Human recombinant interferon α increases oestrogen receptor expression in ZR-75-1 human breast cancer cells and sensitizes them to the anti-proliferative effects of tamoxifen

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In the past ten years the oestrogen antagonist tamoxifen has played an increasingly important role in the management of primary and metastatic breast cancer. Although the mechanism of action of tamoxifen is imprecisely understood, there is considerable evidence that it acts primarily by blocking oestrogen action at its receptor. Interferon, in contrast to tamoxifen, has been disappearing as a single agent in the treatment of breast cancer. Two recent reports have suggested that interferon might enhance oestrogen receptor (ER) expression (Pouillart et al., 1982; Dimitrov et al., 1984). We have therefore investigated the effect of human recombinant interferon α on oestrogen receptor expression by the human breast cancer cell line ZR-75-1 and in addition determined the ability of interferon to potentiate the anti-proliferative effects of tamoxifen.

ER levels were determined in viable cells at 37°C using a whole cell binding assay. Cells (50,000 or 200,000) were plated into a number of microwells and treated with interferon (10–1000 i.u./ml) for 48 h. After removal of interferon, control and treated cells were exposed to [2, 4, 6, 7, 16, 17³H]oestradiol (0.25–3.5 nM, specific activity 140 μCi/mmol) for 1 h. Non-specific binding was determined by simultaneously exposing parallel groups of cells to the radioactive ligand in the presence of a 200-fold excess of diethylstilbestrol. Maximum specific binding capacity (Bmax) and dissociation constant (Kd) were calculated after linearization of data by Woolf or Scatchard analysis.

At the higher cell plating density there was little variability in control cell expression of ER (215 ± 24 fmol/mg of protein, Kd 0.5 ± 0.15 nM), and interferon (10–1000 i.u./ml) had no significant effect on receptor number or affinity for oestradiol. At the lower cell density there was some variation in control receptor levels (Bmax 36–151 fmol/mg of protein), and interferon treatment (100 i.u./ml) consistently resulted in an increase in specific binding (Bmax 178–262 fmol/mg of protein). There was also a small decrease in affinity of oestrogen for its receptor but this effect did not reach significance. Concentrations of interferon of > 100 i.u./ml were ineffective.

Fig. 1(a) shows that interferon at 10 i.u./ml had no significant effect on the proliferation of ZR-75-1 cells during a continuous 6 day treatment. Interferon at 500 i.u./ml markedly inhibited cell proliferation. These data suggest that the effects of interferon on ER expression and cell proliferation are dissociated. Fig. 1(a) also demonstrates that simultaneous exposure of cells to interferon and tamoxifen resulted in a degree of inhibition of cell proliferation greater than that observed with either drug alone, although there was no evidence of synergism. In contrast, a 5 day pre-exposure of cells to interferon (10 i.u./ml) markedly sensitized cells to a subsequent 6 day treatment with tamoxifen (Fig. 1b).

Sica et al. (1986), in a study reported simultaneously with our own preliminary data (van den Berg et al., 1986), demonstrated enhanced ER expression in an oestrogen supersensitive variant of the MCF-7 human breast cancer cell line after treatment with the β subtype of interferon. A combination of tamoxifen and interferon β was also reported to be synergistic in their anti-proliferative effects. Our data are in broad agreement although enhanced ER levels and potentiation of the anti-proliferative effects of tamoxifen towards ZR-75-1 cells are only observed with low doses of α interferon in cells initially plated at low density. The reasons for these constraints are presently unclear. Whatever the precise mechanism, our results to date do suggest that interferon can sensitize breast cancer cells to tamoxifen treatment and that this effect is correlated with the ability of interferon to enhance ER expression. A treatment regimen
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.


Anti-Cancer Res. 6, 396.


Received 26 September 1986

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 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 125I-glucagon with excess unlabelled ligand. Data from the competition binding assay have been subjected to Woolf transformation (Keightley et al., 1983) and values of \( B_{\text{max}} \) and \( K_d \) of 5.18 ± 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.


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.

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