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Effect of tamoxifen and 2-methoxyestradiol alone and in combination on human breast cancer cell proliferation☆

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Abstract

Endocrine therapy is widely accepted for the treatment of hormone receptor-positive breast cancer. However, in many cases eventually resistance will develop and tumor regrows. Combination therapy may be one way to resolve this problem. In the present study we investigated the effect of a combination of the widely used antiestrogen tamoxifen with the endogenous estradiol metabolite 2-methoxyestradiol (2-ME) on the proliferation of human estrogen receptor-positive and receptor-negative breast cancer cells.

The receptor-positive cell line MCF-7 and the receptor-negative cell line BM were treated with 4-hydroxytamoxifen (4-OHTam) and 2-methoxyestradiol in the concentration range of 0.8–25 μM alone and equimolar combinations for 4 days. The proliferation of the cells was determined using the ATP-chemosensitivity test.

4-Hydroxytamoxifen inhibited proliferation of MCF-7 and BM cells with IC_{50} values of 31 and 10 μM, the corresponding figures for 2-methoxyestradiol were 52 and 8 μM. The combination showed IC_{50} values of 6 μM and 4 μM.

These results indicate that a combination of tamoxifen with 2-methoxyestradiol showed an additive inhibitory effect concerning the proliferation of estrogen receptor-positive and receptor-negative breast cancer cell lines. Thus a combination of these substances may allow ameliorating of adverse events of tamoxifen by reducing its concentrations and probably also drug resistance and should be tested in clinical trials.

Keywords: Tamoxifen; 2-Methoxyestradiol; Cancer cell

1. Introduction

Estrogens are known to be a major factor in the etiology of human breast cancer. Therefore, one of the main strategies in the treatment of breast cancer is to prevent the binding of estrogens to its receptor or to reduce systemic or local synthesis of estrogens [1]. Tamoxifen, an antiestrogen, is widely used for endocrine treatment of human breast cancer. Drug resistance, however, remains a significant problem in breast cancer treatment. This is emphasized by the finding that about 15–20% of patients who eventually progress after an initial response to primary treatment are known to respond to second-line therapy with another endocrine agent [2]. Thus new endocrine treatment options are needed.

Recent research has gained evidence that 2-methoxyestradiol (2-ME), an endogenous estradiol metabolite, may be a candidate for treatment of several cancers because of its anti-carcinogenic and anti-proliferative properties [3]. As yet little in vitro data are available investigating the combination of 2-ME with other compounds currently used for breast cancer treatment. Therefore, in the present study we investigated the effect of tamoxifen and 2-ME alone and in combination on the proliferation of a human estrogen receptor-positive and receptor-negative cell line.

2. Material and methods

4-Hydroxytamoxifen (4-OHTam), an active metabolite of tamoxifen and 2-methoxyestradiol were purchased from Sigma and Steraloids, respectively. The compounds were dissolved in ethanol and diluted by ethanol/PBS mixtures to yield a final ethanol concentration of <1% per well.

MCF-7, a human estrogen and progestin receptor-positive breast cancer cell line, was purchased from ECACC, UK. BM, a receptor-negative human breast cancer cell line, was established in our own laboratory and characterized by immunohistochemical methods for the expression of estrogen receptor-α and progestin receptors.

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The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 5% (v/v) fetal calf serum supplemented with 0.3 mg/ml glutamine, 5 ng/ml bovine insulin and 100 U/ml penicillin plus 100 μg/ml streptomycin.

Ninety six well plates were seeded with approximately 1000 MCF-7 or BM cells per well in assay kit medium. Subsequently, the agents were added to the wells in the concentrations of 0.8–25 μM alone and in equimolar combinations. After incubation for 4 days, cell proliferation was measured by a ATP-chemosensitivity test [4].

Statistical analysis was done by ANOVA of the logarithmated values followed by Student's *t*-test.

3. Results

In Fig. 1 the results of cell proliferation of MCF-7 cells are giving as percentage of inhibition compared to the control value. 4-OHTam inhibited cell proliferation between 13% at 0.8 μM and 41% at 25 μM with a calculated IC_{50} value of 31 μM, the corresponding figures for 2-ME were 9% at 0.8 μM and 26% at 25 μM and an IC_{50} value of 52 μM. The inhibition was statistically significant for both compounds at all concentrations tested. The combination of the two compounds showed inhibitory values between 31% at 0.6 μM and 99% at 25 μM and an IC_{50} value of 6 μM. These values were significantly different to the values of each compound alone.

In Fig. 2 the results are illustrated for the BM cell line. Here 4-OHTam showed an inhibitory potency between 2 and 98% whereas the values for 2-ME were between 13 and 69%. The IC_{50} value for 4-OHTam was 10 μM and for 2-ME 8 μM. For 4-OHTam only two concentrations, i.e. 12.5 and 25 μM were significantly different to the control value, whereas for 2-ME all concentrations tested showed a significant inhibition. The efficacy of 2-ME was statistically greater than that of 4-OHTam in the range of 0.8–6.3 μM. The combination of both substances had inhibitory values in the range of 33–99% with an IC_{50} value of 4 μM. The inhibitory values of the combination were statistically significant at the lower concentrations of 0.8–6.3 μM compared to the effect of the monosubstances.

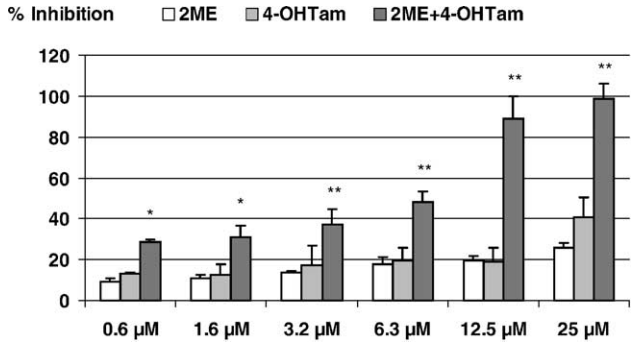

Fig. 1. Changes in proliferation of MCF-7 cells after addition of 2-methoxyestradiol (2-MeOE2), 4-hydroxytamoxifen (4-OHTam) or an equimolar combination of both substances compared to control values (mean ± S.D., $^{*}P < 0.05$, $^{**}P < 0.01$ comparing combination vs. monosubstances).

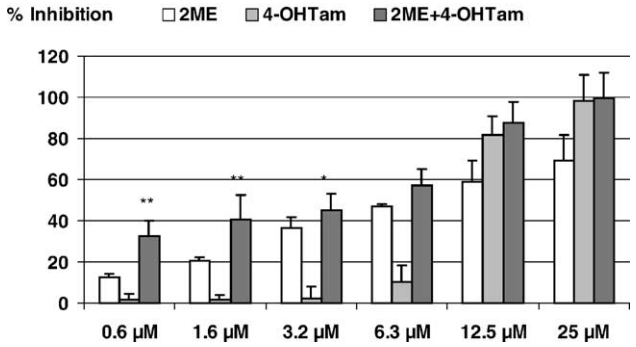

Fig. 2. Changes in proliferation of BM cells after addition of 2-methoxyestradiol (2-MeOE2), 4-hydroxytamoxifen (4-OHTam) or an equimolar combination of both substances compared to control values (mean ± S.D., $^{**}P < 0.01$ comparing combination vs. monosubstances).

4. Discussion

Tamoxifen is an antagonist for the estrogen receptor with respect to human breast cells. Over the past 30 years tamoxifen has been the most widely used endocrine drug for the management of all stages of breast cancer in women with estrogen-dependent tumors [5]. The most serious adverse events in treating patients with tamoxifen are an increased risk of uterine cancer, thromboembolism, hot flushes, sexual dysfunction and cataracts [5]. In contrast, tamoxifen seems to have beneficial effects on the cardiovascular system and bones [5].

The anti-proliferative mechanism of tamoxifen in estrogen receptor-positive breast cancer cells seems to include mainly the inhibition of estrogen binding to its receptor [6]. However, recent data point out that tamoxifen may also inhibit cell proliferation via upregulation of the inhibitory growth factor TGF-β [7] and down regulation of the potent mitogen IGF-II [8], mechanisms which may be independent of its receptor binding properties.

In our experiments, tamoxifen had an IC_{50} value of 31 μM for the MCF-7 cells. In addition, tamoxifen shows an anti-proliferative action in the BM cells, a receptor-negative cell line, with an IC_{50} value of 10 μM.

For the estrogen receptor-negative cell line MDA-MB-231 also an anti-proliferative effect for tamoxifen was observed [9]. In this cell type tamoxifen may down regulate signal transduction [10] and influence telomerase activity [11]. These data indicate an receptor-independent effect of tamoxifen.

It remains unclear, however, why women with receptor-negative breast cancer do virtually not respond to endocrine therapy with tamoxifen [12], although it may inhibit proliferation of receptor-negative breast cancer cells in vitro.

2-Methoxyestradiol is an endogenous estradiol metabolite with a very low binding affinity to the classical estrogen receptor-α [13]. It has been shown to act anti-proliferative in various cancer cells including estrogen receptor-positive and receptor-negative breast cancer cells [3]. Animal experiments and the preliminary data of a clinical phase II study indicate that 2-ME has a very low toxicity and is well tolerated even in high dosages [14,15]. In vitro data indicate that 2-ME may beneficially influence the cardiovascular system by positively modulating the production of vasoactive substances [16]. However, these results await confirmation in clinical studies as well as the effect of 2-ME on menopausal symptoms such as hot flushes. The anti-tumorigenic effect of 2-ME has been well described. However, the molecular basis for it is still unclear. Several mechanisms like the induction of wild-type p53 expression and inhibition of tubulin polymerization are under debate [3]. In addition, 2-methoxyestradiol has been shown to have an anti-proliferative and therefore anti-angiogenetic effect on vascular endothelial cells [17].

Our in vitro results demonstrate that the combination of the two anti-proliferative substances tamoxifen and 2-methoxyestradiol can react in an additive manner in both estrogen receptor-positive as well as receptor-negative human breast cancer cell lines. Thus the mechanism of the anti-proliferative effect of these substances seems to be different and the combination may have some advantages in breast cancer treatment compared to the monosubstances.

In conclusion, the estrogen antagonist tamoxifen and the endogenous estradiol metabolite 2-methoxyestradiol act in an additive manner considering their anti-proliferative effect in both receptor-positive and receptor-negative human breast cancer cell lines. Interestingly we observed that tamoxifen was also effective in vitro in the receptor-negative cell line BM. These is in accordance with results of some other groups. However, these observations are in contrast to the clinical experiences where tamoxifen has not shown activity in patients with receptor-negative tumors. Nevertheless animal experiments and clinical studies are needed to demonstrate the superiority of the efficacy of the combination of tamoxifen with 2-methoxyestradiol for the treatment of breast cancer compared to the monosubstances.

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