Tibolone versus 17β-estradiol/norethisterone: Effects on the proliferation of human breast cancer cells

C. Lippert[1], *B.Sc.*; **H. Seeger**, *Ph.D.*; **D. Wallwiener**, *M.D.*; **A.O. Mueck**, *M.D., Ph.D., PH*

Section of Endocrinology and Menopause, Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen (Germany)

Summary

Tibolone is a synthetic progestin with estrogenic and progestogenic properties, widely used for alleviation of menopausal syndromes and for osteoporosis prophylaxis in postmenopausal women. Since only little data are available on tibolone and breast cancer risk the present study investigates the effect of tibolone on the growth of the human breast cancer cell line, MCF-7. Tibolone is clinically comparable to an estradiol/norethisterone combination, therefore we included this hormone combination in our experiments.

Tibolone was examined alone and in the presence of 0.1 nM estradiol in the concentration range from 0.001 μM to 1 μM. Norethisterone was studied using the same concentration range in combination with 0.1 nM estradiol.

Tibolone led to significant cell growth in the concentration range of 0.01 to 1 μM and was able to significantly stimulate estradiol-induced proliferation at the concentrations 0.01 and 0.1 μM. In contrast, the estradiol/norethisterone combination elicited significant inhibition of cell growth at the concentrations 0.001 and 0.01 μM.

These data suggest that tibolone does have tumor cell-growth promoting effects in vitro whereas the estradiol/norethisterone combination partially inhibits cell growth. Therefore no differences in risk profile are to be expected between conventional hormone substitution using estradiol and norethisterone acetate and tibolone. Drawing a clinical consequence from our experiments would result in not recommending the use of tibolone in postmenopausal women at high risk for breast cancer development until long-term controlled clinical studies have been performed on the effect of tibolone administration and breast cancer risk.

Key words: Tibolone; -17ß-estradiol; Norethisterone; Breast cancer cell growth.

Introduction

Tibolone is a synthetic steroid which elicits estrogenic and progestogenic properties on target cells based on its metabolism to two estrogenic and one progestogenic metabolites [1]. Currently clinical use of tibolone includes alleviation of postmenopausal syndromes as well as prophylaxis of osteoporosis in postmenopausal women; The advantage of tibolone over commonly used hormone replacement therapies is claimed to be the differentiated metabolism in the target cells such as breast and endometrium [1]. In the endometrium tibolone appears to act solely in a progestogenic way resulting in little proliferation under tibolone. In the breast tibolone appears to be converted mainly into progestogenic metabolites which do not trigger proliferation of the breast epithelial cells. However, data on this topic are sparse. Only one in vitro study investigated the effect of tibolone on breast cancer cells [2]. Therefore we examined the effect of tibolone on the proliferation of the human breast cancer cell line, MCF-7. Tibolone was studied alone and in combination with estradiol. In addition we included an E2/NET combination in our study, since tibolone is a norethyndrol derivative, yet possesses estrogenic and progestogenic properties, it therefore seemed appropriate to compare tibolone to hormone replacement therapy using estradiol (E2) and norethisterone (NET).

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Methods

The hormones were dissolved in ethanol. Tibolone was tested in the concentration range of 0.001 μM to 1 μM alone and at each concentration in combination with 0.1 nM E2. NET was tested at the concentrations 0.001 to μM, at each concentration in combination with 0.1 nM E2.

Tissue culture

MCF-7 human breast cancer cells were used from passage 36. Prior to the experiment and for 24 hours after seeding they 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 ug/ml streptomycin.

One day after seeding of the cells and throughout the experiment the same medium was used except for using fetal calf serum treated with dextran coated charcoal to remove any steroids and using DMEM without phenol red.

Hormone treatment

Ninety-six well plates were seeded with 500 MCF-7 cells per well in 5% FCS/DMEM medium. After 24 hours the cells were washed with PBS and the medium was changed to 5% stripped FCS phenol red free DMEM. The cells were stimulated with tibolone alone, with tibolone combined with estradiol or estradiol combined with norethisterone for five days. Tibolone, estradiol and NET were dissolved in 10% ethanol and 90% PBS and were added to the media to give a final ethanol concentration of 0.1%. The controls were treated with 0.1% ethanol.

Proliferation

Proliferation of the MCF-7 cells was measured using a crystal violet staining technique which accordingly stains cell nuclei. In short, the cells were fixed with 11% glutaraldehyde,

washed with distilled water, stained with a 0.1% crystal violet solution, washed with distilled water and solubilised with a 10% acetic acid solution.

After shaking, the plates were read in an ELISA reader at 600 nm. The statistical analysis of the results was performed using the Students't-test (n = 12).

Results

Figure 1 shows the results of the effect of tibolone alone on the proliferation of MCF-7 cells in the absence of estradiol. Tibolone significantly stimulated cell growth in the concentration range of 0.01 to 1 µM compared to control values. The increases were 66.5% (95% confidence interval 49;86) at 0.01 µM, 63.2 (CI 46;82) at 0.1 µM and 66.0% (CL 52;81) at µM compared to the control value = 100%.

Figure 2 illustrates the results of the investigation of tibolone or NET in combination with 0.01 nM µM estradiol. Estradiol alone led to an increase of 92.5% (CI 76;108).

The combination of tibolone and E2 significantly stimulated cell proliferation at the lower concentrations 0.01 and 0.1 µM by 15.7% (CI 6;27) and 12.5%

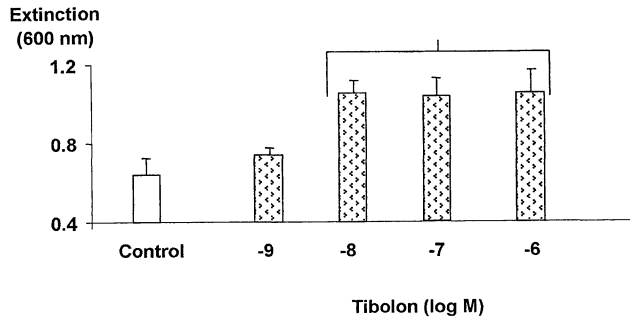

Figure 1. — Changes in proliferation of MCF-7 cells after addition of tibolone compared to control values (mean ± SD, *p < 0.05 vs. control).

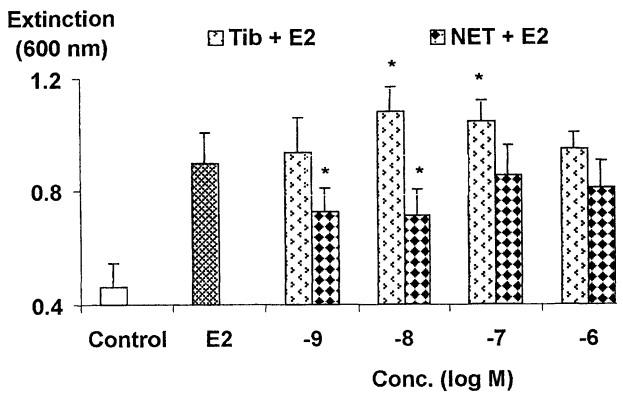

Figure 2. — Inhibition of estradiol (10-10M) induced proliferation of MCF-7 cells after addition of tibolone (Tib) and norethisterone (NET) in the range of 0.001 µ to 1 µM (mean ±SD, * = significance between the combination and estradiol alone induced growth at p = 0.05 level).

(CI 2;24), respectively, compared to the E2 value = 100%. As can be seen in Figure 2 the E2/NET combination triggered inhibition of cell growth which was significant at the concentrations 0.001 and 0.01 µM. Inhibitions in growth observed were 12.5% (CI 7;18) and 10% (CI 2;20) compared to the E2 value.

Discussion

Our results indicate that tibolone is able to stimulate the proliferation of the human breast cancer cell line, MCF-7. However, this occurs at higher concentrations than observed with 17ß-estradiol alone. Under the assumption that tibolone is similarly metabolized in vivo compared to in vitro, the metabolites of tibolone do not appear to have any protective effect on cancer cell growth. These results coincide with the only published experiment so far with breast cancer cell lines [2]. The authors found stimulation of cell growth in the MCF-7 and T-47D cancer cell lines, but only at a tibolone concentration of 0.01 µM compared to an effect of estradiol at 0.1 nM. In addition, they observed differences in the reaction behaviour of some subclones of the cell types used. The differences observed may be attributable to different passages or subclones of the cell types used.

In contrast to tibolone, the results of the E2/NET combination revealed an inhibitory effect on cell proliferation. This was significant at low dosages. We found similar inhibitory behaviour for an estradiol/medroxyprogesterone acetate combination [3].

Breast cancer risk associated with estrogen/progestin replacement therapy is currently widely discussed. So far most epidemiological studies indicate a higher risk for breast cancer in a women using HRT [4] but unfortunately most studies do not clearly distinguish and compare individual progestins used. While the Magnusson study [5] does distinguish between testosterone derived progestins (NET acetate and levonorgestrel) and progesterone derived progestins (MPA) with HRT taken up to five years, and shows a similar increase in relative risk for both, it is based on too small numbers to be statistically reliable, especially as an increase in duration of HRT treatment results in a further decrease in the number of cases. Two recent epidemiological studies on combined HRT versus ERT regarding breast cancer risk have been carried out in the United States. The study of Schairer *et al.* [6], based on over 40,000 women, finds a higher risk for the combined treatment compared with estrogen alone but only in lean women. The study does not distinguish between different types of estrogens and progestins used but states that it is mainly based on conjugated estrogens (Premarin) and to a large extent on medroxyprogesterone acetate. The study by Ross *et al.* [7] reports similar results for combined HRT showing a higher risk compared with ERT. Again the study is based predominantly on conjugated equine estrogens and the great majority of progestin used being medroxyprogesterone acetate. Thus, the role of the progestin addition to ERT is by far not resolved.

Our results are of special interest regarding the effect of tibolone combined with the most effective growth-stimulating estradiol concentrations. Tibolone further increased cell proliferation compared to estradiol alone. This result indicates that further cell growth might be initiated by tibolone under locally present estradiol concentrations. This fact may be of importance since estradiol concentrations have been shown to be higher in breast tissue compared to serum levels, especially in breast cancer tissue [8]. However, some experimental data suggest tibolone and its metabolites to be involved in reducing local estradiol concentrations via positively modulating enzyme systems involved in estradiol metabolism. Tibolone and metabolites have been shown to inhibit the activity of the enzymes sulfatase and 17ß-dehydrogenase and thus prevent the formation of estradiol from the precursor estrone sulfate [9, 10]. In addition tibolone and its metabolites were able to block the activity of the aromatase system which is responsible for the conversion of androstendione and testosterone to estradiol [11]. Furthermore a biphasic action of tibolone on the enzyme sulfotransferase has been observed, i.e., stimulation at the low dosage of 0.01 μM and inhibition at the high dosage of 10 μM [12]. This effect may also have an influence on the inactivation of estradiol.

Other progestins have also been shown to influence enzymatic systems involved in estradiol metabolism. NET, for example, also reduced sulfatase activity, however, it was less active than tibolone [9].

Recently a pro-apoptotic effect of tibolone on two receptor-positive and one receptor-negative mammary cancer cell lines was found [13].

Only one in vivo animal experiment exists on the effect of tibolone on the growth of mammary tumors. Tibolone caused a delay of DMBA-induced tumor growth in rats [2]. However, clinical intervention studies on tibolone and breast cancer risk in postmenopausal women are still lacking. Currently mammographic density has been claimed to be a risk factor for mamma carcinoma. So far several studies have been conducted with tibolone compared to conventional HRT investigating this question. Two studies found a slight increase under tibolone which was, however, not found to be higher than that of the HRT studied [14, 15]. Another study did not find any increase under tibolone whereas HRT induced an increase of about 30% [16].

Two studies with a small number of patients, i.e. four and 11 women, are available on the use of tibolone in women with pre-existing breast cancer [17, 18]. In one study one women developed colateral breast cancer under tibolone administration. In an uncontrolled phase II study on the use of tibolone in women exhibiting tamoxifen resistency in advanced or metastasing mamma carcinoma, the women initially did respond to the tibolone treatment, but later on all patients discontinued tibolone administration because of exacerbation of the disease [19].

Cell culture studies are a means of observing trends and mechanistic effects which are not easily obtained otherwise. Since epidemiological studies are difficult due to the many factors involved, it is important to achieve an understanding of the effect of individual components on certain organs. Cell culture studies have to be interpreted very carefully since culture conditions play an important role in determining the results. A cell culture model cannot reproduce the complex clinical situation but can reflect many characteristics of the original tissue such as enzyme and receptor types so that one can focus on individual factors possibly involved in the in vivo situation. A model can attempt to approximate the clinical situation and help in the elucidation of possible mechanisms involved but never replace prospective clinical or epidemiological studies.

In summary research on the effect of tibolone and cancer cell growth in vitro is still not conclusive. On the one hand tibolone may stimulate cancer cell growth which might even be enhanced in the presence of estradiol, on the other hand tibolone may reduce intracellular estradiol concentrations by positively modulating estradiol metabolism. Therefore, clinical data on tibolone and breast cancer risk are urgently needed. In the meantime, tibolone does not seem to be recommendable for women with enhanced breast cancer risk, at least regarding long-term treatment.

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Address reprint requests to:
ALFRED O. MUECK, M.D., PH.D., PH.
Head of Section of Endocrinology and Menopause
University Hospital
Department of Obstetrics and Gynecology
Schleichstrasse, 4 - 72076 Tuebingen (Germany)

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