Short report

Effect of transforming growth factor β₂ on oestrogen metabolism in the MCF-7 breast cancer cell line

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Abstract
Transforming growth factor β (TGF-β) is a multifunctional regulator of cellular growth and differentiation in many cell types and has a growth inhibitory effect on mammary epithelial cells. The TGF-β₁ isoform has been shown to be present in high concentrations in breast cyst fluid and might have a protective role in breast cancer. In addition, oestrogens play an important role in breast cancer development, and oestrone sulphate (E₁S) might be the main source of active oestrogens in the breast. The aim of this study was to assess the effect of TGF-β₂ on oestrogen synthesis in an attempt to understand the mechanism by which TGF-β₂ may exert a protective effect in breast cancer. In this study, higher concentrations of TGF-β₂ significantly inhibited the conversion of E₁S to oestrone (E₁) and the conversion of E₂ to the potent oestrogen, oestradiol (E₂). TGF-β₂ did not have any effect on MCF-7 cell growth or on E₁ to E₂ conversion. In conclusion, TGF-β₂ might exert a protective role in breast cancer by reducing the amount of active oestrogens present in the breast.

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Keywords: transforming growth factor β₂; oestrone sulphatase; oestradiol-17β hydroxysteroid dehydrogenase; breast cancer

Methods

Cell culture
The hormone dependent MCF-7 breast cancer cell line was a gift from Professor MJ Reed (unit of metabolic medicine, St Mary’s Hospital Medical School, University of London).

MCF-7 human breast cancer cells were maintained in EMEM (3 ml), containing 20 mM HEPES, 5% foetal bovine serum (FBS), 2 mM L-glutamine, non-essential amino acids, and 0.075% NaHCO₃, and grown in 25 cm² tissue culture flasks. For the experiments, 25 cm² tissue culture flasks were seeded with approximately 1 x 10⁶ cells/flask using the medium described above. The cells were allowed to grow for three days at 37°C in a 5% CO₂ incubator. The medium was then removed, and several different concentrations (0.5 ml) of TGF-β₂ and fresh EMEM (2.5 ml) containing 5% dextran charcoal stripped FBS, 20 mM HEPES, 2 mM L-glutamine, non-essential amino acids, and 0.075% NaHCO₃, were added. All treatments were performed in triplicate. MCF-7 cells were exposed to the treatment for 48 hours at 37°C in a 5% CO₂ incubator. At the end of the treatment period.
the monolayers were assayed for E1 or E2DH activity and the number of cell nuclei counted on a coulter counter.

OESTRONE SULPHATASE ASSAY
For the oestrone sulphatase assay, intact monolayers of MCF-7 cells in triplicate 25 cm² tissue culture flasks were washed with phosphate buffered saline (PBS; 2.5 ml) and [3H]-E1S (5 pmol; 90 Ci/mmol) was added to flasks in 2.5 ml of phenol red free medium. Blank incubations, in which labelled steroid was added to cell free flasks, were carried out in parallel. After incubating the cells for four hours at 37°C, 1 ml of medium was pipetted into separate tubes containing [3H]-E1 (5 × 10⁴ disintegrations/minute (dpm)), which were used for recovery correction. The mixture was shaken with toluene (5 ml) for 30 seconds. An aliquot (2 ml) of the organic phase was removed and both ³H and ¹⁴C activity were measured by liquid scintillation spectrometry. The mass of E1S hydrolysed was calculated from the ³H counts obtained, corrected for the volumes of the medium and organic phase used, and for recovery of [¹⁴C]-E1 and the specific activity of the substrate. Results were expressed as fmol/4 hour/10⁶ cells.

E2DH ASSAY
Cells were grown to at least 10⁶ cells/flask before being assayed for E2DH activity. Intact cell monolayers of MCF-7 cells in triplicate 25 cm² tissue culture flasks were washed with PBS at the end of the treatment period. E2DH activity in both the reductive (E₂ to E₁) and oxidative (E₁ to E₂) direction was measured in the intact monolayers. [³H]-E₂ (for reductive activity) or [¹⁴C]-E₂ (for oxidative activity) was added in serum free medium (2.5 ml) to each flask (7 × 10⁶ dpm) and incubated for four hours at 37°C. Medium (2 ml) was then removed from each flask and added to glass tubes containing 50 µl [¹⁴C]-E₁ (for reductive activity) recovery labels (5 × 10⁴ dpm) and unlabelled E₁ and E₂, respectively, at a concentration of 2 mg/ml in ethanol, as carrier steroids for thin layer chromatography and for visualisation of the position of the steroid product under UV light. The oestrogens were extracted into ether (3 ml), dried down under nitrogen, and separated by thin layer chromatography using dichloromethylene/ethyl acetate (4/1 vol/vol) as solvent. The positions of the product (E₁ or E₂) were located by viewing under UV light. The product together with recovery label were eluted with methanol (0.5 ml) from strips cut from the thin layer chromatography plate. ³H and ¹⁴C activities were measured by liquid scintillation spectrometry. Results were expressed as fmol/4 hour/10⁶ cells.

CELL COUNTING
MCF-7 cells were counted by release of cell nuclei. After washing cell monolayers with PBS, cells were incubated with HEPES buffer (20 mM containing 1.5 mM MgCl₂) and a lytic agent, Zap-oglobin (0.2 ml). After 10 minutes, the released cell nuclei were diluted with isotonic solution, Isoton, and an aliquot counted in the coulter counter. One flask in each batch was used to assess cell viability using the trypan blue exclusion method, and 95–98% of the cells were found to be viable.

STATISTICAL ANALYSIS
The effects of TGF-β₁ on cell growth and enzyme activities were compared with controls using the unpaired student’s t test. Results were considered to be significant when p < 0.05.

Results
All cell culture experiments assessing the effect of TGF-β₁ on cell growth and oestrogen metabolism in the MCF-7 cell line were performed in triplicate. The higher concentrations of TGF-β₁ (50 and 100 µg/litre) significantly inhibited E₁S to E₁ (fig 1) and E₁ to E₂ conversion (fig 2) in the MCF-7 breast cancer cell line. TGF-β₁ did not have any effect on MCF-7 cell growth or on E₂ to E₁ conversion over the range of concentrations tested. Similar results were obtained when the experiments were repeated a further two times.

Discussion
Oestrogens have been shown to regulate the production of TGF-β₁ in breast cancer cell lines. Treatment of oestrogen receptor positive
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Tamoxifen has also been shown that tamoxifen, an antioestrogen, can induce autocrine secretion of TGF-β in human breast cancer cell lines, in which it acts as an inhibitor of growth.7 Tamoxifen has also been shown to induce the secretion of active TGF-β from human fibroblasts, despite the demonstrated absence of oestrogen receptors within these cells in vitro and in vivo.8

In our study, high concentrations of TGF-β1 inhibited E1 to E2 conversion in the MCF-7 breast cancer cell line. TGF-β1 has been shown to have a growth inhibitory effect on the oestrogen receptor positive MCF-7 breast cancer cell line and oestrogen receptor negative breast cancer cell lines.9 TGF-β1 has been shown to be at least 10 times more potent than TGF-β2 at inhibiting the growth of MCF-7 cells.9 We found no significant effect of TGF-β1 on MCF-7 cell growth over the concentrations tested. This difference might be the result of differences in the MCF-7 cell line used in different laboratories.

We found recently that TGF-β1 inhibited MCF-7 cell growth in a dose dependent manner over the range of concentrations tested (0.1 to 8 µg/litre).10 TGF-β1 significantly stimulated the conversion of E1 to E2 over the range of concentrations tested (0.1 to 8 µg/litre) and significantly stimulated the conversion of E1 from 2 to 8 µg/litre. TGF-β1 did not have any effect on E2 to E1 conversion. The effect of TGF-β1 on E1 to E2 conversion and E1 to E2 conversion are opposite to those of TGF-β2, and these effects of TGF-β1 occur at lower concentrations than those of TGF-β2. The TGF-β isoforms have been shown to be differentially regulated by E2.1 Conversely, we have shown that the TGF-β isoforms might have different effects on oestrogen synthesis.

The inhibitory effect of TGF-β1 on E1 to E2, and on E1 to E2 conversion is a novel finding, and suggests that TGF-β1 might reduce the amount of the potent oestrogen, E2, present in the breast. In view of the role that oestrogens might have in the development of breast cancer, this action of TGF-β1 could conceivably protect against the development of breast cancer.