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Inhibition of Tumor Necrosis Factor α-Stimulated Aromatase Activity by Microtubule-Stabilizing Agents, Paclitaxel and 2-Methoxyestradiol

A. Purohit, A. Singh, M. W. Ghilchik,* and M. J. Reed
Endocrinology and Metabolic Medicine, Imperial College School of Medicine, and
**The Breast Clinic, St Mary's Hospital, London, W2 1NY, United Kingdom*

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The aromatase enzyme, which converts androstenedione to oestrone, regulates the availability of oestrogen to support the growth of hormone-dependent breast tumours. Cytokines, such as interleukin 6 (IL-6) and tumour necrosis factor α (TNFα) or prostaglandin E_2 (PGE_2), can stimulate aromatase activity. These factors may originate from cells of the immune system that infiltrate breast tumours. Paclitaxel, which is used in the treatment of breast cancer, stabilizes microtubules and has previously been shown to rapidly down-regulate TNF-receptors on human macrophages. The endogenous oestrogen metabolite, 2-methoxyestradiol (2-meOE2), also acts to stabilize microtubules. In this study, we have examined the ability of paclitaxel or 2-meOE2 to antagonise TNFα-stimulated aromatase activity in stromal fibroblasts derived from normal or malignant breast tissues. Paclitaxel inhibited basal and TNFα-stimulated aromatase activities by 88% and 91% respectively. 2-MeOE2 also reduced basal and TNFα-stimulated aromatase activities by 46% and 56% respectively. Both paclitaxel and 2-meOE2 also inhibited stimulation of aromatase activity by IL-6 plus its soluble receptor and PGE_2. The 16α-hydroxylated derivative of 2-meoE2 and 2-meOE3, which does not bind to microtubules, was less effective at inhibiting TNFα-stimulated aromatase activity. Increased 2-hydroxylation of oestrogens, and subsequent formation of their 2-methoxy derivatives, may be associated with a reduced risk of breast cancer. It is possible that the pathway of oestrogen metabolism may influence the ability of stromal cells to respond to cytokine stimulation.

Abbreviations used: IL-6, interleukin 6; TNFα, tumour necrosis factor α; PGE_2, prostaglandin E_2; 2-meOE2, 2-methoxyestradiol; 2-meOE3, 2-methoxyoestriol; Pax, Paclitaxel; CM, conditioned medium; FCS, foetal calf serum; PPARγ, peroxisome proliferator activated receptor γ.

Synthesis of oestrone from androstenedione, by the aromatase enzyme complex, is an important source of oestrogen available to support the growth of hormone-dependent tumours (1). Cytokines, such as IL-6 and TNFα and PGE_2, can all stimulate aromatase activity (2–4). Many breast tumours are infiltrated by macrophages and lymphocytes and there is evidence that these cells may be an important source of the factors that can stimulate aromatase activity (5–6).

The role that the immune system has in the development of cancers remains controversial (7). In women receiving long-term immune suppressive therapy, however, the incidence of breast cancer is reduced (8). This suggests that the immune system may have an immunostimulatory role in the development of breast cancer. Support for such a role, possibly acting via cytokine stimulation of oestrogen synthesis, was obtained by comparing the abilities of conditioned medium (CM) collected from white blood cells of an immunosuppressed subject or woman with breast cancer to stimulate aromatase (9). Stimulation of the activity of this enzyme was greatly reduced by CM collected from cells of the immunosuppressed subject. Furthermore, concentrations of TNFα were barely detectable in CM from cells of the immunosuppressed subject in contrast to the high levels present in CM from cells of a woman with breast cancer. It is likely, therefore, that TNFα has an important role in regulating aromatase activity.

TNFα, like other cytokines, acts by interacting with cell-surface receptors (10). Using human macrophages, paclitaxel, a compound that stabilizes microtubules, was found to rapidly down-regulate TNFα receptors (11). The endogenous oestrogen metabolite, 2-methoxyestradiol (2-meOE2), was recently shown to have a similar effect to that of paclitaxel on microtubule stability (12, 13). In the present investigation we have examined the ability of paclitaxel and 2-meOE2 to antagonise TNFα stimulated activity in cultured fibroblasts derived from breast tissues.

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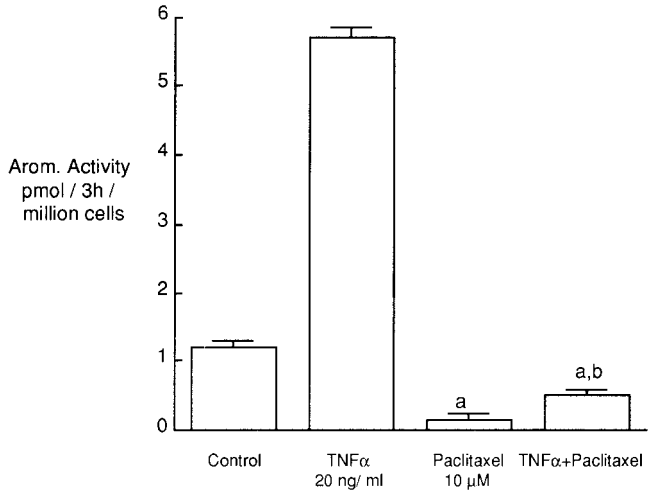

FIG. 1. Inhibition of basal and TNFα-stimulated aromatase activity by paclitaxel (Pax) in breast tissue fibroblasts. Paclitaxel was added to cells which were cultured for 24 h in 2% stripped foetal calf serum. TNFα was added, in the presence of dexamethasone (100 nmol/l) and cells cultured for a further 48 h in the same medium. Controls and cells with paclitaxel, but not TNFα, were also cultured in the presence of dexamethasone for a 48 h period. Aromatase activity was measured in intact monolayers after washing cells with phosphate buffered saline (means ± SD, n = 3; a, $p < 0.001$ versus controls; b, $p < 0.001$ versus TNFα-stimulated aromatase activity).

MATERIALS AND METHODS

Samples of breast adipose or tumour tissue were obtained from women undergoing reduction mammoplasty or lumpectomy after obtaining their informed consent. The study was approved by the hospital Ethics Committee.

Fibroblasts were cultured as previously described (2). Briefly, they were cultured in Eagles' modified minimal essential medium containing Hepes buffer (20 mmol/l), 10% foetal calf serum (FCS) and supplements. Cells were routinely passaged 2–3 times after which replicate 25 cm^2 flasks were seeded with fibroblasts and grown to confluency. For experiments, cells were cultured in phenol red-free medium containing 2% stripped FCS for 24 h in the presence of paclitaxel or 2-meOE2 before the addition of TNFα, IL-6 plus IL-6 soluble receptor (IL-6sR) or PGE_2 and cultured for a further 48 h in this medium. TNFα, IL-6 and IL-6sR (R&D Systems Ltd, Abingdon, Oxford, UK) or PGE_2 (Sigma, Poole, Dorset, UK) were used in the presence of dexamethasone (100 nmol/l, Sigma). Paclitaxel, 2-meOE2 and other chemicals were also obtained from Sigma.

At the end of the treatment period aromatase activity was measured in intact monolayers using [1β-^{3}H] androstenedione (15–30 Ci/mmol, NEN-Du Pont, and Stevenage, Herts, UK) over a 3–20 h period (2, 3). The number of cells was measured by counting cell nuclei using a Coulter counter. Experiments were carried out in triplicate and results shown are representative of 2–3 investigations.

Statistics. Student's t test was used to assess the significance of differences in mean values of treated and control cells.

RESULTS

The ability of paclitaxel to inhibit TNFα stimulated aromatase activity was initially examined using fibroblasts derived from reduction mammoplasty tissue (Fig. 1). In these cells, TNFα, in the presence of dexamethasone, stimulated aromatase activity by 375%. Both paclitaxel and 2-meOE2 inhibited basal aromatase activity by 88% and 46% respectively. In addition, TNFα stimulated aromatase was also significantly reduced by these compounds by 91% and 56% respectively. This ability appears to be specific to agents that stabilize microtubules. Colchicine, which inhibits microtubule polymerization, or Cytocholasin B, which binds to microfilaments, were without effect (data not shown).

As other factors, such as IL-6 and PGE_2, also act via interaction with cell surface receptors, the ability of paclitaxel and 2-meOE2 to antagonise aromatase stimulation by these factors and TNFα was also examined (Fig. 2). TNFα, IL-6 plus IL-6sR or PGE_2 all significantly enhanced aromatase activity in tumour-derived fibroblasts. Both paclitaxel and 2-meOE2 inhibited basal aromatase activity and TNFα stimulated activity. In addition, however, they were also found to antagonise stimulation of aromatase activity by IL-6 plus IL-6sR or PGE_2.

The relative potencies of paclitaxel and 2-meOE2 to antagonise TNFα stimulated aromatase activity were compared in a dose-response study (Fig. 3). In addition, the ability of the 16α-hydroxy derivative of 2-meOE2, 2-meOE3, which does not appear to bind to microtubules (14), to inhibit TNFα stimulated aromatase activity was also examined.

While both paclitaxel and 2-meOE2 inhibited TNFα stimulated aromatase, it was evident that paclitaxel is a more potent antagonist. At 0.1 μM paclitaxel inhib-

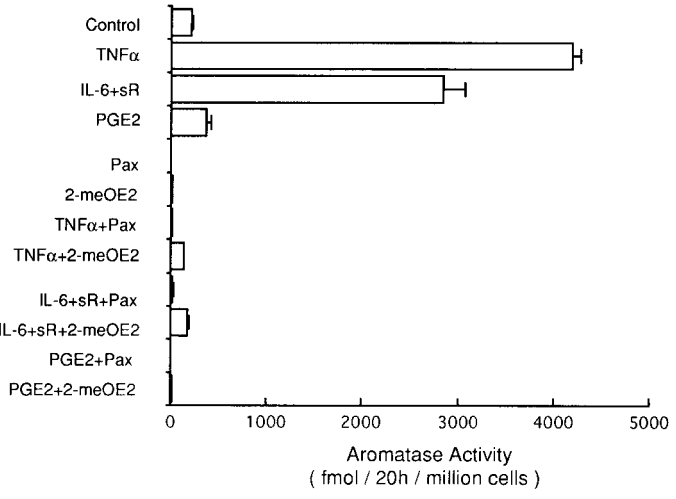

FIG. 2. Inhibition of TNFα (20 ng/ml), IL-6 plus IL-6sR (50 ng + 100 ng/ml) or PGE_2 (10 μM) stimulated aromatase activity in fibroblasts by paclitaxel (Pax, 10 μM) or 2-methoxyestradiol (2-meOE2, 10 μM) (means ± SD, n = 3). The experimental protocol used was as described in the legend to Fig. 1. Aromatase activity in cells treated for 48h with TNFα, IL-6 plus IL-6sR or PGE_2 was significantly higher ($p < 0.01$–$p < 0.001$) than in control cells. All inhibitions were significant ($p < 0.001$) compared with aromatase activity in TNFα-stimulated cells in the absence of paclitaxel or 2-methoxyestradiol.

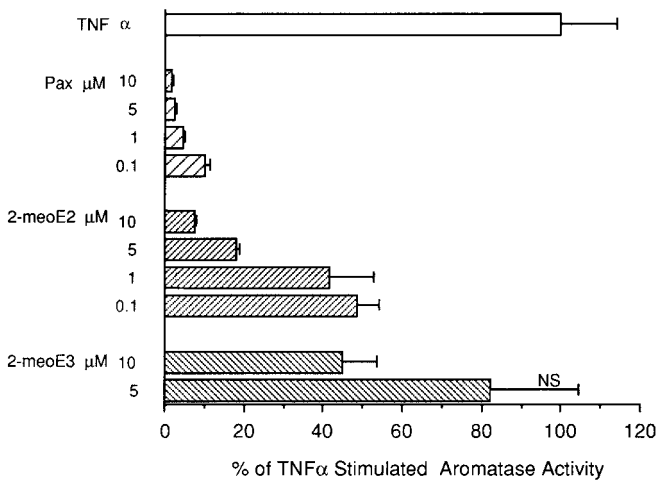

FIG. 3. Dose response for inhibition of TNFα-stimulated aromatase activity (expressed as % of TNFα-stimulated activity) by paclitaxel (Pax), 2-methoxyestradiol (2-meOE2) or 2-methoxyoestriol (2-meOE3) in fibroblasts (means ± SD, n = 3). The experimental protocol used was as described in the legend to Fig. 1. All compounds significantly inhibited TNFα stimulation of aromatase activity ($p < 0.001$) with the exception of 2-methoxyoestriol at 5 μM (NS).

ited stimulation by 90% whereas 2-meOE2 at this concentration only reduced the stimulation by 51%. 2-MeOE3, while showing some inhibitory effect at the highest concentration tested did not significantly reduce TNFα stimulation of aromatase activity at 5 μM.

DISCUSSION

Results from this investigation have revealed that two agents that alter microtubule stability, paclitaxel and 2-meOE2, not only inhibit basal aromatase activity but greatly reduce TNFα stimulated activity. Paclitaxel is used in the treatment of breast cancer but, as far as we are aware, this is the first report demonstrating its ability to inhibit basal and cytokine stimulated aromatase activity. This property is restricted microtubule stabilizing agents as colchicine or cytocholasin B, which have different effects on the microskeleton, were unable to inhibit TNFα stimulated aromatase activity.

TNFα, IL-6 plus IL6sR and PGE2 are the three main factors identified so far that can regulate aromatase activity in fibroblasts derived from subcutaneous adipose or breast tissues. Microtubules may be required for the synthesis of cytokine receptors or for their translocation to the plasma membrane (15). It is likely, therefore, that the effect that paclitaxel and 2-meOE2 have on the ability of TNFα, IL-6 or PGE_2 to stimulate aromatase activity may also result from an effect on the synthesis/translocation of the receptors involved in their signalling. The ability of paclitaxel and 2-meOE2 to reduce basal (i.e. unstimulated) aromatase activity may result from blocking the autocrine/ paracrine action of cytokines and PGE_2, which are known to be produced by these fibroblasts, on aromatase activity (6, 16).

Paclitaxel is used for the treatment of breast cancer but its toxicity precludes its long-term use. The finding that 2-meOE2, an endogenous oestrogen metabolite, may have similar properties to paclitaxel suggests it may have considerable therapeutic potential (17). Oral administration of 2-meOE2 to mice inoculated with B_{16} melanoma, Meth A sarcoma or MDA-MB-435 breast cancer cells significantly reduced tumour growth (18, 19).

The results from this investigation also suggest a possible mechanism by which the immune system could develop an immunostimulatory role. In the presence of adequate production of 2-meOE2, cytokine receptors in breast tissues would be down-regulated and thus cytokine stimulation of aromatase activity inhibited. Reduced production of 2-meOE2 would enable cytokines to stimulate oestrogen synthesis in breast tissues. Bradlow and his colleagues have obtained convincing evidence that a reduction in the formation of 2-hydroxyoestrogens and an increase in synthesis of 16α-hydroxy metabolites is associated with an increased risk of breast cancer (20, 21). The observation in the present study that the 16α-hydroxy derivative of 2-meOE2 had only a limited ability to suppress TNFα stimulated aromatase activity would appear to support Bradlow's findings.

Stromal fibroblasts cultured from adipose tissue have the ability to differentiate into adipocytes. TNFα, while stimulating aromatase activity in fibroblasts, inhibits their differentiation into adipocytes. High concentrations of oestradiol (10–100 μM) can inhibit TNFα stimulated aromatase activity in adipose stromal cells and it has been postulated that a feed-back loop may exist to prevent excessive oestrogen synthesis in these cells (22). PPARγ ligands, such as thiozolidinedione, can also stimulate adipocyte differentiation and also inhibit TNFα stimulated aromatase activity. As high concentrations of oestradiol were required to inhibit TNFα stimulated aromatase activity in human adipose stromal cells it is tempting to speculate that oestradiol may act after conversion to 2-meOE2.

ACKNOWLEDGMENT

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