TAMOXIFEN AS AN ANTI-TUMOUR AGENT: ROLE OF OESTRADIOL AND PROLACTIN

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(Received 15 May 1975)

SUMMARY

Four-day cyclic rats fed 7,12-dimethylbenz(a)anthracene (DMBA) (20 mg) at 50 days of age had peak prolactin, oestradiol and uterine wet weights at pro-oestrus. Tamoxifen (50, 200 and 800 μg daily), administered to ovariectomized rats, produced significant (P < 0.05) decreases in oestrogen-stimulated prolactin levels but was unable to reduce prolactin to control values. Tamoxifen (12.5, 50 and 200 μg daily) produced decreases in size in DMBA-induced rat mammary carcinomata in intact rats although some tumours did not respond to therapy. The ability of the pituitary to produce prolactin was not impaired. Decreases in uterine wet weights and peripheral oestriadiol levels occurred during tamoxifen treatment.

INTRODUCTION

Prolactin has been shown to be intimately involved in the growth of dimethylbenz(a)-anthracene (DMBA)-induced rat mammary carcinomata (Pearson, Molina, Butler, Llerena & Nasr, 1972), although the recent work by Sinha, Cooper & Dao (1973) suggests that simultaneous oestrogen stimulation is also required for effective tumour growth. Since oestrogens are known to raise plasma prolactin levels (Grosvenor & Turner, 1960; Chen & Meites, 1970), non-steroidal anti-oestrogens may exert an anti-tumour effect by impairing oestrogen-stimulated prolactin release. In the rat, U-11,100A (nafoxidide; 1-[2-[(3,4-dihydro-6-methoxy-2-phenyl-1-naphthyl)-phenoxy]ethyl]-pyrrolidine) (Heuson, Waelbroeck, Legros, Gallez, Robyn & L’Hermite, 1971/72), MER 25 (ethamoxytriphetol, 1-(p-β-diethylaminoethoxyphenyl)-2-(p-methoxyphenyl) ethanol) and ICI 46,474 (tamoxifen, trans 1-(p-β-dimethylaminoethoxyphenyl)-1,2-diphenyl but-1-ene) (Jordan, Koerner & Robison, 1975) significantly reduce oestrogen-raised levels of prolactin, thereby suggesting a possible role in anti-tumour activity. Although the DMBA-induced tumour model gives rise to many types of tumours with varying hormonal responses (Mobbs, 1966; Leung, Sasaki & Leung, 1975), this similarity to human breast cancer was considered ideal to determine whether the inhibition of oestrogen-stimulated prolactin release paralleled the anti-oestrogenic and anti-tumour properties of tamoxifen.

MATERIALS AND METHODS

(i) Levels of oestradiol and prolactin during the oestrous cycle

7,12-Dimethylbenz(a)anthracene (Sigma Chemicals) was administered by gavage (20 mg DMBA/2 ml peanut oil) to forty-five 50-day-old female Sprague-Dawley rats (Charles River breeding Laboratories) (Huggins, Grand & Brillantes, 1961). Animals were housed...
under conditions of 12 h light and 12 h darkness and were given food and water ad libitum. Thirty days after DMBA administration rats were smeared daily (between 09.00 and 10.00 h) to establish regular 4-day cycles. Animals were killed by decapitation at known cycle times between 11.00 and 12.00 h. Precautions were taken to reduce noise during the experiment and decapitation was undertaken in a screened area of the laboratory. Blood was collected into ice-cold heparinized tubes and plasma was obtained by centrifugation at 1800 g for 10 min at 4 °C. Plasma was frozen and stored (−10 °C) before radioimmunoassay. Uteri were dissected free of adhering fat, blotted and weighed on a torsion balance.

(ii) Inhibition of oestrogen-stimulated prolactin release

Sprague–Dawley rats (180–200 g) were ovariectomized under ether anaesthesia. Seven days later, animals were divided into six groups of eight rats each. Groups received daily administrations of either oestradiol (5 µg; Sigma Chemicals) alone or in combination with tamoxifen (12.5, 50, 200, 800 µg; Imperial Chemical Industries Ltd). All compounds were administered s.c. in 0.1 ml peanut oil with multiple injections made at separate sites. Ovariectomized control animals received peanut oil alone and vehicle was administered to oestriadiol-treated rats not receiving tamoxifen. Administrations were continued daily for 7 days. On the morning of day 8, animals were treated and placed in a noise-free environment. Plasma and uteri were obtained as described in (i) above between 20.00 and 22.00 h on day 8.

(iii) Anti-tumour activity of tamoxifen

Tumours were induced in thirty 50-day-old Sprague–Dawley rats as previously described (Jordan & Dowse, 1976). Eighty days after DMBA administration, rats with tumours were divided into four groups, each of six rats. Groups received daily s.c. injections of coded solutions: 12.5, 50 or 200 µg tamoxifen (in 0.1 ml peanut oil) or peanut oil alone. Tumour areas were determined for 4 weeks (Jordan & Dowse, 1976). Results were calculated as percentage increase or decrease of the tumour area recorded before treatment. Only tumours present at the start of the experiment were followed, although the occurrence of other tumours in the groups was noted. At the termination of the blind experiment vaginal smears were recorded for a further 3 days and the plasma and uteri were obtained at 11.00–12.00 h on day 3, as described in (i) above.

(iv) Radioimmunoassay

Oestradiol

Peripheral plasma was extracted twice with ethyl acetate–cyclohexane (2:1, v/v) and the pooled extract evaporated to dryness. Samples were dissolved in iso-octane, chromatographed on Celite columns and assayed using the methods presented by Labhsetwar & Watson (1974). Assay sensitivity was 5 pg. Recoveries were in the range 80–85 %. Results were corrected for 100 % recovery by the addition of [3H]oestradiol to plasma before extraction.

Prolactin

Plasma was assayed using the methods described by Odell, Rayford & Ross (1967). Rat prolactin and antibody were obtained from the NIAMDD. Results are expressed as ng NIAMDD-rat prolactin-RP1 standard/ml rat plasma. Sensitivity was 0.25 ng/ml.
RESULTS

Levels of oestradiol and prolactin during the oestrous cycle

The results are summarized in Table 1. Uterine wet weight, oestradiol and prolactin levels were all maximal at pro-oestrus.

Inhibition of oestrogen-stimulated prolactin release

The administration of oestradiol (5 µg/day, s.c., in 0.1 ml peanut oil for 8 days) to 7-day ovariectomized rats produced a sharp increase in uterine weight and plasma prolactin levels (Table 2). A progressive decrease in uterine wet weight was observed with increasing doses of tamoxifen (12.5, 50, 200 and 800 µg/day) but although significant ($P < 0.05$) decreases in prolactin levels were noted this effect plateaued above 50 µg/day and the levels were not reduced to those found in ovariectomized rats. In a parallel experiment tamoxifen alone was administered to ovariectomized rats. Administration of 800 µg tamoxifen/day produced significant ($P < 0.05$) increases in uterine wet weight although no significant rise was observed in the level of plasma prolactin.

Table 1. Mean uterine wet weights, oestradiol and prolactin levels (± S.E.M.) at various stages of the oestrous cycle of rats fed 7,12-dimethylbenz(a)anthracene (20 mg) 35–40 days before death

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>No. per group</th>
<th>Uterine wet wt (mg)</th>
<th>oestradiol (pg/ml)</th>
<th>prolactin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dioestrus</td>
<td>12</td>
<td>301 ± 10***</td>
<td>105.2 ± 17.0*</td>
<td>21.1 ± 4.5*</td>
</tr>
<tr>
<td>Pro-oestrus</td>
<td>9</td>
<td>476 ± 22</td>
<td>130.5 ± 14.2*</td>
<td>81.2 ± 19.2</td>
</tr>
<tr>
<td>Oestrus</td>
<td>13</td>
<td>420 ± 14*</td>
<td>71.2 ± 8.6***</td>
<td>71.2 ± 22.0</td>
</tr>
<tr>
<td>Metoestrus</td>
<td>11</td>
<td>292 ± 10***</td>
<td>64.6 ± 12.7**</td>
<td>39.7 ± 13.7</td>
</tr>
</tbody>
</table>

Number of determinations per group in parentheses.
Pro-oestrus values compared with other cycle stage values, by Student's $t$-test: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; other values $P > 0.05$.

Anti-tumour activity of tamoxifen

Tamoxifen was administered on a blind basis at three dose levels (12.5, 50 and 200 µg/day) to rats with established DMBA-induced rat mammary carcinomata. The percentage change in tumour areas for the three treated groups and the vehicle-treated controls is presented in Fig. 1. The control tumours increased in size over the 4 weeks of the experiment whereas the animals treated with 12.5 and 200 µg tamoxifen/day showed a progressive decline in mean tumour size. After an initial significant ($P < 0.02$) decrease, the mean tumour area of the 50 µg/day treated group increased. In this group 50% of the tumours were unresponsive to tamoxifen therapy and 75% of these were on the same rat. In the other groups 2 out of 15 tumours were unresponsive to 12.5 µg tamoxifen/day and 4 out of 17 tumours were unresponsive in the 200 µg tamoxifen/day group. Five, five, four and nil new tumours occurred in the control, 12.5, 50 and 200 µg/day groups respectively, during the treatment period, but these were not included in the group comparisons.

Control rats were found to display oestrous cycles whereas treatment groups all presented with dioestrous smears. Uterine wet weights of the control rats (Table 3) presented a pattern
Table 2. Mean uterine wet weights and plasma prolactin levels (± S.E.M.) of ovariectomized rats treated for 8 days with s.c. injections of oestradiol alone or in combination with increasing doses of tamoxifen (8 rats per group)

<table>
<thead>
<tr>
<th>Tamoxifen (µg/day)</th>
<th>Oestradiol (µg/day)</th>
<th>Uterine wet wt (mg)</th>
<th>Plasma prolactin levels (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-5</td>
<td>5</td>
<td>152 ± 7***</td>
<td>22-8 ± 5-6***</td>
</tr>
<tr>
<td>50-0</td>
<td>5</td>
<td>515 ± 19</td>
<td>304-4 ± 48-4</td>
</tr>
<tr>
<td>200-0</td>
<td>5</td>
<td>443 ± 19**</td>
<td>240-8 ± 27-3</td>
</tr>
<tr>
<td>800-0</td>
<td>5</td>
<td>378 ± 27***</td>
<td>179-4 ± 16-8*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>290 ± 11***</td>
<td>183-1 ± 4-7*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>252 ± 13***</td>
<td>172-9 ± 27-9*</td>
</tr>
</tbody>
</table>

Level of significance compared with oestradiol-treated rats, by Student's t-test: *P < 0.05; **P < 0.02; ***P < 0.001; other values P > 0.05.

Fig. 1. Mean tumour responses of rats with 7,12-dimethylbenz(a)anthracene-induced mammary carcinomata to the daily administration (s.c.) of vehicle (open triangles) or tamoxifen: 12-5 µg (solid circles), 50 µg (solid triangles), 200 µg (solid squares). Number of tumours per group is shown in parentheses, each group comprised six rats. Tumours were measured once a week and percentage increase or decrease was calculated from the tumour area at the start of the experiment. Statistical significance of the appropriate control v. treated groups was calculated using Student's t-test: *P < 0.05; **P < 0.02. For clarity standard errors of the mean were not included.
similar to that observed in Table 1, with a peak weight at pro-oestrus. Tamoxifen in increasing doses reduced uterine weights in a dose-dependent manner. Oestradiol levels in the plasma (Table 3) were reduced, but statistical analysis by a comparison of treated and control groups was precluded by the variations due to the oestrous cycle. In the control group the highest levels of prolactin were observed at pro-oestrus (Table 3) but increasing doses of tamoxifen did not produce a consistent decrease in prolactin in the circulation. The 200 \( \mu \text{g} \) tamoxifen/day treated group had statistically higher \( (P < 0.05) \) prolactin levels than those of the 12.5 \( \mu \text{g} \) tamoxifen/day group, but this apparent increase was not greater than the prolactin levels observed in the two control rats at pro-oestrus. Tamoxifen did not, therefore, impair the ability of the pituitary to produce high levels of prolactin in the circulation.

Table 3. Cycle stage, uterine wet weight, peripheral oestradiol and prolactin levels of rats with 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinomata (6 rats/group) treated with daily doses of vehicle or tamoxifen for 4 weeks

<table>
<thead>
<tr>
<th>Daily treatment</th>
<th>Cycle stage</th>
<th>Uterine wet wt (mg)</th>
<th>Peripheral plasma levels:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut oil</td>
<td>Pro-oestrus</td>
<td>700</td>
<td>Oestradiol (pg/ml)</td>
</tr>
<tr>
<td></td>
<td>Oestrus</td>
<td>630</td>
<td>169-1</td>
</tr>
<tr>
<td></td>
<td>Metoestrus</td>
<td>595</td>
<td>119-5</td>
</tr>
<tr>
<td></td>
<td>Dioestrus</td>
<td>500</td>
<td>93-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>391</td>
<td>66-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>450</td>
<td>121-9</td>
</tr>
<tr>
<td>12.5 ( \mu \text{g} )</td>
<td>Dioestrus</td>
<td>389±31</td>
<td>109-2±27-9</td>
</tr>
<tr>
<td>50.0 ( \mu \text{g} )</td>
<td>Dioestrus</td>
<td>289±13**</td>
<td>74-7±9-1</td>
</tr>
<tr>
<td>200.0 ( \mu \text{g} )</td>
<td>Dioestrus</td>
<td>258±12***</td>
<td>77-9±5-5</td>
</tr>
</tbody>
</table>

Comparison of 12.5 \( \mu \text{g} \) tamoxifen/day with other tamoxifen-treated group values, by Student's \( t \)-test: \*\( P < 0.05 \); **\( P < 0.02 \); ***\( P < 0.01 \); other values \( P > 0.05 \).

DISCUSSION

Shaikh (1971) demonstrated peak production of ovarian oestradiol at 12.00 h during pro-oestrus with a rapid decrease by 12:00 h at oestrus. In the present study peripheral oestradiol was found to be maximal during pro-oestrus (Table 1) with a significant decrease in levels in the circulation during oestrus and metoestrus. The increased levels of oestradiol result in rapid increases in uterine wet weight (Astwood, 1938) and peripheral prolactin levels (Chen & Meites, 1970). Pro-oestrus rises in prolactin have previously been noted by other workers (Kwa & Verhofstad, 1967; Niswender, Chen, Midgley, Meites & Ellis, 1969; Pearson et al. 1972). Clearly, after DMBA administration, fluctuations in oestradiol, prolactin and uterine wet weights occur consistent with stages of the oestrous cycle, as determined from vaginal smears.

Although tamoxifen significantly \( (P < 0.05) \) reduced the oestrogen-stimulated rise in plasma prolactin levels (Table 2), there was a more profound effect upon oestrogen-stimulated rises in uterine weight \( (P < 0.001) \). We have previously reported this lack of parallelism in the ability of tamoxifen to inhibit oestrogen-stimulated events (Jordan et al. 1975). A similar anti-oestrogenic compound, U-11,100A, was reported by Heuson et al. (1971/72) to inhibit oestrogen-stimulated rises in serum prolactin, but again these workers were unable to reduce the prolactin levels to those found in the ovariecctomized rat.
The inability to demonstrate a dose–response relationship with tamoxifen for anti-tumour effects (Fig. 1) probably reflect the heterogeneous DMBA-induced tumour populations, since each dose was previously shown to be increasingly anti-uterotrophic (Table 2).

Leung et al. (1975) have reported that DMBA-induced tumours in the ovariectomized rat may respond to either oestradiol or prolactin but the non-steroid anti-oestrogen, U-11,100A was unable to control the prolactin-dependent tumours. Although the majority of tumour areas decreased during tamoxifen therapy, the ability of the pituitary to continue to produce high levels of prolactin in the circulation suggests that prolactin-dependent tumours, which may be insensitive to anti-oestrogen therapy, continue to survive by activation of the prolactin receptor (Turkington, 1974). The possibility that insensitive tumours in the various treatment groups had attained hormone independence cannot be excluded.

Although the study did not demonstrate a clear decrease in oestradiol levels in the circulation during tamoxifen therapy (Table 3) the 50 and 200 μg treated groups were only half of the control pro-oestrous levels. In a recent study (Watson, Alain, Anderson & Heald, 1974), tamoxifen (200 μg/kg) was found to inhibit the oestrogen surge before implantation. This effect was thought to be a direct effect upon the ovary since levels of luteinizing hormone in the circulation were unchanged. If in fact oestrogen levels are reduced, either by direct action on the ovary or by reduction in follicle-stimulating hormone levels, then this would enhance the effectiveness of the competitive antagonism at the oestrogen receptor by tamoxifen (Skidmore, Walpole & Woodburn, 1972), and thus the direct effect of oestrogens at the tumour level would be further retarded (Lee & Oyasu, 1974).

In conclusion, tamoxifen was found partially to reduce oestrogen-stimulated prolactin levels in the ovariectomized rat, but although tamoxifen inhibited the growth of DMBA-induced rat mammary carcinomata, the ability of the pituitary to produce prolactin was not impaired. Therefore, tamoxifen may have a direct effect upon the tumour by blocking oestrogen binding (Jordan & Dowse, 1976) rather than an indirect action by completely blocking oestrogen-stimulated prolactin release.

We would like to thank Miss Carol Robinson and Mr David Watson for invaluable technical assistance, and ICI United States for a grant to support S.K. and the study. V.C.J. was supported by contracts AID/CSD 2837 and No. 1-CB-43967. The rat prolactin was a generous gift from the NIAMDD.

REFERENCES


