Eur J Cancer Clin Oncol, Vol. 17, No. 9, pp. 1063–1065, 1981.
Printed in Great Britain.

0277-5379/81/091063-03$02.00/0

Letter to the Editor

Effect of Monohydroxytamoxifen on Mouse Mammary Tumors

MELS SLUYSER*, SIEGINA G. EVERS and CORNELIS C. J. DE GOEIJ

Division of Endocrinology, Antoni van Leeuwenhoekhuis, The Netherlands Cancer Institute, Amsterdam, The Netherlands

TAMOXIFEN, a non-steroidal agent, is effective in inhibiting the growth of hormone-dependent mammary tumors in rats [1, 2] and mice [3, 4]. The drug is used clinically for the treatment of breast cancer (for a review see ref. [5]). Several metabolites of tamoxifen have been identified from studies in animals [6] and humans [7]. One of these metabolites, monohydroxytamoxifen, was found to be more active than tamoxifen as an antiestrogen in the immature rat [8]. Monohydroxytamoxifen is a potent inhibitor of the binding of [^{3}H]-estradiol to estrogen receptors *in vitro* [8], and binds better to this receptor than does tamoxifen [9, 10] or estradiol [10]. Monohydroxytamoxifen is more efficient than tamoxifen in inhibiting the induction of 46K protein by estradiol in MCF-7 cells [11].

These data made it of interest to compare the effects of monohydroxytamoxifen with those of tamoxifen on the growth of hormone-dependent mammary tumors. We have carried out this comparative study using hormone-dependent and -independent mammary tumors of GR mice [3, 12, 13].

Ovariectomized mice of the GRS/A (also called GR) strain were treated continuously with estrone and progesterone, as described previously [12]. One of the hormone-dependent mammary tumors obtained in this way was serially transplanted in hormone-treated castrated (020 × GR)F1 hybrid mice, and tumors of the 2nd transplant generation (which was still hormone-dependent) were pooled and used for investigating the growth inhibition potentials of tamoxifen and monohydroxytamoxifen. Another primary mammary tumor turned out to be hormone-independent, and this tumor was serially transplanted in castrated mice that were not given hormone treatment. Tumors of the 6th transplant generation of this line were also pooled and used for investigating growth inhibition by tamoxifen and monohydroxytamoxifen.

Single-cell suspensions of mammary tumor transplants were prepared by treatment with collagenase, hyaluronidase and pronase [14]. Cell number was counted in a hemocytometer and percentage cell death was determined with Trypan Blue exclusion test. The cell numbers mentioned in the following section are all corrected for cell death.

Tamoxifen base (ICI 46,474; *trans*-1-(4-b-dimethylaminoethoxyphenyl)-1, 2-diphenylbut-l-ene) and monohydroxytamoxifen (ICI 79,280; 1-(4-b-dimethylaminoethoxyphenyl)-1-(4-hydroxyphenyl)-2-phenyl-but-l-ene) were gifts from the Imperial Chemical Industries Ltd., Macclesfield, England. Tamoxifen and monohydroxytamoxifen were administered once a week as a pellet (0.5 mg) s.c. [4]. The first dose was given on day 8 following grafting of the tumor cells.

Tumor size was measured with a vernier calliper. The length (l) width (w), and height (h) were measured, and tumor volume (V) calculated from the formula: $V = 0.5253 \times l \times w \times h$ [15].

Portions of a single-cell suspension from pooled hormone-dependent GR mammary tumors were inoculated into castrated (020 × GR)F1 hybrid mice. Each mouse received 4×10^6 tumor cells in 0.5 ml phosphate buffered saline s.c. in the right flank. Twelve mice were treated

*To whom all correspondence and reprint requests should be addressed, at: Antoni van Leeuwenhoekhuis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

with tamoxifen and 12 mice were treated with monohydroxytamoxifen on days 8, 15 and 22 after grafting of the tumor cells; 35 mice were not treated with these drugs (control group). All mice received estrone and progesterone treatment throughout the whole experiment, as follows: estrone was dissolved in ethanol (2 mg/ml) and the solution was added to the drinking water to give a final concentration of 0.5 μg/ml. Progesterone was administered in pellets introduced s.c. in the neck region of the mouse. The dose was 3 pellets (2.7 mg progesterone per pellet) per animal per week.

Figure 1A shows the growth curves of the tumors. In the control group (untreated), outgrowths measuring 100 mm^3 were obtained after 26 days. Tamoxifen and monohydroxytamoxifen extended the period of time before outgrowths reached this size to 31 and 41 days respectively. On day 30, tumor sizes were 394 ± 327 mm^3 (average volume ± S.D.) for the control group and 75 ± 96 mm^3 and 7 ± 10 mm^3 for the groups treated with tamoxifen and monohydroxytamoxifen respectively. The difference between the controls and the antiestrogen-treated groups were statistically significant ($P < 0.001$). Due to marked differences in the latency periods of the treatment groups, tumors of the control group, the tamoxifen-treated group and the monohydroxytamoxifen-treated group had to be excised after 30, 41 and 47 days, yielding 0.34 ± 0.29 g, 0.39 ± 0.28 g and 0.23 ± 0.16 g tumor yields respectively (tumor weight ± S.D.).

We carried out a similar study with hormone-independent GR mammary tumors. Portions of a single-cell suspension prepared from pooled hormone-independent tumors were injected in castrated (020 × GR) F1 hybrid mice (4×10^6 tumor cells per mouse). Twelve mice were treated with tamoxifen and twelve mice were treated with monohydroxytamoxifen on days 8 and 15; 22 mice were not treated (control group). All mice received treatment with estrogen and progesterone. Figure 1B shows that tamoxifen and monohydroxytamoxifen do not affect the growth curves of the tumors. The mice in this experiment were all killed on day 19; weights of the outgrowths obtained from the tamoxifen-treated group (1.40 ± 0.25 g) and the monohydroxy tamoxifen-treated group (1.59 ± 0.53 g) were not significantly different from those obtained from the controls (1.49 ± 0.48 g).

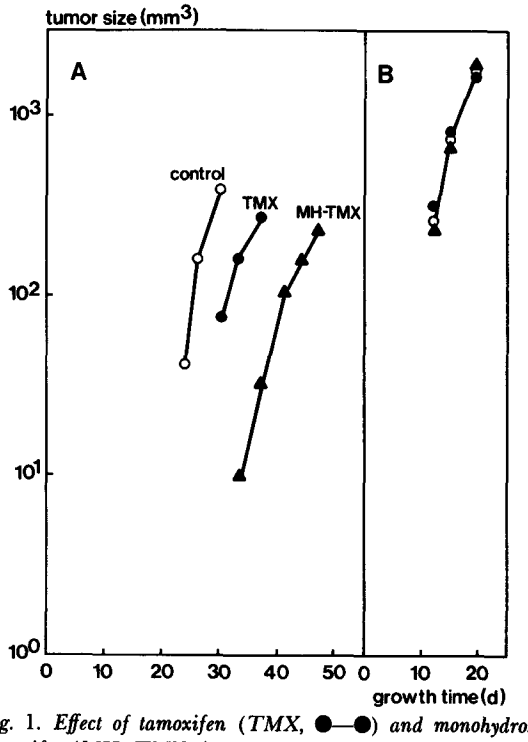

Fig. 1. Effect of tamoxifen (TMX, ●—●) and monohydroxytamoxifen (MH-TMX, ▲—▲) on mouse mammary tumor transplants. Untreated tumors (controls, ○—○). A, hormone-dependent tumors; B, hormone-independent tumors.

Jordan *et al.* [16, 17] report that tamoxifen is a more potent growth inhibitor than monohydroxytamoxifen when tested on DMBA-induced mammary tumors in the rat. They administered these drugs by daily s.c. injection. By contrast, we used mice instead of rats for our experiments and administered the antiestrogens in pellets inserted s.c. in order to ensure a constant flow of the drugs into the animals. It would be of interest to know whether the difference between our results and those of Jordan *et al.* is due to the different way in which we administered the drugs, or to a difference in the relative response to tamoxifen and monohydroxytamoxifen between rat and mouse mammary tumors.

In conclusion, our results show that monohydroxytamoxifen causes more growth inhibition of hormone-dependent GR mouse mammary tumors than tamoxifen when administered in pellets under the skin of GR mice. Neither of these drugs causes growth inhibition of hormone-independent mouse mammary tumors.

REFERENCES

1. NICHOLSON RI, GOLDEN MP. The effect of synthetic antiestrogens on the growth and biochemistry of rat mammary tumors. *Eur J Cancer* 1975; **11**: 571–579.
2. JORDAN VC, JASPAN T. Tamoxifen as an antitumour agent: oestrogen binding as a predictive test for tumour response. *J Endocrinol* 1976; **68**: 453–460.

3. SLUYSER M. Hormone receptors in mouse mammary tumors. *Biochim Biophys Acta* 1979; **560**: 509–529.
4. SLUYSER M. Combined endocrine therapy and chemotherapy of mouse mammary tumors. *Eur J Cancer*, 1981; **17**: 155–159.
5. MOURIDSEN H, PALSHOF T, PATTERSON J *et al.* Tamoxifen in advanced breast cancer. *Cancer Treat Rev* 1979; **5**: 131–141.
6. FROMSON JM, PEARSON S, BRAMAK S. The metabolism of tamoxifen (ICI 46,474) I. In laboratory animals. *Xenobiotica* 1973; **3**: 693–709.
7. FROMSON JM, PEARSON S, BRAMAK S. The metabolism of tamoxifen (ICI 46,474) II. In female patients. *Xenobiotica* 1973; **3**: 711–714.
8. JORDAN VC, COLLINS MM, ROWSBY L, PRESTWICK G. A monohydroxylated metabolite of tamoxifen with potent antiestrogenic activity. *J Endocrinal* 1977; **75**: 305–316.
9. NICHOLSON RI, SYNE JS, DANIEL CP, GRIFFITHS K. The binding of tamoxifen to oestrogen receptor proteins under equilibrium and non-equilibrium conditions. *Eur J Cancer* 1979; **15**: 317–329.
10. BINART N, CATELLI MG, GEYNET C *et al.* Monohydroxytamoxifen: an antiestrogen with high affinity for the chick oviduct oestrogen receptor. *Biochem Biophys Res Commun* 1979; **91**: 812–818.
11. WESTLEY B, ROCHEFORT H. A secreted glycoprotein induced by estrogen in human breast cancer cell lines. *Cell* 1980; **20**: 353–362.
12. SLUYSER M, VAN NIE R. Estrogen receptor content and hormone responsive growth of mouse mammary tumors. *Cancer Res* 1974; **34**: 3253–3257.
13. SLUYSER M, EVERS SG, DE GOEIJ CCJ. Sex hormone receptors in mammary tumors of GR mice. *Nature (Lond)* 1976; **263**: 386–389.
14. SLUYSER M, DE GOEIJ CCJ, EVERS SG. Changes in sensitivity to cyclophosphamide of mouse mammary tumors during serial transplantation. *J Natl Cancer Inst* 1981; **66**: 327–330.
15. JANIK P, BRIAND P, HARTMANN NR. The effect of estrone–progesterone treatment on cell proliferative kinetics of hormone-dependent GR mouse mammary tumors. *Cancer Res* 1975; **35**: 3698–3704.
16. JORDAN VC, ALLEN KE, DIX CJ. Pharmacology of tamoxifen in laboratory animals. *Cancer Treat Rep* 1980; **64**: 745–759.
17. JORDAN VC, ALLEN KE. Evaluation of the antitumour activity in the DMBA-induced rat mammary carcinoma model. *Eur J Cancer* 1980; **16**: 239–251.