PROCEEDINGS OF THE

British Pharmacological Society

13th–15th September, 1978

UNIVERSITY OF NOTTINGHAM

COMMUNICATIONS

Contrasting subcellular responses to monohydroxytamoxifen and oestradiol benzoate in the immature rat uterus

C.J. DIX & V.C. JORDAN

Department of Pharmacology, University of Leeds

Monohydroxytamoxifen is a potent antioestrogen with partial agonist activity in the immature rat uterine wet weight test (Jordan, Collins, Rowsby & Prestwich, 1977). In this study its uterine effects are compared with those of a full agonist, oestradiol benzoate.

Monohydroxytamoxifen (25 μg), oestradiol benzoate (25 μg) or vehicle (0.1 ml arachis oil) was administered s.c. to groups of 8 immature female rats, (Alderley Park strain) which were sacrificed 8, 24, 48 and 72 h later. Uterine cytoplasmic progesterone receptor content was determined, by the method of Vu Hai & Milgrom (1978) using the synthetic progestagen R5020 (Dimethyl-19 norpregna-4,19 diene-3,20-dione, 17α21-[17α-methyl-3H]) and uterine DNA content was determined by the method of Burton (1956). Monohydroxytamoxifen and oestradiol benzoate produced an increase in progesterone receptor content, but only oestradiol benzoate produced a significant increase in uterine DNA at 24, 48 and 72 h (Figure 1).

In a second experiment immature female rats (8 per group) were injected s.c. with monohydroxytamoxifen (25 μg) oestradiol benzoate (25 μg) or vehicle (arachis oil 0.1 ml); colchicine (100 μg in saline) was administered 7 h before sacrifice. The uteri were excised at 24, 48 or 72 hours. Histological sections were prepared and mitotic counts ($n = 50$) and epithelial thickness ($n = 20$) determined for each group. Monohydroxytamoxifen and oestradiol benzoate produced a significant ($P < 0.001$) increase in epithelial thickness compared with controls (14.4 ± 0.39 μm), at 24 (27.1 ± 0.95 μm and 23.5 ± 0.69 μm respectively), 48 (44.3 ± 1.13 μm and 42.9 ± 0.40 μm respectively) and 72 h (55.6 ± 1.06 μm and 25.6 ± 0.66 μm respectively); but only oestradiol benzoate produced an increase in mitoses compared with control (0 mitosis/100 cells) at 48 (22.9 ± 0.93 mitoses/100 cells) and 72 h (9.88 ± 0.79 mitoses/100 cells).

In conclusion monohydroxytamoxifen, like oestradiol benzoate can stimulate a rise in rat uterine progesterone receptor content but, unlike oestradiol benzoate, cannot increase uterine DNA content or epithelial cell division. The separation of uterine protein synthesis and DNA synthesis by an oestrogen antagonist may prove to be useful for investigating the mechanism of oestrogen receptor mediated cell division.

The study was supported by I.C.I. Ltd (Pharmaceuticals Division).

References

BURTON, K. (1956). A study of the condition and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.*, **62**, 315–323.

JORDAN, V.C., COLLINS, M.M., ROWSBY, L. & PRESTWICH, G. (1977). A monohydroxylated metabolite of tamoxifen with potent antioestrogenic activity. *J. Endocr.*, **75**, 305–316.

VU HAI, M.T. & MILGROM, E. (1978). Characterization and assay of the progesterone receptor in rat uterine cytosol. *J. Endocr.*, **76**, 21–31.

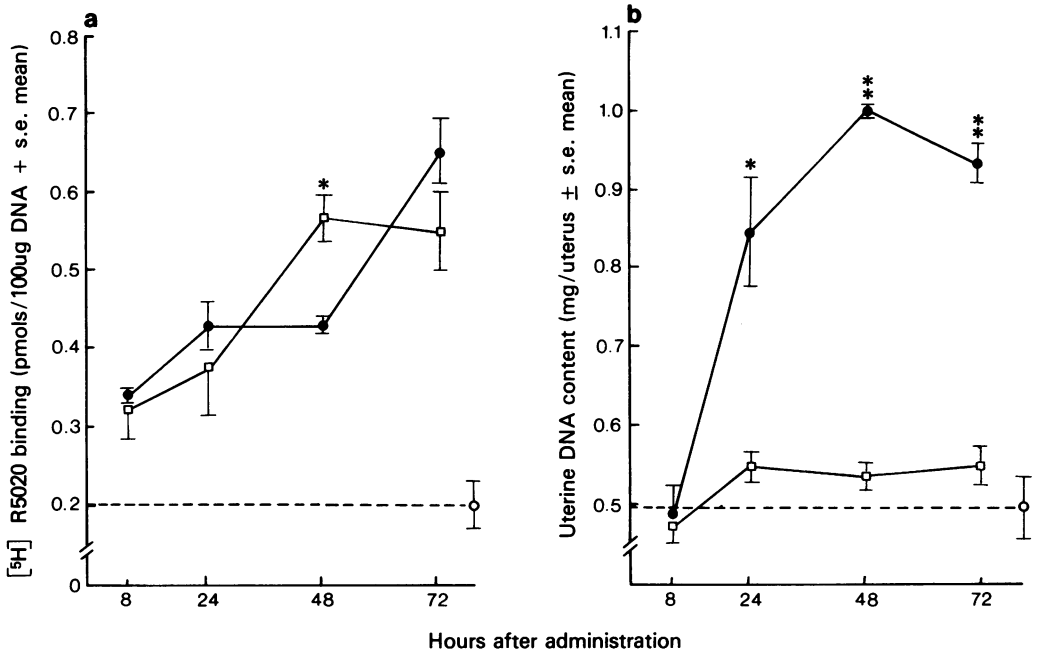

Figure 1 The effect of (□) monohydroxytamoxifen (25 μg) and (●) oestradiol benzoate (25 μg) on (a) the progesterone receptor content and (b) the DNA content, of immature rat uteri. Controls (○) received vehicle (0.1 ml arachis oil). Comparisons of treatment groups at the same time were made by Student's t test. $^{*}P < 0.01$, $^{**}P < 0.001$, all other values $P > 0.05$ ($n = 4$).

The antitumour activity of tamoxifen and monohydroxytamoxifen: a comparative study in the rat

V.C. JORDAN & K.E. NAYLOR

Department of Pharmacology, University of Leeds

Tamoxifen, an antioestrogen used in the treatment of breast cancer, inhibits the initiation (Jordan, 1976*a*) and growth (Jordan, 1976*b*) of hormone-dependent dimethylbenz(*a*)anthracene (DMBA)-induced rat mammary carcinomata. Monohydroxytamoxifen, a metabolite of tamoxifen, has been reported to be a more potent antioestrogen than the parent drug (Jordan, Collins, Rowsby & Prestwich, 1977) and its antitumour activity has now been assessed.

Female Sprague Dawley rats (50 days old) were each given DMBA (20 mg orally in 1 ml arachis oil). Beginning 30 days later, animals (15 per group) were injected s.c. daily with either tamoxifen (0.2, 3, 50 or 800 μg) or monohydroxytamoxifen (0.012, 0.2, 3 or 50 μg) in 0.1 ml arachis oil for 30 days. Controls received vehicle. Eleven weeks after therapy had ceased, tamoxifen had produced a dose-related inhibition of tumour incidence whereas monohydroxytamoxifen was only slightly active (Figure 1). Twenty weeks after therapy 80% of rats previously treated daily with 800 μg tamoxifen had tumours.

In a separate experiment, tamoxifen (800 μg) or monohydroxy-tamoxifen (50 μg) was administered s.c. daily for 30 days to mature female rats ovariectomized 10 days previously (5 per group). Groups were killed on the last day of therapy and 1, 2 and 5 weeks later. Both antioestrogens increased uterine wet weight ($P < 0.001$) and decreased cytoplasmic oestrogen receptor content ($P < 0.001$) when compared with controls. The uterine changes returned to control values 5 weeks after monohydroxytamoxifen whereas the effects of tamoxifen were maintained.

It is concluded that the effectiveness of a 30 day course of antioestrogen therapy in experimental breast cancer is dependent upon prolonged biological activity rather than antioestrogenic potency.

The study was supported by I.C.I. Ltd (Pharmaceuticals Division).