The ability of oestrogen to modulate the effects of cytotoxic drugs in human breast cancer cells: influence of oestrogen receptor status and choice of drug

ROBERT CLARKE,* HENDRIK W. VAN DEN BERG†
and RICHARD F. MURPHY*

*Department of Biochemistry and †Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, N. Ireland, U.K.

We have previously demonstrated that pharmacological concentrations of 17β-oestradiol (E2) increase the cytotoxicity of methotrexate towards hormone responsive MCF-7 human breast cancer cells (Clarke et al., 1985), and this compares with the ability of E2 to potentiate the cytotoxic effects of cytostatic antitumour drugs in these cells (Weichselbaum et al., 1978). However, E2 reduces both the antimitotic and the antiproliferative effects of methotrexate towards E2-unresponsive MDA-MB-436 cells (Clarke et al., 1983). We have subsequently investigated the ability of 10^-9 M-E2 to modulate the effects of clinically achievable concentrations of vincristine (VCR), melphalan (MEL) and adriamycin (ADR) in both MCF-7 and MDA-MB-436 cells.

MDA-MB-436 and MCF-7 cells were maintained as previously described (Clarke et al., 1983, 1985). Cells were seeded into 24 place multiwell dishes at 10^4 cells per well and allowed to attach for 24 h. The growth medium was then replaced with medium containing 10^-9 M-E2 where relevant for a further 24 h. The cells were then exposed to the drug for 24 h in the presence or absence of 10^-9 M-E2 for 24 h. Cell number was estimated on removal of the drug (day 0) and 6 days later (day 6). Cell proliferation is expressed as cell number on day 6 as a percentage of cell number on day 0.

In MCF-7 cells, E2 produces a variable stimulation of cell proliferation (Fig. 1a) (Page et al., 1983; Jakesz et al., 1984; Katzenellenbogen et al., 1984) but does not influence proliferation of MDA-MB-436 cells (Fig. 1b) (Clarke et al., 1985). The inhibitory effects of both VCR and MEL are consistently potentiated in MCF-7 cells (Fig. 1a) but not in MDA-MB-436 cells (Fig. 1b). Thus, the potentiation of the effects of VCR and MEL in the hormone-responsive MCF-7 cells may be the result of the mitogenic potential of E2. However, perturbation of other biochemical pathways not currently identified cannot be precluded.

Whilst the cytotoxic effects of ADR are increased in MCF-7 cells treated in medium containing 10^-7 M-E2, epidermal growth factor, insulin and hydrocortisone (Hug et al., 1986), E2 alone fails to potentiate the cytotoxic effects of ADR (Fig. 1a). The additional mitogens in the medium described by Hug et al. may compensate for any reduction in ADR cytotoxicity induced by E2, for example, by inducing a greater perturbation in cell cycle kinetics. E2 reduces the fluidity of the cellular membranes of both MCF-7 and MDA-MB-436 cells (Clarke et al., 1987). Since ADR may act on the plasma membrane (Tritton & Yee, 1982; Salazar & Cohen, 1984) alterations in membrane fluidity may obscure any increased cytotoxicity resulting from the mitogenic effects of E2 in MCF-7 cells. The inability of E2 to influence ADR-induced cytotoxicity in MDA-MB-436 cells may reflect their intrinsically more fluid membranes (Clarke et al., 1987).

These results suggest that the ability of E2 to potentiate the cytotoxic effects of antineoplastic drugs may be influenced by both the ER content of the target cell and the choice of drug.

Abbreviations used: E2, 17β-oestradiol; VCR, vincristine; MEL, melphalan; ADR, adriamycin.

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Pharmacological and suprapharmacological concentrations of both 17β-oestradiol and tamoxifen reduce the membrane fluidity of MCF-7 and MDA-MB-436 human breast cancer cells

ROBERT CLARKE,* HENDRIK W. VAN DEN BERG,† JOHN NELSON* and RICHARD F. MURPHY*

*Department of Biochemistry and †Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, N. Ireland, U.K.

Over 50% of postmenopausal patients respond to high doses of oestrogenic hormones (Kennedy, 1974) and over 30% of breast cancers respond to tamoxifen (TAM) (Rose et al., 1982). Whilst the ability to respond to endocrine manipulation is related to the oestrogen receptor (ER) content of the tumour, some tumours which do not have ER respond to these therapies (Patterson et al., 1982). We have examined the ability of 17β-oestradiol (E2) and TAM to influence the membrane fluidity of both MCF-7 (E2-responsive) and MDA-MB-436 (E2-unresponsive) cells as determined by the steady-state polarization of fluorescence (P) of 1,6-diphenylhexatriene. P was determined by measuring the emission intensities through an analyser orientated perpendicular to (I₀) and then in parallel with (I∥) the direction of polarization of the excitation light:

$$P = \frac{I₀ - I∥}{I₀ + I∥}$$

An increase in P indicates a decrease in membrane fluidity. Table 1 shows that MCF-7 cells have intrinsically less fluid membranes than MDA-MB-436 and this could be partly due to ER-mediated functions since the cells may not be fully depleted of endogenous steroids (Strobl & Lippman, 1979). However, the observed P values for both cell lines are within the ranges reported for other cell types (Dave et al., 1981; Parola et al., 1981; Sakamoto et al., 1981; Strulovici et al., 1981).

10^-6 M-E2 produces an equivalent increase in P in both cell lines but oestrogenic effects are induced only in MCF-7 cells. In both MCF-7 and MDA-MB-436 cells, 10^-3 M-E2 is approximately equitoxic and produces similar perturbations in membrane fluidity (Table 1). 10^-5 M-TAM also decreases the membrane fluidity of both MCF-7 and MDA-MB-436 cells but anti-oestrogenic effects (i.e. inhibition reversible by E2) are observed only in MCF-7 cells. 10^-6 M-TAM is less cytotoxic (i.e. inhibition not reversible by E2) towards MDA-MB-436 cells than MCF-7 cells, cell number being reduced to 45% and 31% of starting cell number respectively, and produces less perturbation of membrane fluidity. Therefore, the inhibitory effects of high doses of both E2 and TAM are unlikely to be mediated through the ER and may be the result of altered membrane function.

E2 is highly lipophilic and would partition predominantly into the bilipid layer of the cellular membranes. Wilmer (1961) proposed that steroid molecules align themselves with their cyclopentano end inserted into lipid and the more hydrophilic portion seeking the aqueous phase or any polar groups in the vicinity. However, steroids may not be incorporated into the membrane structure but lie flat or on their edge along the surface of the phospholipid polar heads.

Abbreviations used: TAM, tamoxifen; ER, oestrogen receptor; E2, 17β-oestradiol.

Table 1. Effect of E2 and TAM on the steady-state polarization of fluorescence (P) of 1,6-diphenylhexatriene in MCF-7 and MDA-MB-436 human breast cancer cells

<table>
<thead>
<tr>
<th></th>
<th>10^-3 M-E2 (mean ± S.E.)</th>
<th>10^-6 M-E2 (mean ± S.E.)</th>
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<tbody>
<tr>
<td>MCF-7</td>
<td>+0.014 ± 0.004</td>
<td>+0.023 ± 0.003</td>
</tr>
<tr>
<td>MDA-MB-436</td>
<td>+0.013 ± 0.004</td>
<td>+0.032 ± 0.006</td>
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</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>10^-5 M-TAM (mean ± S.E.)</th>
<th>10^-6 M-TAM (mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>+0.015 ± 0.007</td>
<td>+0.056 ± 0.033</td>
</tr>
<tr>
<td>MDA-MB-436</td>
<td>+0.006 ± 0.001</td>
<td>+0.016 ± 0.005</td>
</tr>
</tbody>
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(Duval et al., 1983). The ability of TAM (a triphenylethylene anti-oestrogen) to perturb membrane fluidity may be the result of a similar association with the cellular membranes. Steroid association with the membrane would be expected to alter membrane properties. A decrease in the fluidity of the environment of a membrane protein could inhibit its mobility and thereby influence functional capabilities. Thus, the ability of E2 to reduce the steady-state levels of methotrexate in both MDA-MB-436 (Clarke et al., 1983) and MCF-7 cells (Clarke et al., 1985) and of [3H]folate in chick intestinal cells (Eilam et al., 1982) may be due to a physical restriction of the folate membrane transport system.

The cytotoxic effects of E2 and TAM correlate with their ability to perturb the fluidity of the cellular membranes of both E2-responsive and E2-unresponsive cells and may, therefore, be mediated through altered membrane function.

Rose, C., Theidale, K., Boesen, E., Salimtschik, M., Dombernowski,