Eur J Cancer Clin Oncol, Vol. 19, No. 8, pp. 1179–1181, 1983.
Printed in Great Britain.

0277-5379/83$3.00+0.00

Letter to the Editor

N-Desmethyltamoxifen Inhibits Growth of MCF 7 Human Mammary Carcinoma Cells *in Vitro*

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TAMOXIFEN (trans 1-(4-β-dimethylaminoethoxyphenyl)-1,2-diphenylbut-1-ene), a non-steroidal antioestrogenic compound, is used in the treatment of human breast cancer [1]. Several metabolites of this drug have been described [2]. Although the major metabolite was initially misidentified as monohydroxytamoxifen (*trans* 1-(4-β-dimethylaminoethoxyphenyl)-1-(4-hydroxyphenyl)-2-phenylbut-1-ene), it has recently been demonstrated to be *N*-desmethyltamoxifen (trans 1-(4-β-methylaminoethoxyphenyl)-1,2-diphenylbut-1-ene), and its steady-state plasma levels are 1.5-fold higher than those of the parent drug [3, 4]. Since there have been very few studies of the biological activity of *N*-desmethyltamoxifen [5, 6], we compared the effects of tamoxifen and *N*-desmethyltamoxifen on the proliferation and cell-cycle kinetics of MCF 7 human breast cancer cells [7] *in vitro*. This cell line was chosen because it metabolizes tamoxifen to a negligible extent [5, 8].

The sources of materials and the experimental techniques (cell culture and flow cytometry) were as previously described [9] except that 5% untreated foetal calf serum was used. Exponentially growing cells were plated in 25-cm^2 flasks (Corning, NY) at a density of 5×10^4 cells in 5 ml medium. When cell numbers reached approximately 1×10^5 per flask (30 hr) the medium was replaced and drugs (tamoxifen or *N*-desmethyltamoxifen, with or without a 10-fold lower concentration of oestradiol) were added from ethanolic stock solutions so that the final ethanol concentration was 0.1% in all flasks. The experimental medium was changed daily thereafter. Cells were harvested and viable cells counted after 144 hr.

The control cells exhibited exponential growth with a mean doubling time of approximately 27 hr. At concentrations of 1–5 μM *N*-desmethyltamoxifen and tamoxifen were almost equipotent in causing a dose-dependent inhibition of cell number increase (Fig. 1). The concentration required to produce almost static cell numbers over the 114-hr drug-treatment period was 5 μM for *N*-desmethyltamoxifen and 7.5–10 μM for tamoxifen. At the higher drug concentrations (7.5–10 μM) *N*-desmethyltamoxifen was much more potent than tamoxifen in causing a decrease in cell numbers (Fig. 1).

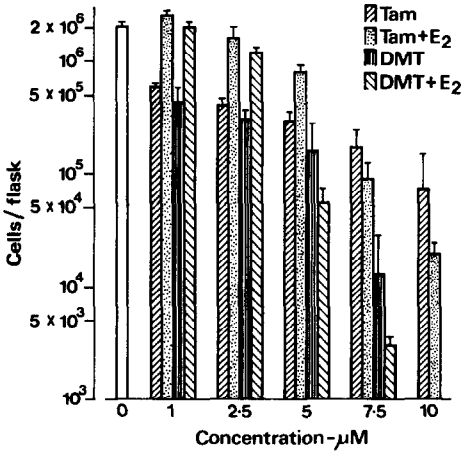

Fig. 1. Effect of tamoxifen and N-*desmethyltamoxifen with or without oestradiol on MCF 7 cell growth. Cells were grown for* 114 hr *in the presence of tamoxifen or* N-*desmethyltamoxifen with or without a* 10*-fold lower dose of oestradiol. Bars represent mean* ± *S.D. of triplicate flasks from* 2–3 *separate experiments. There were no cells remaining in flasks treated with* N-*desmethyltamoxifen* 10 μM.

Accepted 17 March 1983.
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The effects of 1–2.5 μM tamoxifen and *N*-desmethyltamoxifen were completely reversed by oestradiol. However, at the higher doses (5–10 μM) the drug-induced inhibition of cell proliferation was only partially reversed by oestradiol or not reversed at all (Fig. 1). Treatment with oestradiol alone (0.1–1 μM) produced a small but significant increase in cell numbers (data not shown).

Analysis of the DNA histograms obtained by flow cytometry of cells treated with either tamoxifen or *N*-desmethyltamoxifen showed a dose-dependent reduction in the percentage of cells in S phase (Fig. 2) with a concomitant rise in the percentage of cells in G_0/G_1 phase (data not shown). The cell-cycle kinetic effects produced by 1–5 μM tamoxifen and 1–2.5 μM *N*-desmethyltamoxifen were completely reversed by the simultaneous administration of a 10-fold lower concentration of oestradiol, but at higher concentrations reversibility by oestradiol did not occur (Fig. 2).

These results indicate that tamoxifen and its major metabolite, *N*-desmethyltamoxifen, are both potent growth inhibitors of the MCF 7 human breast cancer cell line *in vitro*, with *N*-desmethyltamoxifen being much more potent than tamoxifen in the higher concentration range. It has previously been reported that tamoxifen-induced growth inhibition of MCF 7 cells has both oestrogen-reversible antiproliferative and oestrogen-irreversible cytotoxic components [9], and this observation has now been extended to *N*-desmethyltamoxifen. Rapid decrease in cell numbers occurred on exposure to 7.5 and 10 μM *N*-desmethyltamoxifen, indicating that an increase in cell death rate is involved at the higher, oestrogen-irreversible doses.

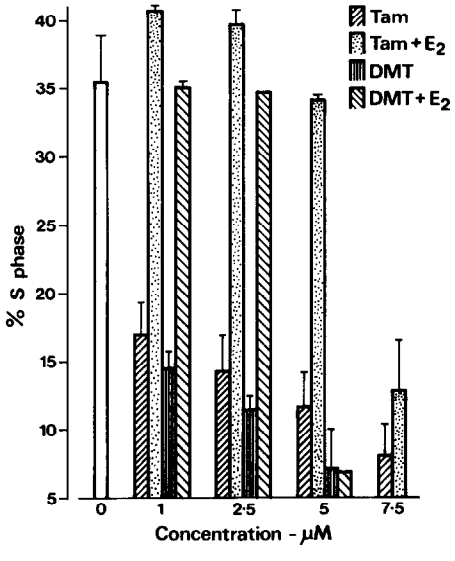

Fig. 2. Effect of tamoxifen and N-desmethyltamoxifen with or without oestradiol on the percentage of MCF 7 cells in S phase of the cell cycle. Cells were grown as in Fig. 1, and the percentage of S-phase cells was calculated from DNA histograms obtained by flow cytometry. Bars represent mean ± S.D. of duplicate flasks from 2–3 separate experiments. There were insufficient cells for analysis in flasks treated with N-desmethyltamoxifen ⩾7.5 μM or tamoxifen 10 μM.

The cell-cycle kinetic data indicate that both tamoxifen and *N*-desmethyltamoxifen are cell-cycle phase-specific agents, causing a dose-dependent reduction in the percentage of cells in S phase with a corresponding rise in the percentage of cells in G_0/G_1. Such kinetic changes may have important implications for the design of treatment regimens in which antioestrogens are combined with other phase-specific cytotoxic chemotherapeutic agents.

In plasma from patients receiving long-term tamoxifen treatment the concentration ratio *N*-desmethyltamoxifen:tamoxifen was 1.5:1, while in tumour homogenates the ratio was 2:1 [3]. Thus the finding that *N*-desmethyltamoxifen is no less potent than tamoxifen in inhibiting human breast cancer cell growth in culture suggests that *N*-desmethyltamoxifen may play a major role in the tumour regression produced by tamoxifen treatment of women with breast cancer. This has implications for interpreting the pharmacokinetics of tamoxifen, since tamoxifen reaches steady-state levels after 4 weeks, in contrast to the 8 weeks required for *N*-desmethyltamoxifen steady-state levels to be attained during the chronic administration of tamoxifen [4], suggesting that the full antitumour effect of tamoxifen may not be achieved until somewhat later than previously realised. A detailed understanding of the relative contributions of the antitumour effects of tamoxifen and its metabolites in this situation is thus of potential importance in the design of more efficacious therapy of human breast cancer.

Acknowledgements—The authors thank ICI Ltd., Pharmaceutical Division for supply of tamoxifen and *N*-desmethyltamoxifen, and Professor M. H. N. Tattersall for his comments on the manuscript.

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