PROLONGED OESTROGENIC AND MITOGENIC ACTIVITY OF TAMOXIFEN IN THE OVARIECTOMIZED MOUSE

L. MARTIN AND E. MIDDLETON
Department of Hormone Physiology, Endocrine Group, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX

(Received 8 November 1977)

SUMMARY

A dose of tamoxifen (ICI 46,474), which has been found to inhibit the vaginal smear response to oestrogens, exerted prolonged oestrogenic effects in mice and increased the rate of cell proliferation in both the vagina and uterus. The vaginal epithelium became multilayered with stratified or cornified surface layers and the uterus developed gross cystic glandular hyperplasia. This demonstrates that ICI 46,474 is simply a weak oestrogen in the mouse.

INTRODUCTION

Emmens (1971), using the mouse vaginal smear test, found that subcutaneous injections of tamoxifen (ICI 46,474) had prolonged effects, which were initially oestrogenic but later antioestrogenic. Jordan (1975) confirmed Emmens's findings that soon after injection vaginal smears are cornified but later they are negative and oestrogen treatment fails to induce positive smears. However, in Jordan's experiments the vaginal and uterine weights of treated animals were always much higher than those of the controls suggesting that the drug exerted prolonged oestrogenic effects. Therefore, we gave ovariectomized mice doses of ICI 46,474 identical to those used by Jordan (1975) and examined the histological structure and rates of cell proliferation in the reproductive tract.

ANIMALS AND METHODS

Six-week-old randomly bred mice of the strain used previously (Emmens, 1971; Martin, Finn & Trinder, 1973) and weighing 25–30 g were ovariectomized using tribromoethanol (Avertin, Winthrop) anaesthesia. Two weeks later they were 'primed' with a single s.c. injection of 100 ng oestradiol-17β in arachis oil. Six days later they were given one or two injections, 24 h apart, of 0-05 ml arachis oil s.c. with or without 1.5 mg ICI 46,474 (batch ADM 56110/75). The solution of ICI 46,474 was made up immediately before use from a freshly prepared ethanolic solution. The dose schedule was that used by Jordan (1975).

Animals were killed at various times up to 60 days after injection. Smears taken from the dorsal vaginal surface immediately before autopsy were stained with methylene blue and classed as positive or negative according to Emmens (1960). Uteri and vaginas were weighed and fixed in Bouin's fluid with the uteri pinned out to constant length. Transverse wax sections (5 μm) were prepared according to Martin et al. (1973). Vaginal mitotic indices were determined from counts of 2000 basal cells/organ. Uterine luminal and glandular epithelial cell numbers, and mitotic and dead cell indices were determined as described by Martin et al. (1973).
RESULTS

Effects on the vagina

Four days after the injection of ICI 46,474, vaginal weights had increased from 40 to 70 mg and remained significantly higher than those of the control groups for the 60 days of the experiment (Table 1). Smears became cornified at 48–96 h but thereafter increasing numbers became negative with many leucocytes, although often with many cornified cells as well. These results are similar to those obtained by Jordan (1975). However, histological sections showed that from 48 h onwards the epithelia of the treated animals were multilayered with stratified or cornified surface layers (Pl. 1, fig. 1) whereas the epithelia of control animals were thin and atrophic (Pl. 1, fig. 2).

The mitotic index of the basal cells quadrupled within 24 h of the injection of ICI 46,474; thereafter it fluctuated but remained two to three times higher than that of the controls throughout the experiment.

Effects on the uterus

Weights increased rapidly and remained high for 28 days in treated mice; thereafter they fell, but were double those of control animals at the end of the experiment.

The initial pattern of luminal cell proliferation induced by ICI 46,474 was similar to that induced by oestradiol-17β (Martin et al. 1973): a rapid fall in the proportion of dead cells, a rapid rise in the proportion of mitotic cells, with cell numbers increasing to a maximum by 48 h. As cell numbers increased the mitotic index fell toward control values, as it does in mice treated continuously with oestrogen (Lee, 1972).

After day 3, the proportion of dead luminal cells increased and remained at values well above those of control uteri. At day 60, mitotic and dead cell indices of treated uteri were respectively 4 and 16 times greater than those of controls. Thus, although cell numbers were not greatly increased by ICI 46,474 at this time, cell proliferation and turnover rates were increased.

At autopsy 28–60 days after injection, uteri from treated mice appeared to contain ‘bubbles’: histological examination showed these to be grossly cystic glands (Pl. 2, figs 3 and 4). Development of cystic hyperplasia was preceded by a steady increase in gland cell numbers which was first evident 24 h after the injection of ICI 46,474 (Table 1). By day 13 they had increased to almost six times the control values; up to this time gland structure, though hyperplastic (Pl. 2, fig. 3a), was normal. At 60 days, gland cell numbers though declining were three times greater than those of control animals and some uteri still showed signs of cystic hyperplasia (Pl. 2, fig. 3c).

In the first week after treatment, gland mitotic indices rose two to three times above those of the initial control group. This response was similar to that induced by oestrogen (Finn & Martin, 1973). Thereafter the mitotic indices of treated mice fluctuated about lower values, but even at 60 days were nine times higher than those of the controls killed at the same time.

Like oestradiol-17β (Martin et al. 1973; Martin, Pollard & Fagg, 1976) ICI 46,474 rapidly reduced the number of dead cells visible in the glands and values remained low for 72 h. Thereafter, and during the phase of cystic hyperplasia, the proportion of dead cells increased to values well above those of the initial controls and at 60 days was 20 times higher than in controls at that time. Thus ICI 46,474 increased the proliferation and turnover rates of the glandular cells throughout the experiment.

DISCUSSION

Most studies have shown that compounds which are effective partial antagonists of oestrogen in the rat are totally ineffective in the mouse where they are weakly oestrogenic.
Table 1. Effects of tamoxifen on cell proliferation in the mouse uterus and vagina (means ± S.E.M., n = 5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Autopsy at</th>
<th>Wet wt (mg)</th>
<th>No. of positive smears</th>
<th>Mitotic index (%)</th>
<th>Wet wt (mg)</th>
<th>No.</th>
<th>% Mitotic</th>
<th>% Dead</th>
<th>No.</th>
<th>% Mitotic</th>
<th>% Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>AO × 1</td>
<td>24 h</td>
<td>40 ± 4</td>
<td>0</td>
<td>0.4 ± 0.2</td>
<td>39 ± 2</td>
<td>277 ± 27</td>
<td>0.7 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>432 ± 45</td>
<td>0.7 ± 0.2</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>T × 1</td>
<td>24 h</td>
<td>48 ± 2</td>
<td>2</td>
<td>1.6 ± 0.2</td>
<td>100 ± 4</td>
<td>683 ± 37</td>
<td>3.1 ± 0.8</td>
<td>0.1 ± 0.1</td>
<td>610 ± 44</td>
<td>0.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>T × 2</td>
<td>48 h</td>
<td>59 ± 5</td>
<td>4</td>
<td>1.4 ± 0.1</td>
<td>139 ± 18</td>
<td>908 ± 30</td>
<td>0.7 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>677 ± 122</td>
<td>1.7 ± 0.4</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>T × 2</td>
<td>72 h</td>
<td>66 ± 6</td>
<td>5</td>
<td>0.9 ± 0.1</td>
<td>121 ± 9</td>
<td>875 ± 105</td>
<td>0.7 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>963 ± 45</td>
<td>2.3 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>T × 2</td>
<td>96 h</td>
<td>70 ± 6</td>
<td>4</td>
<td>1.7 ± 0.2</td>
<td>135 ± 8</td>
<td>987 ± 100</td>
<td>0.2 ± 0.1</td>
<td>1.8 ± 0.4</td>
<td>1106 ± 138</td>
<td>1.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>T × 2</td>
<td>7 days</td>
<td>73 ± 7</td>
<td>3</td>
<td>0.7 ± 0.2</td>
<td>148 ± 9</td>
<td>687 ± 49</td>
<td>0.5 ± 0.3</td>
<td>5.9 ± 1.1</td>
<td>1702 ± 301</td>
<td>1.8 ± 0.2</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>T × 2</td>
<td>14 days</td>
<td>71 ± 6</td>
<td>1</td>
<td>1.0 ± 0.1</td>
<td>123 ± 6</td>
<td>680 ± 85</td>
<td>0.5 ± 0.2</td>
<td>2.9 ± 0.4</td>
<td>2616 ± 410</td>
<td>0.7 ± 0.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>T × 2</td>
<td>21 days</td>
<td>69 ± 4</td>
<td>2</td>
<td>1.2 ± 0.2</td>
<td>125 ± 7</td>
<td>523 ± 77</td>
<td>0.7 ± 0.2</td>
<td>2.2 ± 0.5</td>
<td>1643 ± 111</td>
<td>1.4 ± 0.2</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>T × 2</td>
<td>28 days</td>
<td>81 ± 6</td>
<td>0</td>
<td>0.8 ± 0.1</td>
<td>135 ± 6</td>
<td>666 ± 88</td>
<td>0.5 ± 0.2</td>
<td>4.1 ± 0.4</td>
<td>1788 ± 212</td>
<td>0.8 ± 0.2</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>T × 2</td>
<td>49 days</td>
<td>63 ± 1</td>
<td>0</td>
<td>0.9 ± 0.2</td>
<td>79 ± 9</td>
<td>491 ± 48</td>
<td>0.6 ± 0.2</td>
<td>6.4 ± 2.2</td>
<td>1841 ± 526</td>
<td>1.0 ± 0.3</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td>T × 2</td>
<td>60 days</td>
<td>62 ± 3</td>
<td>0</td>
<td>0.9 ± 0.2</td>
<td>75 ± 16</td>
<td>459 ± 63</td>
<td>0.4 ± 0.1</td>
<td>1.6 ± 0.9</td>
<td>1368 ± 316</td>
<td>0.9 ± 0.2</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>T × 2</td>
<td>60 days</td>
<td>45 ± 4</td>
<td>0</td>
<td>0.3 ± 0.1</td>
<td>42 ± 7</td>
<td>278 ± 12</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>414 ± 83</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.05</td>
</tr>
</tbody>
</table>

Mice were given one or two injections of arachis oil (AO) or 1·5 mg tamoxifen in arachis oil (T). The time of autopsy in hours or days relates to the time of the first or only injection. Differences between the luminal and glandular mitotic and dead cell indices of the 24 h and 60 day control groups were due to the higher turnover rates of cells shortly after priming.
This is true of U,11100A (Emmens & Martin, 1965; Terenius, 1970; Lee, 1974) and, at least in short-term tests, of ICI 46,474 (Harper & Walpole, 1967; Terenius, 1970; Emmens, 1971; Lee, 1974; Jordan, 1975). The present results suggest that the long-term inhibition of vaginal cornification by ICI 46,474 described by Emmens (1971) and Jordan (1975) is an artifact arising from the method of evaluating vaginal smears.

Emmens, Cox & Martin (1960) found that progestins inhibit vaginal cornification evaluated by smears, but have no effect on cornification evaluated in histological sections. This group also found that many compounds which inhibit cornification evaluated by smears do not suppress the proliferative responses to oestrogens (Emmens et al. 1960, 1962).

In our study, histological examination showed that many leucocytes were present in the vaginae of treated animals even when the epithelium was stratified or cornified; such leucocytosis could account for the ‘inhibition’ observed by Emmens (1971) and Jordan (1975).

In classifying a vaginal smear as positive, equal importance is assigned to the absence of leucocytes as to the presence of cornified cells (Emmens, 1960). The basis for assigning such importance to leucocytes stems from their disappearance at oestrus (Allen, 1922) and in short-term oestrogen assays which do not involve antagonists (Biggers & Claringbold, 1954). Their validity as criteria of assessment in long-term tests has never been established. Prolonged, continuous treatment with oestrogens produces uterine leucocytosis (Burrows, 1945) and it seems likely that similar effects could be generated in the vagina particularly if submaximal stimulation was involved.

Cystic glandular hyperplasia of the type found here develops in ovariectomized mice treated continuously with oestrogen (Parkes, 1935) or with long-acting triarylalkenes similar to ICI 46,474 (Emmens & Carr, 1973). It is also produced by continuous treatment with oestradiol-17\(\beta\) in mice of the strain used here and in C57 Black and BR6 inbred mice (L. Martin & A. E. Lee, unpublished observations). Such hyperplasia bears a striking resemblance to that found in human endometria (Vellios, 1974).

We are grateful to Rosemary Johnson for expert histological assistance, and to Dr B. Furr of ICI for the gift of ICI 46,474.

REFERENCES

Mitogenic effects of ICI 46,474


**DESCRIPTION OF PLATES**

**PLATE 1**
(Stained with haematoxylin and eosin; × 100.)

Fig. 1. Transverse sections showing the vaginal epithelia of mice treated with 2 × 1·5 mg doses of ICI 46,474 and killed (a) 7 days or (b) 60 days later.

Fig. 2. Transverse section through the vagina of an untreated animal killed at the same time as that in Fig. 1b.

**PLATE 2**
(Stained with haematoxylin and eosin; × 50.)

Fig. 3. Transverse sections through the uteri of mice treated with 2 × 1·5 mg doses of ICI 46,474 and killed (a) 4 days, (b) 21 days or (c) 60 days later.

Fig. 4. Transverse section through the uterus of an untreated mouse killed at the same time as that in Fig. 3c.