Is the combination with 2-methoxyestradiol able to reduce the dosages of chemotherapeutices in the treatment of human ovarian cancer? Preliminary in vitro investigations

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Summary

Purpose of investigation: The endogenous estradiol metabolite, 2-methoxyestradiol (2ME), has been shown to be a potent inhibitor of cell growth and a strong anti-angiogenic substance. We investigated for the first time whether in vitro combinations of 2ME with various chemotherapeutic compounds may result in an additive inhibitory effect on the proliferation of human ovary cancer cells.

Method: As a model two different human ovary cancer cell lines were used. All cell lines were incubated with equimolar concentrations of 2ME (0.8-25 μM) and the chemotherapeutics epirubicine, doxorubicine, paclitaxel, docetaxel, carboplatin, vinorelbine, 5-fluorouracil and mafosfamide. Proliferation was measured after four days using the ATP-chemosensitivity test.

Results: For both ovary cancer cell lines a significant additive effect of 2ME with epirubicine and carboplatin was observed at the lower concentration range of these chemotherapeutic substances.

Conclusion: 2ME is able to enhance the antiproliferative activity of certain chemotherapeutics at pharmacological relevant concentrations. This estradiol metabolite is currently in a phase II trial in patients with refractary metastatic breast cancer and the tolerability has been shown to be very good. The combination of 2ME with chemotherapeutics may therefore offer a new clinically relevant treatment regimen for hormone-dependent cancer.

Key words: 2-Methoxyestradiol; Chemotherapeutics; Ovary cancer cells; Proliferation.

Introduction

2-Methoxyestradiol (2ME), an endogenous estradiol metabolite, elicits an antiproliferative potency in various tumor cell lines and endothelial cells independent of the hormone receptor status [1-3]. The mechanism(s) responsible are not fully elucidated, however, different actions such as disrupting of microtubules and thereby inhibition of HIF-1a, a key angiogenic transcription factor [4], are active. Furthermore upregulation of the extrinsic and intrinsic apoptotic pathway have been demonstrated [4].

In the present study we investigated whether combinations of 2ME with various chemotherapeutic compounds may result in an additive effect on the proliferation of human ovary cancer cells.

Material and Methods

2-Methoxyestradiol (2ME) was purchased from Steraloids, USA. The cytostatica used were epirubicine (Epi, Pharmacia), daunorubicine (Dau, Pharmacia), paclitaxel (Pac, Bristol-Myers Squibb), docetaxel (Doc, Aventis), carboplatin (Car, Bristol-Myers Squibb), vinorelbine (Vin, Pierre Fabre Pharma), 5-fluorouracil (FU, Hexal) and mafosfamide (Maf, Baxter).

The compounds were dissolved in ethanol or PBS where appropriate and diluted with an ethanol/PBS mixture to yield a final ethanol concentration of 0.01% per well or with PBS alone.

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Two ovarian cancer cell lines were used, i.e. FRAWU and ERGR, which were established in our laboratory. 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 cells per well in assay kit medium. Subsequently, the agents were added to the wells in the concentrations of 0.8 to 25 μM alone and in equimolar combinations. After incubation for four days, cell proliferation was measured by an ATP-chemosensitivity test (5). In brief, proliferation is quantified by measuring light which is emitted during the bioluminescence reaction of luciferine in the presence of ATP and luciferase.

Statistical analysis was done by ANOVA with the logarithmated values followed by Dunnett's procedure from quadruplicates of two independent experiments. The overall alpha level was set at 0.05.

Results

In both cell lines 2ME alone showed a dose-dependent similar antiproliferative activity which was in the range of 15 to 45% inhibition as compared to control values.

The antiproliferative effect of all chemotherapeutics alone was similar in both cell lines and was in the range of 20% inhibition at the lowest concentration up to nearly 100% inhibition at the highest concentration.

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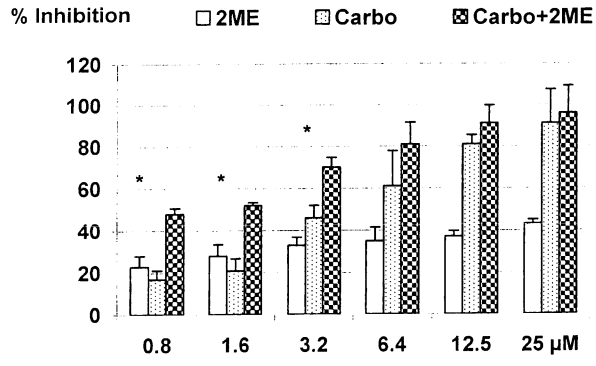

Figure 1. — Changes in proliferation of the ovarian cancer cell line ERGR after addition of 2-methoxyestradiol (2ME) and carboplatin (Carbo) alone and in equimolar combinations (means ± SD, each concentration in quadruplicates from two independent experiments; * $p < 0.05$ comparing combination vs monosubstances).

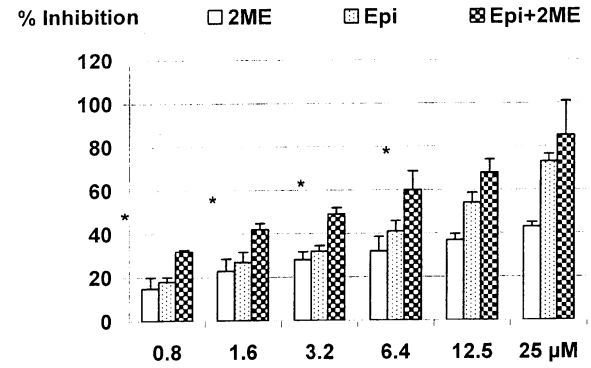

Figure 2. — Changes in proliferation of the ovarian cancer cell line ERGR after addition of 2-methoxyestradiol (2ME) and epirubicine (Epi) alone and in equimolar combinations (means ± SD, each concentration in quadruplicates from two independent experiments, * $p < 0.05$ comparing combination vs monosubstances).

2ME with carboplatin and 2ME with epirubicine an additive antiproliferative effect was observed, but only at the lower concentration range of 0.8-3.2 µM for carboplatin and at 0.8 to 6.4 µM for epirubicine. For example, in Figures 1 and 2 the inhibition of proliferation of the ovarian cell line ERGR is illustrated after addition of 2ME and carboplatin or epirubicine, respectively.

For all other combinations with 2ME the effect of the chemotherapeutic substances was more dominant than the effect of 2ME.

Discussion

Estrogens are known to be a major factor in the etiology of breast cancer. However, corresponding data for ovarian cancer are controversial. Recent data indicate that hormone therapy may increase ovarian cancer risk [6]. In the discontinued 2002 WHI trial the risk under combined estrogen/progestin therapy was enhanced, but not statistically significant [7].

Estrogens are responsible for cell proliferation and may increase cancer incidence by enhancing the number of errors occurring during cell replication. Evidence is accumulating that estradiol metabolites may influence carcinogenesis whereby some metabolites may possess procarcinogenic and others anti-carcinogenic properties [8]. 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 anticarcinogenic and antiproliferative properties [9]. As yet little in vitro data are available investigating the combination of 2ME with other compounds currently used for hormone-dependent cancer treatment.

The present data indicate that certain combinations of 2ME with chemotherapeutic substances may have an additive antiproliferative effect, thus allowing the reduction in the concentration of the cytostatics which usually have serious side-effects.

In a previous work we investigated the effect of a combination of 2ME with various chemotherapeutics in human breast cancer cell lines [10] and also found an additive effect for certain combinations.

These data emphasize that different mechanism(s) by 2ME and by certain therapeutic substances are working and thus may enhance the effectiveness of ovarian cancer treatment.

Enhancing the efficacy of conventional cancer therapies such as chemo- or hormonal treatment by new compounds are promising ways in the gynecological oncology. Animal experiments and the data of a clinical phase II study indicate that 2ME has a very low toxicity and is well tolerated even in high dosages [2, 11]. The first results of a phase I study of the combination of 2ME with docetaxel revealed a good tolerability [12]. 2ME did not significantly alter the pharmacokinetics of docetaxel and vice versa. Serum levels of 2ME achieved after treatment with a dosage of 1 mg were in the range of 100 to 600 nM. Preliminary experiments with a new 2ME formulation demonstrated plasma concentrations up to 10 µM [12], thus approximating the effective concentrations in our experiment. For most chemotherapeutics used in the present work the concentration range tested correlated with therapeutic concentrations. Thus the combination of 2ME with different chemotherapeutics may provide new clinical options in the treatment of hormone-dependent cancers.

In vitro data indicate that 2ME may beneficially influence the cardiovascular system by positively modulating the production of vasoactive substances [13-15]. However, these results await confirmation in clinical studies as well as the effect of 2ME on menopausal symptoms such as hot flushes.

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