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The effects of progesterone on breast tissue

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J. Desreux, F. van den Brûle, A. Noël, R. Delansorne, J.-M. Foidart

THE EFFECTS OF PROGESTERONE ON BREAST TISSUE

Summary

The control of the number of mammary cells depends on an equilibrium between cell multiplication by mitosis, and apoptotic cell death. It has been described that an apoptotic surge occurs at the end of the luteal phase. In this work, we demonstrate that the interruption of a progestin treatment (nomegestrol acetate) induces a phase of increased apoptotic death in the normal mammary epithelial cells, as demonstrated by TUNEL staining and biochemical determination of caspase-3 activity. Continuous treatment by the progestin does not increase apoptotic rates. The apoptotic rates of epithelial cells from fibroadenoma or the adenocarcinoma cell line T-47D are not affected by either the continuous or discontinuous treatment. Our data indicate apoptotic induction in normal mammary epithelial cells by interruption of a progestin treatment, a fact that could constitute an important parameter for developing benign, preneoplastic or neoplastic mammary disease.

INTRODUCTION

The control of cell number during processes involving the renewal of tissues is essential in embryos and adults. This control relies on a delicate balance between proliferation and cell death. Proliferation and apoptosis (programmed cell death) have indeed been implicated in several normal processes, such as tissue development and homoeostasis but also in many pathological states.

In the "resting" human breast, epithelial cell proliferation has been reported to peak during the follicular [1, 2] and luteal phases of the menstrual cycle [2–5]. On the other hand, Ferguson and Anderson [2] noted the highest percentage of apoptotic cells during menstruation. The expression of Bcl-2, which inhibits apoptosis, is maximal in breast epithelial cells during ovulation and then dramatically declines as apoptosis rises [6].

Moreover, variations of Bcl-2 expression are correlated with seric progesterone levels [7]. This sequence of events suggests that Bcl-2 expression and apoptosis are directly or indirectly influenced by ovarian steroid hormones.

Understanding the regulation of mammary tissular homoeostasis by the ovarian steroid hormones during the menstrual cycle is an important step for managing the risks of the hormonal replacement therapy (HRT). To date, epidemiological studies are lacking to demonstrate a link between continuous or discontinuous progestin administration in HRT and the risk of breast cancer [8, 9].

Therefore, in the present study, we examined the effects of a progestin (nomegestrol acetate or NOMAC) on the apoptosis of normal breast epithelial cells, both *in vitro* and *in vivo*, and of fibroadenoma *in vivo* and cancerous cells *in vitro*.

MATERIALS AND METHODS

In vitro experimentations

HMEC (human mammary epithelial cells) were isolated from tissues obtained from reduction mammoplasties and processed immediately after surgery as previously described [10]. Culture medium was changed every 3 days and the primary cells used in the experiment were harvested after trypsinization of 15-day-old cultures. Human mammary tumour cell line T-47D was obtained from American Type Cell Culture and cultured according to their recommendations.

Hormonal treatments

After 48 h, medium was replaced with fresh steroid-free medium (without red phenol and supplemented with dextran-charcoal-treated serum). Then semi-confluent (50 %) cultures were treated as follows:

1. NOMAC withdrawal: 10^{-7} M NOMAC (kindly provided by Theramex, Monaco) was added for 3 days and then the medium was replaced with fresh steroid-free medium supplemented with 0.1 % ethanol (used as the vehicle) and incubated for 3 days;
2. continuous NOMAC: 10^{-7} M NOMAC was present during all 6 days;
3. untreated control: cells were grown in a steroid-free medium containing 0.1 % ethanol for 6 days. Media were changed once a day.

In situ detection of apoptosis

TUNEL (terminal dUTP nick-end labelling) [11] was used to detect free 3'-end OH groups *in situ* in fragmented DNA according to the manufacturer's instructions (In Situ Cell Death Detection Kit™, Boehringer Mannheim) Automated quantitative immunolabelling analysis was performed using a computer assisted image processor (CAS 200), as previously described [11]. At least 1000 nuclei were counted for each well. Appropriate positive and negative controls were included.

Caspase-3 activity

Caspase-3 (Yama/apopaïn/CPP32) is an enzyme like interleukin 1b-converting enzyme implicated in apoptosis [10, 11]. Its activity was measured as previously described [12].

Caspase-3 inhibition

The inhibitor DEVD-CHO (Z-Asp-Glu-Val-Asp-CH2F, Enzyme Systems

Products) was added to the medium (50 µl/ml of medium) simultaneously with NOMAC withdrawal. Cells were fixed and analysed using the TUNEL technique described above.

In vivo experimentations

Patients

Forty premenopausal women (mean age: 32.8 ± 11.6 years, range: 16–53 years) with documented spontaneous regular menstrual cycles were enrolled in this study. Previous hormonal contraception was discontinued. Twenty of these women had requested a reduction mammoplasty and had no known breast pathology (normal breast group). Other twenty underwent removal of a fibroadenoma, which had been diagnosed clinically and radiologically and confirmed histologically (fibroadenoma group). In each group, the women were randomly assigned to one of two treatment groups and received a hydro-alcoholic gel (2 g/day), containing either NOMAC 2.2 g/day or a placebo. Each gel formulation was applied daily to both breasts for 14 days prior to surgery, from the 1st to the 14th (± 1) day of the menstrual cycle. Surgery was performed on the 15th day of menstrual cycle. The study design was approved by the University of Liège Ethics Committee and oral informed consent was obtained from each woman.

Study design

Surgery was performed on the 15th day of menstrual cycle. The gel was applied for the last time 24–30 hours before surgery. During surgery, a blood sample was taken for plasma level measurements of E2 (estradiol), P (progesterone), SHBG (sex hormone binding globulin) and NOMAC. Two samples of normal breast tissue or fibroadenoma were set aside for this study. The first was stored at –20 °C and processed for measurement of tissue E2 and NOMAC concentrations using specific RIA. The second sample was fixed and used for semi-quantitative evaluation of ER (estradiol receptors) and PR (progesterone receptors) using ER-RIA and PR-RIA kits and for detection of apoptosis by TUNEL, as described above with some modifications.

RESULTS

In vitro studies

Regulation of HMEC apoptosis by a synthetic progestin

HMEC were cultured with NOMAC or after its withdrawal and apoptosis was evaluated using the TUNEL technique. Untreated control cells showed low and constant levels of apoptosis (8 ± 5 %; n = 4). Similar apoptotic levels (9 ± 6 %; n = 4) were observed when cells were exposed continuously for 72 hours to 10^{-7} M NOMAC. In contrast, the apoptosis rate was higher and clearly affected by NOMAC withdrawal, with HMEC apoptosis peaking (41 ± 14 %; n = 4) 24–30 h after NOMAC withdrawal. Subsequently, the number of cells undergoing apoptosis returned to low levels comparable to those of control cultures (untreated cells or cells subjected to continuous treatment) (9 ± 6 %; n = 4) (Fig. 1).

To further characterize the biochemical characteristics of the apoptotic

Figure 1: HMEC apoptosis after NOMAC withdrawal and effect of caspase-3 inhibitor DEVD-CHO. Cells cultured as described under Materials and Methods were either untreated (●), continuously treated with NOMAC (■), or NOMAC was withdrawn at 0 h (▲). Caspase-3 inhibitor was able to block the apoptotic response observed after NOMAC withdrawal (◆).

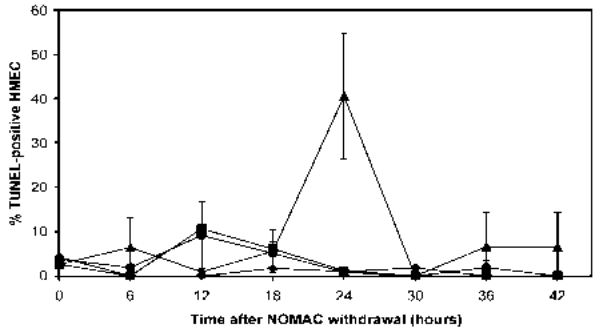
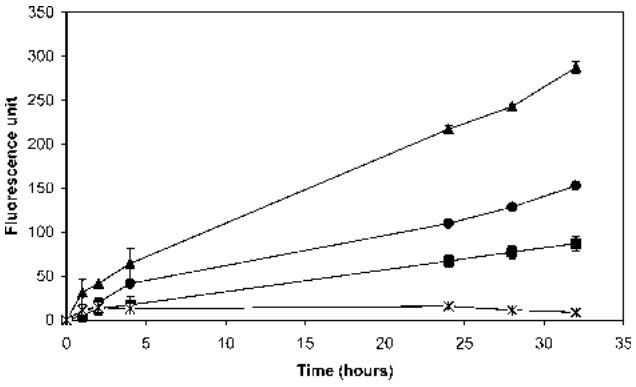


Figure 2: Influence of NOMAC withdrawal on caspase-3 activity in HMEC. Cultured cells were untreated (●), continuously exposed to NOMAC (■), or NOMAC was withdrawn after 24 h (▲). Some wells were incubated with 0.9 % NaCl (✕).Caspase-3 activity was measured in cell lysates incubated with the specefic fluorogenic substrate, Z-DEVD-AFC.



response, caspase-3 activity was analysed in cell lysates by using a specific fluorigenic substrate. Caspase-3 activity was consistently increased in cultures from which NOMAC had been withdrawn for 24–32 h in comparison to those of untreated controls or cultures continuously exposed to NOMAC (Fig. 2).

We therefore tested the effect of a caspase-3 inhibitor on the apoptosis of cells from which NOMAC had been withdrawn. When the caspase-3 inhibitor DEVD-CHO was added to the culture medium at the time of progestin withdrawal, apoptosis remained at control levels, as assessed by TUNEL (Fig. 1). As expected, this caspase-3 inhibitor did not affect cell apoptosis under control or under continuous NOMAC conditions (data not shown).

Absence of NOMAC-induced apoptosis in tumoural breast T-47D cells

To determine whether or not this apoptotic response to NOMAC withdrawal was specific to normal epithelial cells, human breast adenocarcinoma T-47D cells expressing PR were subjected to NOMAC treatment and withdrawal, and apoptosis was evaluated using TUNEL technique. Unlike that of HMEC, T-47D apoptosis remained below 1 ± 0.5 % (n = 4) even 24 h after NOMAC withdrawal (Fig. 3). These findings suggest that the influence of progestin withdrawal on apoptosis observed *in vitro* is specific to normal mammary epithelial cells.

In vivo studies

Women with no known pathology (normal breast group) who had requested reduction mammoplasty and women undergoing fibroadenoma removal (fibroadenoma group) were enrolled in the present *in vivo* study. 25 (12 from the normal breast group and 13 from the fibroadenoma group) among the 40 women initially enrolled, completed the study. The 15 others were excluded because of protocol irregularities. In the normal breast group, six patients received the NOMAC gel, and six received the placebo gel. In the fibroadenoma group, six applied the NOMAC gel and seven the placebo gel. Owing to the large interindividual variations, plasma E2, P and SHBG and tissue E2 levels did not vary significantly among the groups.

In situ apoptosis detection

Apoptosis was detected in breast sections using the TUNEL technique. The stromal cells of normal breast tissue or fibroadenoma did not differ significantly in their apoptosis levels, regardless of the treatment. The epithelial cells of NOMAC-treated normal breast exhibited significantly higher levels of apoptosis compared with their placebo-treated counterparts ($p < 0.01$) (Fig. 4). In fibroadenomas, the rate of epithelial cell apoptosis was higher than in normal breast, but did not vary according to the breast exposure to progestin (Fig. 4).

Discussion

These data demonstrate that stopping the administration of a progestin (like NOMAC) to normal epithelial mammary cells *in vitro* and *in vivo* induces a significant increase of cell

Figure 3: Effect of NOMAC withdrawal or continuous treatment on HMEC and T47D tumor cell apoptosis. Cells cultured as described under Materials and Methods were either untreated (*white columns*), treated continuously with NOMAC (*hatched columns*), or NOMAC was withdrawn (*black columns*).

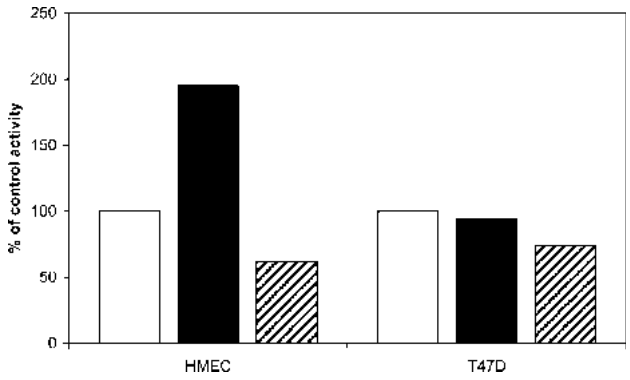
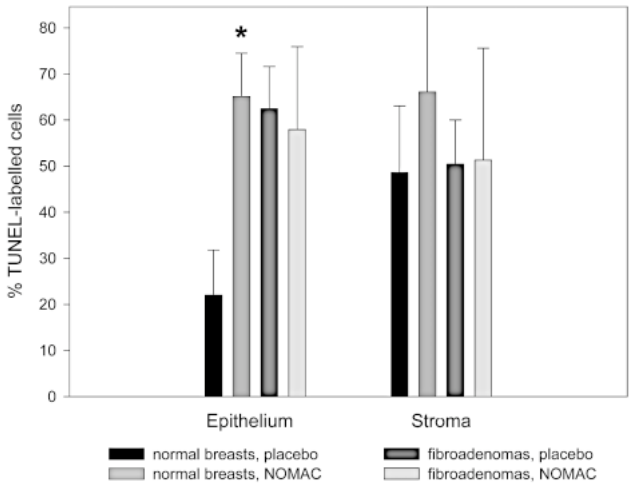


Figure 4: NOMAC percutaneous mammary treatment before reduction mammoplasty (normal breasts) induces a significant increase (*, $p < 0.05$) of apoptotic cell death percentage (TUNEL labelled) in normal breasts, compared with placebo percutaneous administration. No difference in apoptosis levels was observed in stromal cells (stroma) in normal breasts or in fibroadenoma, nor in epithelial cells (epithelium) in fibroadenoma, compared with placebo treatment.

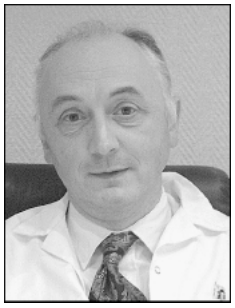


death by apoptosis. This phenomenon was demonstrated by two independent methods: the TUNEL labelling, which detects free 3′-end OH groups *in situ* in fragmented DNA, and the measurement of caspase-3 activity, which is the main enzyme implicated in the apoptosis. This apoptosis induction seems to be specific of normal cells, as shown by the absence of modulation of apoptosis by progestin in fibroadenomas *in vivo* and cancerous cells T-47D *in vitro*. These data suggest a dysregulation of apoptosis in benign and malignant tumoural cells.

Moreover, data *in vitro* suggest that a continuous administration of progestin may not modify apoptosis rates of normal epithelial mammary cells. This information could be interesting in the context of HRT, because a lack of apoptosis can lead to a tissular homoeostases dysfunction, leading to the emergence of benign or neoplastic diseases. Nevertheless, this interpretation must be qualified by the absence of specific clinical data confirming this hypothesis, and by the influence of other factors implicated in the modulation of the steroids effects on the epithelial mammary cell, like the extracellular matrix, the fibroblasts, the adipocytes, the lymphocytes and other endocrine factors.

Conclusion

The NOMAC withdrawal triggers *in vivo* and *in vitro* a peak of apoptosis in normal mammary epithelial cells, but does not in fibroadenoma nor in cancerous cells T-47D. This phenomenon could explain the peak of apoptosis observed on day 28 of menstrual cycle, after the fall of the progesterone seric level. The apoptosis in the breast would be the equivalent of the menstruations in the endometrium. Indeed, if the proliferative effect of estrogens alone remains discussed, our data show that a sufficient progesterone level maintained during a sufficient length of time and followed by a sudden fall of this level, triggers a peak of apoptosis. This could prevent the emergence of dysplasia and cancer. This hypothesis confirms the data of Allan et coll. [13], which have shown a reduction in apoptosis relative to mitosis in normal epithelium in the neighbourhood of fibrocystic changes and carcinoma in premenopausal human breast.



Prof. Dr. Jean-Michel Foidart

Professor Jean-Michel Foidart was born in Liège (Belgium) in 1949. He received his M.D. degree from the University of Liège in 1974 and passed the examination of the Educational Council for Foreign Medical Graduates successfully. From 1976 to 1979 he became a fellow at the Women's Clinic at John Hopkins Hospital (Baltimore, MD, USA). He became a Ph.D. in Cell Biology and Biochemistry in 1983. Upon his return in Belgium, he was appointed Chairman of the Department of Obstetrics and Gynecology at the University Clinic at Bois de l'Abbaye in Seraing, from 1985 to 1987. In 1988, he became Professor of Biology at the University of Liège, up to 1996 when he was appointed Professor of Obstetrics and Gynecology, and chairman of the Department of Obstetrics, Gynecology and Senology at the same University. He has been an invited lecturer at the Universities of Paris VII and Paris XI since 1994 and received several international teaching awards in Belgian, French and US Universities. He has received many honorary and scientific distinctions and organized more than 15 international congresses in the field of placentation, gynecology, and gynecologic oncology. He is a member of the editorial board of 8 different international peer-reviewed periodics and is a member of many national and international scientific committees, and a correspondant of the Belgian and French Academies of Medicine. His research concerns mainly experimental oncology. He is heading a laboratory with 30 scientists who are mainly conducting basic research in the field of proteases and angiogenesis during tumor development and metastases formation. His fellowship at the John Fogarty International Research Center from 1977 to 1978, then as "visiting associate" at the National Institutes of Health (Bethesda) and as "special postdoctoral fellow" at John Hopkins Hospital (Baltimore, USA), as well as other long term stays in Paris and at the University of Oulu (Finland) allowed him to have both a basic and a clinical training in the field of reproductive medicine.

He is the author and co-author of more than 300 publications with high impact factor (> 3). He is currently President of the Belgian Society of Gynecology.

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References:

1. Vogel PM, Georgiade NG, Fetter BF, Vogel FS, McCarty KS. The correlation of histologic changes in the human breast

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