Is there a protective role of progestogens on the proliferation of human ovarian cancer cells in the presence of growth factors?

H. Seeger, D. Wallwiener, A.O. Mueck

Section of Endocrinology and Menopause, University Women's Hospital, Tuebingen (Germany)

Summary

Purpose of investigation: The role of progestogens in the genesis of ovarian cancer remains unclear although a rather protective behaviour has been suggested. Epidemiological studies indicate a possible increase in the risk for combined estrogen/progestin as compared to estrogen alone. It is ambigious whether a difference exists within the various progestogens. Apart from sex steroids, growth factors play a crucial role in the genesis of ovarian cancer, although as yet little investigated. In the present study we have explored the effect of progesterone (P), medroxyprogesterone acetate (MPA) and norethisterone (NET) on the proliferation of human ovarian cancer cells alone and in the presence of growth factors.

Methods: For the experiments human ovarian cancer cells (OVCAR-3) were used. The progestogens were tested at the concentrations of 0.01 to 10 μM. The growth factor mixture consisted of EGF, FGF und IGF-I, each at a concentration of 10 pM. The incubation time was three or seven days. Proliferation rate was measured by an ATP-assay.

Results: After three days' incubation the growth factors induced an increase in the proliferation rate of about 50%. Progesterone alone did not show any significant change as compared to the control values, whereas NET and MPA elicited a significant increase at 1 and 10 μM and at 1 μM, respectively. In the presence of growth factors none of the progestogens was able to inhibit the proliferative stimulation. After seven days' incubation the growth factors still showed an increase of about 50%. MPA alone had an inhibitory effect at 10 μM, for NET and P no effects were observed. Again in the presence of growth factors no progestogen was able to show an inhibitory effect.

Conclusion: Our results indicate that progestogens do not have a protective role on the growth of pre-existing ovarian cancer cells, at least in the presence of growth factors. Further investigations are worthwile to evaluate possible differences between the effect of the various progestogens.

Key words: Progesterone; Norethisterone; Medroxyprogesterone acetate; Growth factors; Proliferation; Human ovarian cancer cells.

Introduction

The role of progestogens in the genesis of ovarian cancer still remains unclear; up to now rather protective effects have been assumed [1]. In the combined estrogen/progestin arm of the Women's Health Initiative (WHI) study, however, a risk increase was observed, although it was not statistically significant [2]. This could point to a proliferative effect of progestogens on pre-existing ovarian cancer cells. However, the results of the monoarm of the WHI are still awaited.

As in the case of breast cancer, it remains unknown if there is a difference between the various progestogens available for hormone therapy. Apart from the sex steroids, growth factors clearly are involved in the survival of normal and malignant ovarian epithelial cells [3]. In the present study we have investigated the effect of progesterone, medroxyprogesterone acetate and norethisterone on the proliferation of human ovarian cancer cells alone and in the presence of growth factors.

Revised manuscript accepted for publication November 12, 2005

Material and Methods

Progesterone (P), medroxyprogesterone acetate (MPA) and norethisterone (NET) were purchased from Steraloids, USA. The test substances were dissolved in ethanol and diluted with an ethanol/buffer mixture to the appropriate test concentrations.

Epidermal growth factor (EGF), fibroblast growth factor-basic (bFGF) and insulin-like growth factor (IGF-I) were purchased from Sigma Chemicals. The compounds were reconstituted according to the manufacturer's instructions stated on the package insert and were stored in aliquots at -20°C.

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 U/ml penicillin plus 100 μg/ml streptomycin.

Ninety-six well plates were seeded with approximately 1,000 cells per well in assay kit medium. To test the proliferation activity, the cells were first incubated for three days with charcoal/dextrane-treated FCS and then the progestogens were added in a concentration range of 1 and 10 μM alone or in the presence of a growth factor mixture (each growth factor 10 pM) followed by incubation for three or seven days. After incubation, cell proliferation was measured using an ATP-chemosensitivity test [4]. 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 triplicates of three independent experiments. The overall alpha level was set at 0.05.

Results

In Figure 1 the results for an incubation period of three days is shown. The growth factor mixture elicited an increase in the proliferation rate of about 50%. P alone had no effect, whereas NET significantly increased proliferation at both concentrations and MPA at 1 µM. In combination with growth factors none of the progestogens was able to reduce the effect of the growth factors, however, MPA at a concentration of 1 µM further enhanced the effect of the growth factors.

In Figure 2 the results for an incubation period of seven days is depicted. Again the growth factor mixture stimulated proliferation of the cells by about 50% as compared to control values. P alone had no significant effect, whereas NET stimulated proliferation at 1 µM, but not at 10 µM. MPA showed no effect at 1 µM, but inhibited proliferation of the cells by about 50% at 10 µM. In combination with the growth factor mixture the following picture was observed: None of the progestogens had any significant inhibitory effect on the growth factor-induced stimulation of the proliferation of ovarian cancer cells.

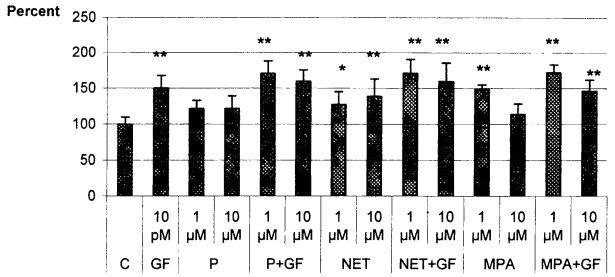

Figure 1. — Percent changes of proliferation of OVCAR-3 cells after addition of growth factor mixture (GF), progesterone (P), norethisterone (NET) and medroxyprogesterone acetate (MPA) compared to control values (= 100%). Incubation time was three days.
(means ± SD, * $p < 0.05$; ** $p < 0.01$ compared to control).

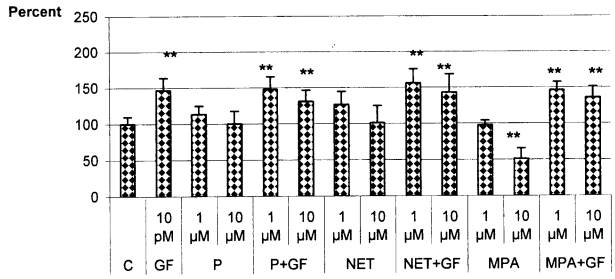

Figure 2. — Percent changes of proliferation of OVCAR-3 cells after addition of growth factor mixture (GF), progesterone (P), norethisterone (NET) and medroxyprogesterone acetate (MPA) compared to control values (= 100%). Incubation time was seven days.
(means ± SD, ** $p < 0.01$ compared to control).

Discussion

Ovarian cancer is the fourth-ranking cause of cancer death in women from Western countries [5]. Approximately 90% of ovarian cancers arise from ovarian surface epithelial cells [3]. 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.

Little data are available concerning a possible impact of growth factors on the risk of ovarian cancer. It is evident that normal ovarian epithelial cells can secrete and have receptors for agents such as growth factors that stimulate cell growth and differentiation; especially EGF and FGF seem to be of importance [3].

Indeed, our data indicate that growth factors such as EGF, FGF and IGF-I are strong survival factors for human ovarian cancer cells at very low concentrations, having a two- to three-fold higher activity than estradiol, at least in our cell model (data not shown).

The role of progestogens is still unclear, but experimental studies have suggested an inhibitory effect on the proliferation of normal and cancerous ovarian cells [1]. Levonorgestrel, a synthetic progestogen, induces differential regulation in the ovarian epithelium of TGF-β in monkeys, a change in the expression of which is highly associated with apoptosis [6]. Progesterone significantly inhibited cell proliferation and induced apoptosis in ovarian carcinoma cell lines, whereby an up-regulation of p53 expression was observed [7]. In human ovarian surface epithelium cells and in malignant cells progesterone was able to induce apoptosis via the Fas/FasL signalling pathway [8].

However, in our cell model using OVCAR-3 cells progesterone had a neutral effect on the proliferation, whereas NET and MPA elicited a stimulatory effect. Only MPA inhibited cell proliferation at the highest concentration after seven days of incubation. Certainly more reflecting the in vivo situation is the investigation of the effect of progestogens in the presence of growth factors. In this case none of the investigated progestogens was able to trigger a major inhibitory effect on the growth factor stimulated cell proliferation. These data indicate that in vivo a protective role of progestogens on the proliferation of pre-existing ovarian cancer cells cannot be awaited. On the other hand a further risk increase for ovarian cancer seems questionable.

Recently, two large prospective studies have provided evidence of a significant increased risk of ovarian cancer in hormone users [9, 10]. The first one [9] was conducted on 211,500 American women indicating an increased risk of mortality from ovarian cancer. The second one [10], 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 hormone therapy 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 [11]. So far meta-analyses do not indicate a positive correlation between hormone therapy and ovarian cancer and the results are often inconsistent [12, 13].

Despite their widespread use, in vitro models have certain limitations: the choice of culture conditions can unintentionally affect the experimental outcome, and cultured cells are adapted to grow in vitro; the changes which have allowed this ability may not occur in vivo. The homogenicity of cell lines can be viewed as an advantage or disadvantage. It allows the study of cells which represent a tissue population, however, responses may not fully mimic those of the complex in vivo situation. Limitations of this in vitro study might be the high concentrations needed for an effective antiproliferative effect. We only present results of rather high progestogen concentrations of 1 and 10 μM, since lower in vitro concentrations did not show any relevant effect. The clinically relevant blood concentrations for the progestogens most commonly used for hormone therapy, MPA and NET, are in the range of 4 x 10^{-9}M to 10^{-8}M for MPA [14] and around 10^{-8}M for NET [15]. However, higher concentrations may be required in vitro in short-time tests in which the reaction threshold can only be achieved with supraphysiological dosages. Higher concentrations may also be reached in vivo in the vessel wall or organs compared to the concentrations usually measured in the blood.

A further limitation of our work is the short incubation period of the cells with the substrates under investigation, in comparison to the longer time period for which hormone therapy is usually prescribed. In vitro experiments can support, but not replace clinical trials, and therefore, further clinical studies are needed to determine which progestogens, if any, have an influence on ovarian cancer risk when combined with estrogens.

References

[1] Ho S.-M.: "Estrogen, progesterone and epithelial ovarian cancer". *Reprod. Biol. Endocrinol.*, 2003, *1*, 73.

[2] Writing Group for the Women's Health Initiative Investigators: "Risks and benefits of estrogen plus progestin in healthy postmenopausal women". *JAMA*, 2002, *288*, 321.

[3] Auersperg, N., Wong, A.S.T., Choi K.-C., Kang S.K., Leung P.C.K.: "Ovarian surface epithelium: Biology, endocrinology and pathology". *Endocrine Reviews,* 2001, *22,* 255.

[4] Andreotti P.E., Thornthwaite J.T., Morse I.S.: "ATP tumor chemosensitivity assay". In: Stanley P., Kricka L.J. (eds.). "Bioluminescence and Chemoluminescence: Current Status". Chichester, J. Wiley & Sons, 1991, 417.

[5] Greenlee R.T., Murray T., Bolden S., Wingo P.A.: "Cancer statistics, 2000". *Cancer J. Clin.,* 2000, *50,* 7

[6] Rodriguez G.C., Nagarsheth N.P., Lee K.L., Bentley R.C., Walmer D.K., Cline M. *et al.*: "Progestin-induced apoptosis in the Macaque ovarian epithelium: differential regulation of transforming growth factor-β". *J. Natl. Cancer Inst.,* 2002, *94,* 50.

[7] Bu S.-Z., Yin D.-L., Ren X.-H., Jiang L.-Z., Wu Z.-J., Gao Q.-R., Pei G.: "Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines". *Cancer,* 1997, *79,* 1944.

[8] Syed V., Ho S.-M.: "Progesterone-induced apoptosis in immortalized normal and malignant human ovarian surface epithelial cells involves enhanced expression of FasL". *Oncogene,* 2003, *22,* 6883.

[9] Rodriguez C., Patel A.V., Calle E.E., Jacob E.J., Thun M.J.: "Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women". *JAMA,* 2001, *285,* 1460.

[10] Lacey Jr. J.V., Mink P.J., Lubin J.H., Sherman M.E., Troisi R., Hartge P. *et al.*: "Menopausal hormone replacement therapy and risk of ovarian cancer". *JAMA*, 2002, *288,* 334.

[11] Anderson G.L., Judd H.L., Kaunitz A.M., Barad D.H., Beresford S.A., Pettinger M. *et al.*: "Effects of estrogen plus progestin on gynaecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial". *JAMA,* 2003, *290,* 1739.

[12] Coughlin S.S., Giustozzi A., Smith S.J., Lee N.C.: "A meta-analysis of estrogen replacement therapy and risk of epithelial ovarian cancer". *J. Clin. Epidemiol.*, 2000, *53,* 367.

[13] Negri E., Tzonou A., Beral V., Lagiou P., Trichopoulos D., Parazzini F. *et al.*: "Hormonal therapy for menopause and ovarian cancer in a collaborative re-analysis of European studies". *Int. J. Cancer*, 1999, *80,* 848.

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

[15] 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.

Address reprint requests to:
A.O. MUECK, M.D., Pharm.D., Ph.D.
Head of Section of Endocrinology and Menopause
University Women's Hospital
Calwerstrasse 7
72076 Tuebingen (Germany)