Modulation of transforming growth factor beta expression and induction of apoptosis by tamoxifen in ER positive and ER negative breast cancer cells

Sir – We read with interest the paper by Perry *et al.* (1995) reporting pharmacological modulation of transforming growth factor β_1 (TGF-β_1) expression in MCF-7 cells by mechanisms that appear to be independent of the conventional oestrogen receptor (ER) and that may involve either transcriptional or post-transcriptional events.

Our group have previously reported induction of this potent epithelial growth inhibitor by tamoxifen in fetal fibroblasts *in vitro* and proposed a negative paracrine hypothesis (Colletta *et al.*, 1990). Consistent with this concept of a direct action of tamoxifen upon fibroblasts, we subsequently demonstrated stromal induction of TGF-β_1 in both ER-positive and ER-negative breast cancers following primary tamoxifen therapy (Butta *et al.*, 1992). Up-regulation was seen prodominantly between and around stromal fibroblasts, with little increased immunoreactivity in the vicinity of epithelial cells. In this respect, these results were at variance with previous *in vitro* studies showing induction of TGF-β by tamoxifen in MCF-7 cells (Knabbe *et al.*, 1987). The present study concurs with the latter observations and supports the existence of both functional autocrine and paracrine inhibitory loops involving TGF-β_1 (Benson and Colletta, 1995). However, previous work on induction of TGF-β_1 in breast carcinoma cell lines revealed that this response to anti-oestrogens was confined to ER-positive (MCF-7) cells, and growth inhibition of ER-negative cells was only observed when these were co-cultured in the presence of MCF-7 cells, which alone could respond to tamoxifen and produce TGF-β, which acted in a negative paracrine manner upon ER-negative cells (Knabbe *et al.*, 1987). These results were consistent with tamoxifen acting via the ER with TGF-β_1 being a proximate growth modulator.

We have recently confirmed induction of TGF-β_1 in the breast tumour fibroblasts *in vitro* in the absence of any detectable ER protein (Benson *et al.*, 1995). The modulation of TGF-β_1 expression in both ER-positive and ER-negative breast cancer cells observed in the present study may reflect a common ER-independent mechanism of action that is operative in both epithelial cells and fibroblasts.

However, it should be noted that the concentrations of tamoxifen employed in this study were relatively high. In our own *in vitro* experiments, maximal induction was observed at tamoxifen concentrations of between 500 and 1000 nM, and similar concentrations induced TGF-β_1 in MCF-7 cells. A biphasic pattern of growth inhibition of breast cancer cells by anti-oestrogens has been observed, with an E2 reversible effect at concentrations of 10–1000 nM, and an E2 irreversible inhibition at concentrations of 1–10 μM (Sutherland *et al.*, 1986). Although specific non-ER-mediated mechanisms cannot be excluded, it is possible that growth inhibition at higher concentrations represents a direct cytotoxic action of tamoxifen upon cells. Indeed, Bronzert *et al.* (1985) reported the maximal non-cytotoxic growth-inhibitory doses of anti-oestrogens to be <1 μM for MCF-7 cells.

These considerations raise the issue of whether the concentrations of tamoxifen used in the present study exert non-specific cytotoxic effects rather than formally activating pathways leading to enhanced TGF-β production. Programmed cell death could result from such non-specific action, and indeed may be a final common pathway for many cytotoxic agents. Any apparent increases in cytosolic TGF-β_1 protein could be a consequence of defective secretion with intracellular retention secondary to cytotoxic effects of tamoxifen. It is noteworthy that the induction of TGF-β was relatively modest, with 2–3 times probably being the minimal fold induction with physiological consequence. Much greater magnitudes of induction have been witnessed *in vitro* (Colletta *et al.*, 1990; Benson *et al.*, 1995) and *in vivo* (Butta *et al.*, 1992).

The suggestion that tamoxifen may act by a post-transcriptional mechanism at lower concentrations and a transcriptional one at higher concentrations of anti-oestrogen is intriguing; we have confirmed that induction of TGF-β_1 in breast tumour fibroblasts is not associated with elevated levels of transcript. Moreover, tamoxifen may enhance rates of translation by overcoming the inhibitory influence of stem-loop structures in the long 5′ untranslated region of TGF-β_1 mRNA (Kim *et al.*, 1992). Such structures may interfere with ribosomal binding directly or via a cytoplasmic protein that could be displaced by tamoxifen. This mechanism may become saturated at higher concentrations of tamoxifen, when increased levels of TGF-β_1 mRNA must precede augmentation of protein production. Several studies have suggested that modulation of TGF-β isoforms *in vivo* in response to tamoxifen occurs at the transcriptional level (Thompson *et al.*, 1991; MacCullum *et al.*, 1994). Perhaps dual mechanisms are operative depending upon the nuances of pharmacokinetics and the precise concentrations of tamoxifen in the immediate cellular environment.

JR Benson,
M Baum,
Academic Department of Surgery,
The Royal Marsden Hospital,
Fulham Road,
London SW3 6JJ
UK

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Association of TGF-β_1 expression with the effects of tamoxifen on estrogen receptor positive and negative breast cancer cells

Sir – We thank Dr Benson and Dr Baum for their comments regarding our paper (Perry *et al.*, 1995*a*). We are familiar with their studies on the potential role of transforming growth factor (TGF)-β_1 produced by stromal fibroblasts in tamoxifen-induced growth inhibition. As our work, and the work of others (Knabbe *et al.*, 1987), indicates that tamoxifen has direct effects on epithelial cells, we agree that tamoxifen may act via both functional autocrine and paracrine inhibitory loops involving TGF-β_1. The relative importance of autocrine and/or paracrine TGF-β_1 secretion in tamoxifen-induced growth inhibition may be dependent on the cell line and the dose and duration of tamoxifen treatment.

Knabbe *et al.* (1987) have shown that oestrogen receptor (ER)-positive MCF-7 cells respond to anti-oestrogen (100 nM 4-hydroxytamoxifen), but ER-negative MDA-231 cells do not. However, other studies have shown that ER-negative cells, including MDA-231, respond to tamoxifen concentrations of 1 μM or greater (Taylor *et al.*, 1984). We have also noted that, although MCF-7 cells are more sensitive to tamoxifen than MDA-231 cells, MDA-231 cells do respond to micromolar concentrations of tamoxifen (Perry *et al.*, 1995*b*). The response of ER-negative cells to tamoxifen is not surprising as several clinical studies have shown that the effectiveness of tamoxifen is independent of ER status (for review see Jaiyesimi *et al.*, 1995). As we were interested in studying the potential role of TGF-β_1 in ER-negative as well as ER-positive cells, we chose a concentration of tamoxifen (10 μM) that possessed significant activity against both cell lines. Although this tamoxifen concentration is relatively high compared with the serum levels achieved in patients on standard dose (20–80 mg day^{-1}) therapy, anti-oestrogens accumulate in human tissues and tumours at levels 10–60 times higher than in serum (Lien *et al.*, 1991). Thus, intratumoral tamoxifen concentrations may be in the micromolar range. We agree that a 10 μM tamoxifen concentration is likely in a range in which the effects are no longer solely dependent on ER. Indeed, this is supported by our results, whereby 10 μM tamoxifen had similar effects on TGF-β_1 expression in both the ER-positive MCF-7 and ER-negative MDA-231 cell lines. Also, the addition of oestrogen failed to inhibit tamoxifen-induced TGF-β_1 expression in either cell line, again indicating that tamoxifen at this concentration appears to induce TGF-β_1 through an ER-independent mechanism. We concur with the conclusions of Taylor *et al.* (1984) and Sutherland *et al.* (1986), that tamoxifen appears to have both ER- and non-ER-dependent activity.

Many, if not all, cytotoxic agents are capable of inducing programmed cell death (apoptosis). This includes agents with different mechanisms of action and that cause damage to different targets. However, we do not agree with Dr Benson and Dr Baum that the apoptotic activity of tamoxifen at a 10 μM concentration was strictly due to non-specific damage unrelated to enhancement of TGF-β_1 production. As our work demonstrates, the amount of TGF-β_1 induced by tamoxifen highly correlated with the amount of DNA cleavage. In addition, tamoxifen induced DNA cleavage was abrogated by the addition of an anti-TGF-β_1 antibody. This demonstrates that TGF-β_1 does have an important role in tamoxifen-induced apoptosis in both ER-positive and ER-negative cells.

We agree that current evidence suggests that tamoxifen may act via a post-transcriptional mechanism at lower concentrations and a transcriptional mechanism at higher concentrations.

Whether such mechanistic differences are important in determining the therapeutic effectiveness *in vivo* remain to be determined.

Roger R Perry,
Yuan Kang,
Bridget Greaves,
Division of Surgical Oncology,
Eastern Virginia Medical School,
Norfolk, Virginia 23507, USA

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