in vivo, combining low dose interferon and tamoxifen, has considerable attractions, not least because such a regimen would be expected to be relatively non-toxic.


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The response of human breast cancer cells to glucagon

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Oestrogens stimulate the growth of about 30% of human breast cancers and increase the synthesis of several secreted and intracellular proteins in MCF-7 human breast cancer cells in vitro (Westley & Rochefort, 1979). Many human breast cancer cells specifically bind insulin (Benson & Holdaway, 1982) and there appears to be a relationship between the concentration of insulin and oestrogen binding in mammary tumour systems, both in vivo and in vitro. Rhomberg (1975) reported that a higher proportion of diabetic women with advanced breast cancer respond to hormonal therapy than non-diabetic patients with comparable advanced disease. Butler et al. (1980) reported that insulin reduces the sensitivity of MCF-7 human breast cancer cells in vitro to the effects of oestrogens and antioestrogens.

As there is interplay between the actions of insulin and glucagon in target tissues, glucagon may also be involved in the growth of insulin-responsive breast cancer. We have examined the effects of glucagon on three human breast cancer cell lines MCF-7, ZR-75-1 and MDA-MB-436. Both MCF-7 and ZR-75-1 cells respond to insulin with increased rates of protein and DNA synthesis (Osborne et al., 1978) and so the cells were examined for a similar biological response to glucagon.

In contrast to results obtained with insulin, glucagon treatment has no effect on the rate of DNA synthesis. However, glucagon induces a significant increase in the rate of protein synthesis in each of the cell lines. Fig. 1(a) typifies results obtained with ZR-75-1 cells.

The presence of saturable binding sites was demonstrated by displacement of $^{125}$I-glucagon with excess unlabelled ligand. Data from the competition binding assay have been subjected to Woolf transformation (Keightley et al., 1983) and values of $B_{max}$ and $K_d$ of $5.18 \pm 0.68$ fmol/10$^6$ cells and 1.02 nm respectively were obtained (Fig. 1b). Although two distinct classes of binding sites have been reported in hepatocytes (Bonnevie-Nielsen & Tager, 1983), we have only observed one class in these cells.

We have observed considerable variation in the extent of glucagon binding with each of the cell lines. This may be due to variations in the cell culture conditions. Preliminary results indicate that if cells are incubated in medium with no Phenol Red or hormones for more than a week, receptor number rises significantly. This is in agreement with findings of Hilf et al. (1978), who have shown that insulin receptor number is dependent on oestrogen concentration.


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Fig. 1. Data for ZR-75-1 cells

(a) Effect of glucagon on protein synthesis. (b) Woolf plot of data from the competition binding assay $F$, free glucagon; $B$, bound glucagon.
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

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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 cytotoxic arabinoside in these cells (Weichselbaum et al., 1978). However, E2 reduces both the antimitogenic 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^-8 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 lo4 cells per well and allowed to attach for 24 h. The growth medium was then replaced with medium containing 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^-8 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. Cell proliferation (Fig. 1) (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.