# Estradiol metabolites are potent mitogenic substances for human ovarian cancer cells

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**Summary**

*Purpose of investigation:* The etiology of ovarian cancer appears to be associated with a long-term influence of estrogens. However, evidence is accumulating that certain estradiol metabolites may play a decisive role in the carcinogenesis of estrogen-dependent diseases. In the present study we examined the effect of estradiol metabolites on the proliferation and apoptosis of human ovarian cancer cells in comparison to the effect of their parent substance.

*Methods:* The ovarian cancer cell line OVCAR-3 was used for the experiments. 17ß-estradiol (E2), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2) and 16α-hydroxyestrone (16-OHE1) were incubated for seven days in the concentration range of 0.01 nM to 10 nM. Proliferation and apoptosis were measured by commercially available assay kits.

*Results:* E2 enhanced proliferation rate and concomitantly reduced apoptotic rate of the ovarian cancer cells at physiological concentrations. 2-OHE2 had no significant effect, whereas 4-OHE2 elicited similar effects on proliferation and apoptotic rate as E2. The greatest proliferative and antiapoptotic effect was observed for 16-OHE1, the values being significantly different to the effects of E2.

*Conclusion:* The pattern of endogenous estradiol metabolism may play a role in defining ovarian cancer risk. This may be of importance in certain predisposed women who are treated with hormone therapy in postmenopause.

*Key words:* Estradiol metabolites; Ovarian cancer cells; Proliferation.

## Introduction

Ovarian cancer is the fourth-ranking cause of cancer death in women from Western countries [1]. Approximately 90% of ovarian cancers arise from ovarian surface epithelial cells [2]. The etiological factors involved in ovarian epithelial carcinogenesis have not yet been clearly defined, but recent epidemiological studies have pointed out that estrogens could be responsible for promoting ovarian tumor progression in postmenopausal women. There is also growing experimental evidence that estrogens may play an important role in ovarian carcinogenesis [3, 4].

There are clinical and experimental indications that estradiol metabolites may play a decisive role in the carcinogenesis of estrogen-dependent cancers [5]. In this respect intensive research has been conducted concerning the association of estradiol metabolites and breast cancer [5]. Estradiol metabolites appear to elicit proliferative as well as antiproliferative actions on human breast cancer cells [6]. Therefore in the present study we have investigated the effect of various estradiol metabolites on the proliferation and apoptosis of human ovarian cancer cells in comparison to the effect of the parent substance 17ß-estradiol.

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## Material and Methods

17ß-Estradiol (E2), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2) and 16α-hydroxyestrone (16-OHE1) were purchased from Steraloids, USA. The test substances were dissolved in ethanol and diluted with an ethanol/buffer mixture to the appropriate test concentrations.

OVCAR-3, a human estrogen receptor-positive ovarian cancer cell line obtained from ATCC, USA, was used for the experiments. The cells were maintained in RPMI 1640 medium containing 20% (v/v) fetal calf serum supplemented with 2mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 1 mM sodium pyruvate, 0.01 mg/ml insulin and 100 µ/ml penicillin plus 100 µg/ml streptomycin.

Ninety-six well plates were seeded with approximately 1,000 cells per well in assay kit medium. For testing the proliferation activity, the cells were first incubated for three days with charcoal/dextrane-treated FCS and then the estrogens were added in a concentration range of 0.01 to 10 nM followed by incubation for seven days. After incubation, cell proliferation was measured using an ATP-chemosensitivity test [7]. In brief, proliferation is quantified by measuring light which is emitted during the bioluminescence reaction of luciferine in the presence of ATP and luciferase.

Apoptosis was measured using the Cell Death Detection ELISA (Roche). One thousand cells were incubated with various estrogen concentrations for seven days at 37°C. Afterwards the cells were lysed, centrifuged and the supernatant was transferred to a 96 plate.

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

## Results

In Figure 1 the results of the effect of estradiol and estradiol metabolites on the proliferation of OVCAR-3 cells are illustrated. 17ß-Estradiol (E2) induced a significant increase in the proliferation rate at 0.01 and 0.1 nM with values of 13 and 20%, respectively. 2-Hydroxyestradiol (2-OHE2) had no significant effect over the tested concentration range. In contrast 4-hydroxyestradiol (4-OHE2) stimulated proliferation at 0.01 and 0.1 nM with increases of about 12 and 26%, respectively. For 16-hydroxyestrone (16-OHE1) the highest increases in proliferation was observed out of all estrogens tested. This metabolite significantly stimulated the proliferation of OVCAR-3 cells at 0.01, 0.1 and 1 nM with values of about 53, 48, 36% which were statistically significant compared to the effect of 17ß-estradiol.

In Figure 2 the results of the apoptosis assay are depicted. E2 elicited a reduction of apoptosis of the OVCAR-3 cells at the concentrations of 0.01 and 0.1 nM. For 2-OHE2 no significant effect was observed. 4-OHE2 was able to reduce apoptosis at the concentrations 0.01 and 0.1 nM. 16-OHE1 had the strongest antiapoptotic effect of all estrogens tested with reductions of about 30-60% at the concentrations 0.01, 0.1 and 1 nM.

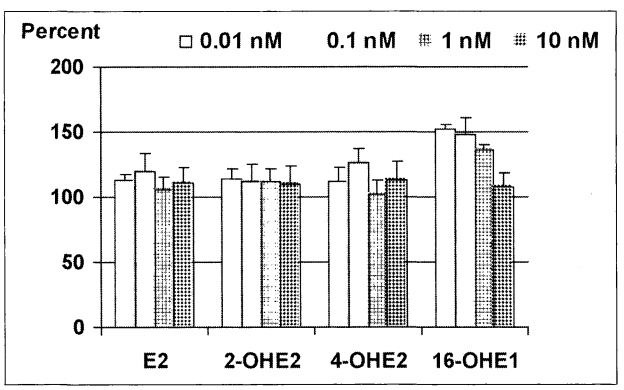


Figure 1. — Percent changes of proliferation of OVCAR-3 cells after addition of estradiol (E2), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2) and 16a-hydroxyestrone (16-OHE1) compared to control values (= 100%).
(means ± SD, * $p < 0.05$; ** $p < 0.01$ compared to control).

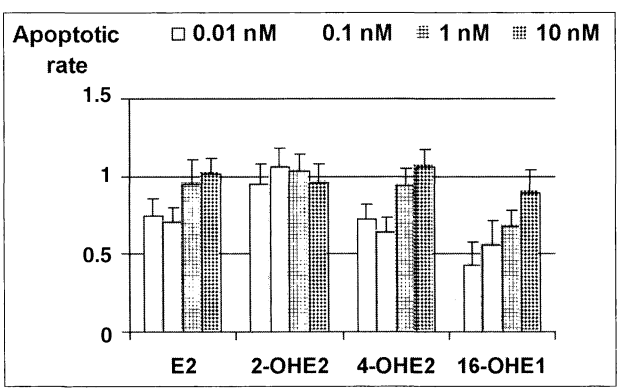


Figure 2. — Apoptotic rate of OVCAR-3 cells after addition of estradiol (E2), 2-hydroxyestradiol (2-OHE2), 4-hydroxyestradiol (4-OHE2) and 16a-hydroxyestrone (16-OHE1) compared to control values (= 1).
(means ± SD, * $p < 0.05$; ** $p < 0.01$ compared to control).

## Discussion

The balance between proliferation and apoptosis is crucial for the survival of cells. Estrogens are survival factors for both human breast and ovarian cells. It appears that the etiology of breast and ovarian cancer is associated with estrogens. Epidemiological data suggest that exogenous estrogens may increase breast cancer as well as ovarian cancer in postmenopausal women [8-12]. However, in terms of ovarian cancer the data are still controversial. Recently, two large prospective studies provided evidence of a significant increased risk of ovarian cancer in HRT users [8, 9]. The first one [8] was conducted on 211,500 American women indicating an increased risk of mortality from ovarian cancer. The second one [9], conducted on 44,241 women, confirmed this trend and demonstrated that the risk is time-dependent. In this study, the relative risk of ovarian cancer incidence was 3.2 in women who had used HRT for 19 years or more. The recent results of the Women's Health Initiative Trial also showed that continuous combined estrogen plus progestin therapy may increase the risk of ovarian cancer [10]. So far meta analyses do not indicate a positive correlation between hormone therapy and ovarian cancer and the results are often inconsistent [11, 12].

Experimental data also suggest that ovarian tumor growth may be estrogen-sensitive. The proliferation effect of estrogens has been demonstrated in different estrogen receptor-positive (ER+) ovarian cancer cell lines [3, 4]. Antiestrogens such as tamoxifen [13] can inhibit the mitogenic effect of estradiol on these cells. Estrogens could affect tumoral progression by increasing cell proliferation as well as promoting invasion or cell mobility.

Our data confirmed that estradiol is able to increase the proliferation rate of ovarian cancer cells at physiological concentrations. Concomitantly there is a reduction of the apoptosis rate. Thus the net effect is stimulation of the growth of ovarian tumor cells. Concerning the estradiol metabolites different effects have been observed. 2-hydroxyestradiol has, as already shown in human breast cancer cells, no significant effect on the proliferation and apoptosis of ovarian cancer cells. In contrast, 4-hydroxyestradiol has similar effects on proliferation and apoptosis to its parent substance. Surprisingly the greatest effect on the increase of proliferation and decrease of apoptosis is elicited by 16α-hydroxyestrone. This metabolite induces a nearly two- to fourfold further increase of proliferation as compared with 17ß-estradiol.

The results of the estradiol metabolites may reflect their binding properties to the estradiol receptor-α. 2-hydroxyestradiol has nearly no affinity to this receptor, whereas the binding property of 4-hydroxyestradiol resembles those of 17ß-estradiol [14]. In contrast, 16α-hydroxyestradiol has a similar affinity to the receptor to 17ß-estradiol, but it appears to bind irreversibly to the

receptor and thus a prolonged activation may occur [15].

In the last decade a possible role of estradiol metabolites emerged concerning breast carcinogenesis. In vivo, 17ß-estradiol, the most potent human estrogen, is mainly metabolized by hydroxylation at the A- or the D-ring leading to either 2- and 4-hydroxyestrogens or 16α-hydroxyestrone and estriol, respectively [16]. Certain metabolites such as the 4-hydroxyestrogens and 16α-hydroxyestrone appear to be able to act as procarcinogenic substances whereas 2-methoxyestradiol, a conjugated compound of 2-hydroxyestradiol, acts anticarcinogenically [6]. The prevailing theory postulates that the former metabolites, particularly 16-OHE1, increase the rate of cell proliferation by stimulating estrogen receptor (ER)–mediated transcription, thereby increasing the number of errors occurring during DNA replication [15]. An alternative theory suggests that the 4-hydroxyestrogens especially are metabolized to quinone derivatives, which directly remove base pairs from DNA through a process called depurination [17]. Error-prone DNA repair then results in point mutations.

In a previous study we found that 2-methoxyestradiol in high supraphysiological dosages can inhibit the proliferation of human ovarian cancer cells and is able to have an additive action with certain chemotherapeutics [18].

Our data indicate that the tendency of endogenous or exogenous estradiol metabolism towards an increased D-ring metabolism may increase ovarian cancer risk. This may be of concern in the long-term hormone treatment of predisposed postmenopausal women. Genetic polymorphism and/or life-style may be responsible for a shift of estradiol metabolism and should be considered when treating postmenopausal women with hormones.

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