The effect of medroxyprogesterone acetate and norethisterone on the estradiol stimulated proliferation in MCF-7 cells: comparison of continuous combined versus sequential combined estradiol/progestin treatment

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Summary

Objective: Little is known on the type of progestin and regimen type in relation to breast cancer risk. We have compared the effect of medroxyprogesterone acetate (MPA) and norethisterone (NET) on the estradiol stimulated proliferation in MCF-7 cells with respect to different regimens used in combined hormone replacement therapy (HRT).

Design: To approximate the *in vivo* conditions in HRT, MCF-7 cultures were pretreated with estradiol followed by estradiol/progestin treatment to represent the sequential combined model and compared with non pretreated cultures followed by estradiol/progestin treatment for the continuous combined model.

Results: When using progestins in the continuous combined form with estradiol (10^{-10} M) both progestins showed a significant reduction in the estradiol stimulated proliferation of the MCF-7 cells. In the sequential combined model the addition of MPA led to a stronger significant reduction of MCF-7 proliferation but in a narrower concentration range (from 10^{-8} to 10^{-6} M) compared to the continuous treatment. NET did not show any significant effect on proliferation in the SC model.

Conclusion: Different regimen types and different progestins do lead to significantly different effects on the proliferation of a breast cancer cell line. These findings might be useful in the elucidation of potential mechanisms involved in the clinical situation.

Key words: Hormone replacement therapy; Medroxyprogesterone acetate; Norethisterone; MCF-7 cells; Regimen types.

Introduction

Hormone replacement therapy has only in recent years started to widely include progestins since long-term mono-estrogen replacement therapy (ERT) was connected with an increase in endometrial cancer which could be prevented by the addition of a progestin to ERT. When epidemiological studies indicated that long-term ERT was possibly related to a slight increase in the risk of breast cancer, the addition of a progestin raised the question what kind of effect the progestin would have on breast cancer risk. In particular, an important question asked was whether different individual progestins had different effects on the risk.

Epidemiological studies so far have mostly not addressed the question of different types of progestins used, and are therefore interpreted according to the major progestin used in the specific country in which the study took place. A very recent epidemiological study carried out in the United States by Schairer *et al.* including over 40,000 women came to the conclusion that long-term combined HRT did lead to a higher risk of breast cancer even when compared with estrogen replacement alone [1]. The primary progestin used in the combined HRT in the Schairer study was MPA. In a similar study, also carried out in the United States, and also using mainly MPA in combination HRT, by Ross *et al.* [2] an even more pronounced increase in breast cancer risk was found than in the Schairer study for combined HRT compared with ERT with duration of time. A Swedish study by Magnusson *et al.* in which both MPA and NET were used in combined HRT, the increase in breast cancer risk was similar for combined HRT and ERT [3]. When comparing progesterone-derived progestins with testosterone-derived progestins used in combined HRT, both showed a similar risk in breast cancer for up to five years. Use for over five years led to a substantially higher risk for testosterone-derived progestins while progesterone-derived progestins even seemed to be connected with a decrease in breast cancer risk.

In addition to the type of progestin used, the type of regimen used, such as continuous combined (CC) versus sequential combined (SC), is also being discussed with respect to its potential for increasing or reducing the risk of breast cancer. Only very few epidemiological studies have addressed the breast cancer risk in different regimens used and often lack statistical power since they are based on too few cases. While Ross *et al.* showed the sequential combined treatment to have a higher risk compared to CC, based on the use of mainly MPA, Magnusson *et al.* showed the contrary i.e. a higher risk for CC but based on testosterone-derived progestins only. Interestingly Magnusson did show a higher risk for SC compared with CC for testosterone-derived HRT use of up to two years.

In the present study we were interested in the effects of the two most common progestins used in HRT, MPA, a C21 progestin, and NET, a C19 progestin, on the proliferation of a human breast cancer cell line. In particular we focused on the effect of regimen on cell proliferation. Since breast cancer initiation usually occurs many years before presentation of the disease, it is important to understand what effect different types of progestins and

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different regimens used in HRT might have on pre-existing breast cancer cells. Since clear epidemiological studies giving an indication of which regimen might be associated with a lower breast cancer risk for different progestins are lacking, we tried to modulate the situation in an *in vitro* model in order to find out more about possible mechanisms and effects involved.

MCF-7 cells were originally derived from a pleural effusion of a postmenopausal patient with metastatic breast carcinoma [4, 5]. This cell line is frequently used and well studied, and was chosen since it is estrogen positive, the ER regulation having been well studied [6, 7] and because MCF-7 cells retain characteristics of differentiated mammary epithelium.

Since cell lines per definition proliferate indefinitely, a cell line can be described as being on the way towards becoming the culture equivalent of a neoplastic cell and therefore could be defined as being abnormal. Nevertheless cell lines do retain many characteristics of their original tissue, such as enzyme and receptors types and patterns and are therefore useful for studying effects of compounds on cell behaviour. They offer many advantages to non-transformed cells such as being easily cultivatable in the numbers required. Non-transformed cells from biopsies have the advantage of being derived directly from the *in vivo* organism and being closer in their DNA composition. On the other hand the cells of individual biopsies lose enzyme activity and die over time due to having a short life spell during *in vitro* cultivation outside of the organism. Cells from a biopsy are not so well characterised, and are harder to cultivate. In general they require higher concentrations of serum and higher cell numbers are needed for seeding due to those cells being less well adherent than the MCF-7 cells.

A model using a cell line can not reflect the clinical situation but can offer the possibility to concentrate on certain factors which are suspected to be important in the *in vivo* situation in order to try to elucidate certain mechanisms and effects which might play a role in the *in vivo* situation. This might be helpful in cases where it is difficult to study the effect of individual factors in a complex situation, or when results from epidemiological studies are still being awaited. A model can try to come close to approximating the clinical situation in as many factors as possible, but cannot replace clinical or epidemiological studies.

Materials and Methods

Chemicals

17ß-estradiol, MPA and NET were purchased from Sigma, Munich, Germany and dissolved in 0.1% ethanol. Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco BRL, Eggenstein, Germany and fetal calf serum (FCS) from Seromed Biochrom KG, Berlin, Germany.

Tissue culture

The MCF-7 cells used were from passage 55 and cultured throughout the experiment in 5% (v/v) dextran charcoal stripped fetal calf serum, DMEM without phenol red, L-glutamine (4mM), bovine insulin (5 ng/ml), and 100 U/ml penicillin plus 100 µg/ml streptomycin. Non-stripped FCS and phenol red containing DMEM medium was used for the seeding of the cells.

Hormone treatment

Five-hundred cells per well were seeded into 96 well plates in 5% FCS DMEM medium. After 24 hours the cells were washed with PBS and the medium was changed to stripped FCS, phenol red free DMEM. The cells were pre-incubated for three days prior to treatment in both models to increase the sensitivity of the cells to estradiol. The cells used for sequential combined treatment were stimulated with estradiol (10^{-10}M) for five days and then incubated with estradiol (10^{-10}M) and either MPA or NET (in different concentrations, from 10^{-11} to 10^{-6}M) for a further five days. For the continuous combined model the cells were incubated for five days without estradiol, and then for the next five days with estradiol (10^{-10}M) and either MPA or NET (in different concentrations, from 10^{-11}M to 10^{-6}M). The estradiol concentration chosen for pre-stimulation and stimulation was based on previous studies showing this concentration to be the most effective for stimulation of MCF-7 cells [8]. The concentration range for the progestins was chosen to include supra- and sub-physiological conditions in addition to described *in vivo* serum concentrations. The *in vitro* culture conditions of five days only, instead of using 12±2 days for the SC model, which would be closer to the clinical setting for endometrial protection, had to be chosen due to experimental restrictions concerning maximum confluency acceptable within the wells. Estradiol and the progestins were dissolved in 10% ethanol and 90% PBS and were added to the media to give a final ethanol concentration of 0.1% ethanol. The controls were treated with 0.1% ethanol for ten days. Medium and test substances were changed every 48 hours.

Proliferation

Proliferation was measured using the crystal violet staining technique according to Kueng *et al.* [9] which 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.

Statistics

All experiments were carried out for n=12 wells for each experimental condition, and using the same passage of MCF-7 cells. The statistical analysis of the results was performed using the Students' t-test.

Results

As can be seen in Figure 1, the continuous combined treatment model of MPA leads to a significant reduction of estradiol-induced growth of the MCF-7 cells from 10^{-10} to 10^{-6} M. Growth was inhibited from 42.4%±8.4 to 63.6%±9.5 compared with the growth induced in the estradiol-stimulated controls. The highest growth inhibition was seen at the highest MPA concentration tested, i.e. 10^{-6}M. NET also showed a significant inhibitory effect on MCF-7 cell growth at a smaller range of concentrations 10^{-9} and 10^{-8} M, with 21.8%±6.9 and 26.0%±7.0 growth inhibition, respectively while 10^{-10} and 10^{-11}M were close to significance at α=5% (p=0.057 and 0.060, respectively). MPA and NET both showed a significant growth reduction at the pharmacologically relevant concentrations with MPA being about twice as effective at inhibiting cell growth of MCF-7 cells compared with

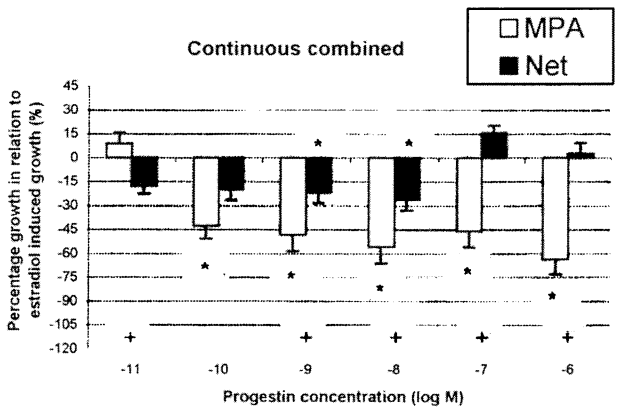

Figure 1. — Continuous-combined model: Changes in cell numbers of estradiol-stimulated MCF-7 cultures (10^{-10}M) treated with MPA or NET in combination with estradiol using cell cultures without estradiol pretreatment.
The bars represent percentage growth compared with growth in the estradiol-induced (10^{-10}M) controls = 0% (means ± SEM; n = 12; * = $p<0.05$ for one progestin at one concentration, + = $p<0.05$ between the two progestins for one concentration).

NET. A significant difference between MPA and NET for each concentration was shown at all concentrations except for 10^{-10}M which is probably due to a greater standard deviation in growth between the individual wells.

In the sequential combined treatment (Figure 2), MPA only showed significant anti-proliferative potential at the higher concentrations of MPA tested, i.e. 10^{-8}, 10^{-7} and 10^{-6} M. But the inhibitory effect itself, i.e. 63.1±8.2, 80.3±9.4 and 101.7±7.2%, respectively, was much greater than for the continuous combined model. At 10^{-6} M the inhibitory effect even completely suppressed estradiol-induced growth. And at 10^{-7} M the growth suppression of the sequential combined model was twice the amount seen in the continuous combined model, i.e.

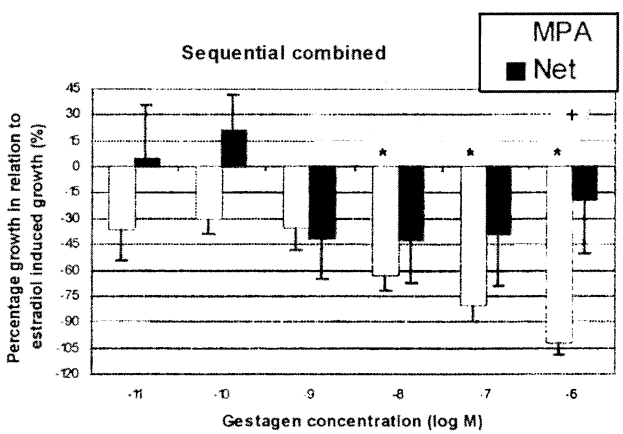

Figure 2. — Sequential-combined model: Changes in cell numbers of estradiol-stimulated MCF-7 cultures (10^{-10}M) treated with MPA or NET in combination with estradiol using cell cultures which were pretreated with estradiol for 3 days.
The bars represent percentage growth compared with growth in the estradiol-induced (10^{-10}M) controls = 0% (means ± SEM; n = 12; * = $p<0.05$ for one progestin at one concentration, + = $p<0.05$ between the two progestins for one concentration).

80.3±9.4% estradiol-induced growth inhibition compared with 46.0±10.1%. Although NET also showed some growth inhibition from 10^{-9} to 10^{-6} M, with the strongest growth inhibitory effect at 10^{-9}M with 41.6% growth inhibition and at 10^{-8} M with 42.1%, this inhibition was not found to be significant at α=5%, which is due to the strong variation in growth inhibition between the individual experiments. Interestingly the non-significant growth inhibition for NET at 10^{-9} and 10^{-8} M for the sequential combined model was also about twice the amount compared with the continuous combined model, i.e. 41.6±22.7 and 42.1±24.9% compared with 21.9±6.9 and 26.0±7.0%. Significance between the two progestins at each concentration was only shown for the highest concentration 10^{-6}M. The lack of significance at the lower concentrations was probably due to the high standard deviations of the individual treatment. A high degree of variability between the experimental treatments arose even though all experiments were carried out using the same batch and passage number of MCF-7 cells under the same experimental conditions. A slight variation in number of MCF-7 cells per well is probably responsible for the variation within one experimental condition tested but should not have affected the results between treatments.

Discussion

So far most epidemiological studies indicate a higher risk for breast cancer in women using HRT [10] but unfortunately most studies do not clearly distinguish and compare individual progestins used. While the Magnusson study [3] 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 duration of HRT treatment increases with the number of cases decreasing even further. Two recent epidemiological studies on combined HRT versus ERT regarding breast cancer risk have been carried out in the United States. The study by Schairer *et al.* [1], based on over 40,000 women, found 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.* [2] reported 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 was medroxyprogesterone acetate.

Very few studies have included a comparison between the regimen types cyclic versus continuous combined. The Ewertz study [11] found the sequential combined (SC) treatment to have a higher breast cancer risk compared to continuous combined (CC), the conclusion being based on only eight breast cancer cases in the continuous-combined group. The Magnusson study [3], which compares SC

versus CC including only testosterone-derived progestins, also shows a higher breast cancer risk in the SC group for up to two years HRT treatment. Although this conclusion might again be based on too few cases (51 per regimen group), it might nevertheless give an indication of possible differences between the two regimens. Ross *et al.* [2] interestingly also found a higher risk for SC compared with CC, however, without any statistical significance. These studies therefore indicate that there might be a difference between the two regimens regarding breast cancer risk. It has to be remembered, however, that these differences might be related to the different doses of progestins used in the different regimens or to a specific interaction related to the type of estrogen used.

Estrogens while most likely not involved in the initiation of breast cancer, have been shown to promote the proliferation of breast cells as well as breast cancer cells which possess functional estrogen receptors [12]. Progesterone leads to inhibition of estradiol-induced proliferation by down-regulating estrogen and progesterone receptors, and is involved in the differentiation of the terminal ductuli in the mamma. There is still controversy about the exact role of progestins on breast tissue. High-dose MPA treatment in breast cancer patients was shown to increase the number of cells in the G0/G1 phase and to reduce the number of cells in the S and G2/M phase [13]. The transit time in the G1 phase was also shown to be increased in MCF-7 cells [14, 15]. In contrast, two studies, one based on female monkeys and one on obese postmenopausal women, both found a higher proliferation in breast epithelial cells in the groups that had taken combined estrogen/MPA HRT compared with estrogen alone [16, 17].

NET has been shown to have a growth-stimulating effect on MCF-7 cells when used alone by acting on ER [8, 18], and by differential regulation of TGF-ß expression [19]. On the other hand, treatment of breast cancer with high dose NET was shown to lead to partial remission or stabilisation in many cases [20].

There are only a few *in vitro* studies so far which have investigated the effect of MPA and NET on estradiol induced proliferation. Jeng/Jordan [19] showed MPA and NET to have a similar proliferative effect on the proliferation of MCF-7 cells compared to the control, but unfortunately they did not provide an estradiol control for comparison. Interestingly they also studied NET together with estradiol in a further experiment using a smaller starting number of MCF-7 cells and including an estradiol control which resulted in NET having an antiproliferative effect on the estradiol-induced cells compared with the estradiol control alone. In the study by Schoonen *et al.* [8], MPA plus estradiol significantly inhibited cell proliferation from 10^{-9} to 10^{-6} M with NET not showing any significant inhibition of the MCF-7 cells. The different results of Schoonen *et al.* and Jeng and Jordan are probably due to different culture conditions such as differences in the media for seeding of the cells as well as for their cultivation and a different number of days of preculture before the test substances were added.

Our results show that in continuous-combined treatment MPA has a stronger antiproliferative effect on estradiol-induced MCF-7 cells compared with NET, as well as a wider range of concentrations at which MPA significantly reduces cell proliferation. Sequential combined treatment seems to result in a narrowing of the range of significant inhibition seen in CC on the one hand but also in a strong increase of antiproliferative potential for both MPA and NET. The stronger inhibition could be shown to be significant in the case of MPA but not for NET due to the larger standard deviation seen for the individual experiments.

In HRT the commonly used dosages of 5 mg (CC) to 10 mg (SC) per day of MPA lead to serum concentrations of $4x10^{-9}$M to 10^{-8}M [21, 22]. Treatment of breast cancer with high dose MPA of commonly used dosages of around 1000 mg results in serum concentrations of around 10^{-6}M [23]. For NET, usual dosages of 1 mg/day (for CC and SC) result in serum concentrations of around 10^{-8}M [24, 25]. As the oral dose is usually taken once a day, an initial rise in serum concentration is seen within the first few hours, followed by a subsequent decline.

Our results show that different regimens do have a distinctly different effect on estradiol-stimulated MCF-7 proliferation in this *in vitro* model. It seems that the continuous-combined form of HRT treatment is effective over a broader range of concentrations, especially also at lower progestin concentrations which might be more relevant to the mean plasma concentrations seen in patients. The broader range might be an advantage concerning inter-patient plasma variations and for possibly different progestin concentrations reached within different tissues. In the SC model only MPA was able to significantly inhibit MCF-7 proliferation, and only at the higher concentrations tested. Although the inhibition was significantly higher compared to the CC model, up to complete suppression of estradiol-induced breast cancer cell proliferation at 10^{-6} M MPA, this might imply that possibly this form of regimen would only be advantageous for women with very high MPA plasma concentrations. NET did not show any significant inhibition in the SC model which could be an indication that the CC form of treatment might have a greater effect on the MCF-7 cells. This lack of inhibitory effect of NET in the SC model goes with the observation in the Magnusson study that a cyclic regimen with testosterone-derived progestins leads to a higher breast cancer risk within the first two years of treatment compared to a continuous regimen and might be due to the interaction of NET with the estrogen receptor after pre-stimulation of the cells with estradiol.

We defined our SC model as cells pretreated with estradiol, while the CC model was defined as cells without estradiol pretreatment. The effect a CC type regimen would have on women who had previously undergone ERT or for women with elevated endogenous estrogens such as seen in clinical obesity are important questions which cannot be answered with our model. The exact clinical setting of 12±2 days of progestin could not be reproduced with our *in vitro* model due to experimental restrictions. Nevertheless our model does give the possibility to study potential effects of progestins after an initial period of estradiol stimulation. Regarding the

effect of progestins on breast tissue, there has been no large epidemiological study so far which has indicated a protective effect of combined HRT for protection against breast cancer. The only well documented effect of progestins in combined HRT has been in their antiproliferative effect on endometrial cells [26, 27]. These *in vitro* experiments do show that the type of regimen is important for the outcoming results and that they do have different effects on the proliferation of the breast cancer cell line chosen here, MCF-7 cells. Since the exact experimental conditions used, such as passage number of the cell line used, starting number of cells, length of experiment and medium used can have a strong effect on the result of the experiment, it is important to be cautious with deductions of the *in vivo* situation. *In vitro* experiments are useful for concentrating on single factors in order to study them and to further our understanding of potential mechanisms which might play a role in the clinical situation.

Our experimental results seem to indicate that it might be possible that different regimens are more suitable for different patients, for example with different plasma estradiol/progestin levels. In order to evaluate which regimen type is more protective with respect to breast cancer, prospective clinical studies are needed which take into account a more individualised form of treatment for each patient.

References

[1] Schairer C., Lubin J., Troisi R., Sturgeon S., Brinton L., Hoover R.: "Menopausal estrogen and estrogen-progestin replacement therapy and breast cancer risk". *JAMA*, 2000, *283*, 485.

[2] Ross R. K., Paganini-Hill A., Wan P. C., Pike M. C.: "Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin". *J. Natl. Cancer Inst.*, 2000, *92*, 328.

[3] Magnusson C., Baron J. A., Correia N., Bergström R., Adami H. O., Persson I.: "Breast-cancer risk following long-term oestrogen- and oestrogen-progestin-replacement therapy". *Int. J. Cancer*, 1999, *81*, 339.

[4] Soule H. D., Vazquez J., Long A., Albert S., Brennan M.: "A human cell line from a pleural effusion derived from a breast carcinoma". *J. Natl. Cancer Inst.*, 1973, *51*, 1409.

[5] Pourreau-Schneider N., Martin P. M., Charpin C., Jacquemier J., Saez S., Nandi S.: "How culture conditions modulate the morphofunctional differentiation of the human estradiol-sensitive mammary cell line (MCF-7)". *J. Steroid. Biochem.*, 1984, *20*, 407.

[6] Saceda M., Lippman M. E., Chambon P., Lindsey R. L., Ponglikitmongkol M., Puente M., Martin M. B.: "Regulation of the estrogen receptor in MCF-7 cells by estradiol". *Mol. Endocrinology*, 1988, *2*, 1157.

[7] Katzenellenbogen B. S., Kendra K. L., Norman M. J., Berthois Y.: "Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absense of estrogens". *Cancer Res.*, 1987, *47*, 4355.

[8] Schoonen W. G. E. J., Joosten J. W. H., Klooosterboer H. J.: "Effects of two classes of progestagens pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: I. MCF-7 cell lines". *J. Ster. Biochem. Mol. Biol.*, 1995, *55*, 423.

[9] Kueng W., Silber E., Eppenberger U.: "Quantification of cells cultured on 96-well plates". *Anal. Biochem.*, 1989, *82*, 16.

[10] Collaborative Group on Hormonal Factors in Breast Cancer: "Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer". *Lancet*, 1997, *350*, 1047.

[11] Ewertz M.: "Influence of non-contraceptive exogenous and endogenous sex hormones on breast cancer risk in Denmark". *Int. J. Cancer*, 1988, *42*, 832.

[12] Husmann F.: "Hormone und Krebs: in wieweit erhöhen Estradiol und insbesondere Testosteron das Mammakarzinom-Risiko". *Gyne.*, 1998, *11*, 293.

[13] Doihara H., Takashima S., Saeki H., Takiyama W., Kurita A., Soga H. *et al.*: "Effect of medroxyprogesterone acetate on the cell kinetics in primary breast cancer". *Gon To Kagaku Ryoho*, 1990, *17*, 2057.

[14] Sugiyama K., Shimizu M., Akiyama T., Ishida H., Okabe M., Tamaoki T., Akinaga S.: "Combined effect of navelbine with medroxyprogesterone acetate against human breast carcinoma MCF-7 cells in vitro". *Brit. J. Cancer*, 1998, *77*, 1737.

[15] Sutherland R. L., Hall R. E., Pang G. Y., Musgrove E. A., Clarke C. L.: "Effect of medroxyprogesterone acetate on proliferation and cell cycle kinetics of human mammary cell". *Cancer Res.*, 1988, *48*, 5084.

[16] Cline J. M., Soderqvist G., von Schoultz E., Skoog L., von Schoultz B.: "Effects of hormone replacement therapy on the mammary gland of surgically postmenopausal cynomolgus macaques". *Am. J. Obstet. Gynecol.*, 1996, *174*, 93.

[17] Hofseth L. J., Raafat A. M., Osuch J. R., Pathak D. R., Slomski C. A., Haslam S. Z.: "Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast". *J. Clin. Endocrin. Metab.*, 1999, *84*, 4559.

[18] Catherino W. H., Jeng M. H., Jordan V. C.: "Norgestrel and gestodene stimulate breast cancer cell growth through an oestrogen receptor mediated mechanism". *Br. J. Cancer*, 1993, *67*, 945.

[19] Jeng M. H., Jordan V. C.: "Growth stimulation and differential regulation of transforming growth factor-ß1 (TGFß1), TGFß2, and TGFß3 messenger RNA levels by norethindrone in MCF-7 human breast cancer cells". *Mol. Endocr.*, 1991, *5*, 1120.

[20] Clavel B., Pichon M. F., Pallud C., Milgrom E.: "Estradiol and progesterone receptors content and response to norethisterone treatment in advanced breast cancer". *Eur. J. Cancer Clin. Oncol.*, 1982, *18*, 821.

[21] Svensson L. O., Johnson S. H., Olsson S. E.: "Plasma concentrations of medroxyprogesterone acetate, estradiol and estrone following oral administraton of Klimaxil®, Trisequence®/Provera®, and Divina®. A randomised, single-blind, triple cross-over bioavailability study in menopausal women". *Maturitas*, 1994, *18*, 229.

[22] Stanczyk F. Z., Brenner P. F., Mishell D. R., Ortiz A., Gentzschein E. K. E., Goebelsmann U.: "A radioimmunoassay for norethindrone (NET): measurement of serum net concentrations following ingestion of NET-containing oral contraceptive steroids". *Contraception*, 1978, *18*, 615.

[23] Blossey H. C., Wander H. E., Koebberling J., Nagel G. A.: "Pharmakokinetic and pharmacodynamic basis for the treatment of metastatic breast cancer with high dose medroxyprogesterone acetate". *Cancer*, 1984, *54*, 1208.

[24] Klehr-Bathmann I., Kuhl H.: "Formation of ethinylestradiol in postmenopausal women during continuous treatment with a combination of estradiol, estriol and norethisterone acetate". *Maturitas*, 1995, *21*, 245.

[25] Andreasen E. E., Madsen L. G., Jensen H. K.: "Absorption of norethisterone acetate from tablets with normal and coarse particle size". *Ugeskr Laeger*, 1989, *151*, 1109.

[26] Grady T., Gebretsadik T., Kerlikowske L., Ernster V., Petitti D.: "Hormone replacement therapy and endometrial cancer risk: A metaanalysis". *Obstet. Gynecol.*, 1995, *85*, 304.

[27] Persson I., Adami H. O., Berkvist L., Lindgren A., Petterson B.: "Hoover Risk of endometrial cancer after treatment with oestrogens alone or in conjunction with progestogens: results of a prospective study". *BMJ*, 1989, *298*, 147.

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