies or ovariectomy, causing 78% inhibition. A similar potentiation of antitumor effect by the combination was manifested in the dimethylbenzanthracene-induced mammary tumor of the rat when ON (5 mg/kg) and ICI (30 mg/kg) were administered once daily for 4 weeks (s.c.). The remission rates of tumors found after treatment with ICI, ON, the combination and ovariectomy (complete and partial remission) were 15%, 46%, 71% and 100% respectively. In the animals bearing DMBA-induced tumors, treatment with ON alone significantly increased the serum levels of luteinizing hormone and prolactin, but caused only a slight increase in the peripheral levels of estradiol and progesterone. ON had no appreciable effect on the uterine and ovarian weights. ICI reduced the uterine weight and the serum progesterone level. In the combination with ON, ICI reversed the effect of ON on the progesterone level without influencing the luteinizing hormone and prolactin levels. These findings suggest that the augmentation of antitumor effectiveness by the combination of two antihormones can be ascribed not only to their effects at estrogen- and progesterone-receptor-binding sites, but also to the decrease in the peripheral level of progesterone. Thus, an appropriate combination of antiprogestin and pure antiestrogen may be useful in the management of breast cancer.

Key words: Antiprogestin-antiestrogen-endocrine combination therapy-experimental mammary tumor

Introduction

Endocrine therapy of advanced breast cancer is chosen in patients whose tumors contain estrogen receptors (ER). The antiestrogen, tamoxifen, – because of its low side-effects – is preferably used for first line treatment and is known to be effective clinically in ER-positive tumors. Tamoxifen is not, however, an ideal antiestrogen, because it has partial estrogenic properties (Furr and Jordan 1984) which may be related to the increase in the risk of endometrial carcinoma during adjuvant therapy of breast cancer (Gottardis et al. 1990). In the years, efforts have been made to find a pure estrogen antagonist with no agonistic effects. Thus, Wakeling and Bowler (1987) and Wakeling et al. (1991) have found some 7α-substituted estradiols that are pure antiestrogens, able to cause complete inhibition of the uterotrophic effect of estradiol. A non-steroidal compound, ZK 119010, has also been characterized as a pure antiestrogen (von Angerer et al. 1990; Nishino et al. 1991). These so-called pure antiestrogens seem, however, to be weakly effective in inhibiting the growth of some experimentally-induced mammary tumors and are not able to exert an ovariectomy-like effect (Wakeling and Bowler 1988). Although pure antiestrogens significantly inhibit the growth of (MNU)-induced mammary tumors and EnDA-endometrial carcinoma in rats, they exert only a weak antitumor effect on rat dimethylbenzanthracene (DMBA)-induced and mouse MXT-mammary tumors (Schneider et al. 1992 a). On the other hand, the tumors often contain not only ER but also progesterone receptor (PR), and patients with both ER- and PR-positive tumors are known to have an especially high response rate to endocrine therapy (McGuire 1980).

The antitumor effect of the progesterone antagonist, mifepristone, has been well documented (Bakker et al. 1987; Romieu et al. 1987; Klijn et al. 1989). In recent publications, we reported on the antitumor effect of antiprogestins and the
mechanisms in experimental mammary tumor models (Schneider et al. 1989; Michna et al. 1992 a, b; Schneider et al. 1992 b). It has been established that the main antitumor mechanism of antiprogestins depends neither on non-hormone-receptor-mediated cytotoxic effects nor on a blockade of the ovarian and pituitary gland functions. Antiprogestins inhibit cell proliferation and induce cell differentiation leading to terminal cell death (apoptosis). Now it is reasonable to expect that combination therapy with an appropriate antiestrogen and antiprogestin, which exert their antitumor effects independently through interactions with different receptors, may be more effective than the corresponding monotherapies. The in vitro-findings of Thomas and Monet (1992) indicate a synergistic inhibitory effect of the combination of mifepristone and tamoxifen on the proliferation of MCF-7 cells under certain experimental conditions. An additive antitumor effect of mifepristone and tamoxifen on rat DMBA-induced mammary tumors was also found in an in vivo-study (Bakker et al. 1989). The present study demonstrates that, in comparison with the monotherapies, treatment with a combination of the antiprogestin onapristone (ON) and the pure antiestrogen ICI 164384 (ICI) exerts a more pronounced antitumor effect on the mouse MXT and rat DMBA-induced mammary tumors containing ER and PR.

Materials and methods

The animals used were kept in a room maintained at constant temperature (21±1°C) and humidity (50±5%) on a 14 h light/10 h darkness schedule (light on at 6:00 a.m.). They received water and commercially available food (Altromin, Altreger/Lippe, Germany) ad libitum.

Chemicals and formulations

DMBA (9,10-dimethyl-1,2-benzanthracene) was purchased from Serva (Heidelberg, Germany) and dissolved in peanut oil at the concentration of 10 mg/0.5 ml. Antiestrogens, onapristone [11β-(4-dimethyl-amino-propyl)-17α-hydroxy-17β-(3-hydroxypropyl)-13α-methyl-4,9-gonadatrien-3-one] and ICI 164384 [11-3,17β-dihydroxy-13,5(10)-trien-7α-y1]-N-n-butyl-N-methylundecanamide] were synthesized in the laboratories of Schering AG. The daily doses of both antiestrogens were dissolved in 0.2 ml castor oil containing 20% benzyl benzoate.

Animals and hormone-sensitive mammary tumors

Mouse MXT-mammary tumors. The MXT line M 3.2 was kindly provided by Dr. A. E. Bogden (EG+G Bogden Laboratories, Worcester, Mass., USA). Two pieces of tumor (about 1-2 mm in diameter) were implanted subcutaneously in intact, female 8- to 10-week-old BDF 1 mice.

Rat DMBA-induced mammary tumors. Tumors were induced by a single oral administration of 10 mg DMBA in female Sprague-Dawley rats (55-57 days old).

Treatment of animals

Mice bearing MXT-tumors were palpated for tumors 20 days after the implantation. Only animals with two palpable tumors were used for the experiment. On the following day, one group of animals was ovariectomized and the daily treatment of other groups of animals with ON (5 mg/kg) or ICI (30 mg/kg) or the combination of the two antiestrogens (6 times a week, s.c.) was started and continued for 3 weeks. The control animals received only the vehicle. The tumor area was determined by caliper measurements once weekly. The tumor area is the product of the longest diameter and its perpendicular. At the end of the experiment the animals were sacrificed and the tumors were removed and weighed.

Rats treated with DMBA were examined for tumors by palpation once weekly. The tumor size was determined by caliper measurements of the longest diameter and its perpendicular. In animals with at least one tumor with a size of 1.5x1.5 cm, either ovariectomy was performed or the daily treatments with ON (5 mg/kg, s.c.), ICI (30 mg/kg, s.c.) or the two antiestrogens were initiated and continued for 4 weeks. At the start of therapy, the total size of all tumors in each animal was termed 100% and changes in the size during the experiment were calculated in terms of this value. The response of tumors to the treatments was also measured according to the usual criteria, i.e. as the percentage of animals showing complete remission, partial remission or progression of tumors. The day after the last treatment all animals were sacrificed and the genital organs (ovary, uterus) excised and weighed. The blood was collected for the radioimmunoassay determinations of estradiol, progesterone, luteinizing hormone, prolactin.

Radioimmunoassay

Serum levels of estradiol and progesterone were measured using the kits supplied by Diagnostic Products Co. (Los Angeles, USA). Serum luteinizing hormone and prolactin were determined radioimmunoassay using reagents, which were kindly provided by Dr. A. F. Parlow (NIAMDD).

Statistics

The data obtained were subjected to a variance analysis (Dunnett test) for multiple comparisons among groups. The results were considered to be significant if p<0.05.

Results

MXT-mammary tumors of the mouse

As is seen from Table 1, ovariectomy led to significant inhibition of tumor growth compared to the control, inducing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose s.c. (mg/kg)</th>
<th>Number of animals</th>
<th>Tumor area (mm²)</th>
<th>Inhibition (%)</th>
<th>Tumor weight (mg)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(Vehicle)</td>
<td>8</td>
<td>215±134</td>
<td>0</td>
<td>2199±1185</td>
<td>0</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>(Vehicle)</td>
<td>5</td>
<td>113± 61*</td>
<td>55</td>
<td>941±368*</td>
<td>57</td>
</tr>
<tr>
<td>ON</td>
<td>30</td>
<td>9</td>
<td>120± 62*</td>
<td>52</td>
<td>976±513*</td>
<td>56</td>
</tr>
<tr>
<td>ICI</td>
<td>30</td>
<td>9</td>
<td>168± 41</td>
<td>33</td>
<td>1579±389</td>
<td>28</td>
</tr>
<tr>
<td>ON+ICI</td>
<td>5±30</td>
<td>9</td>
<td>71± 23*</td>
<td>72</td>
<td>487± 153*</td>
<td>78</td>
</tr>
</tbody>
</table>

The animals were treated six times a week for 3 weeks. Tumor area and weight were evaluated after 3 weeks of therapy

* Significant difference in comparison to the control (P<0.05)