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The effect of continuous combined 17β-oestradiol and dihydrodydrogesterone on apoptotic cell death and proliferation of human breast cancer cells in vitro

H.R. Franke[a,*], I. Vermes[b]

[a]*Department of Obstetrics and Gynecology, Medisch Spectrum, Twente Hospital Group, Enschede, The Netherlands*
[b]*Department of Clinical Chemistry, Medisch Spectrum, Twente Hospital Group, Enschede, The Netherlands*

1. Introduction

In vitro and *in vivo* studies showed that unopposed oestrogens induce proliferation while progestogens promote apoptosis in human breast epithelial and breast cancer cells [1]. The balance between programmed cell death (apoptosis) and cell proliferation determines the tumour growth rate and any alteration between the two factors may be a key element for the uncontrolled expansion of malignant tumours.

2. Material and methods

17β-Oestradiol and dihydrodydrogesterone, the major metabolite of dydrogesterone, were incubated in concentration of 10^{-6} M for 144 h *in vitro* under standard conditions in MCF-7 cells, an oestrogen receptor-positive human breast cancer cell line.

Apoptosis was measured using a DNA fragmentation assay according to a previously published protocol in Ref. [2]. Proliferation was determined by measuring the mRNA expression of Mucine 1 and Cyclin D1. Mucine 1 is a high molecular protein, present in glandular epithelial cells and overexpressed in malignant cells. Cyclin Dl controls cell-cycle progression by activating cyclin-dependent kinase partners and is overexpressed in over 50% of human breast cancer cells [3]. Both Mucine 1 and Cyclin Dl were measured by using the quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) technique.

* Corresponding author. Tel.: +31-534-872330; fax: +31-534-339571.

E-mail address: hfranke@xs4all.nl (H.R. Franke).

3. Results

Table 1 summarises the results of the apoptotic, and proliferation assays and the apoptotic/proliferation ratios.

3.1. Conclusions

Women previosuly treated for breast cancer should not use unopposed low dosages of oestrogens. However, epidemiological, laboratory and clinical studies suggest that certain combinations of oestrogens and progestogens are more likely to be beneficial than harmful [4]. In a retrospective study, lower risks of recurrences and mortality were found in women who used hormone replacement therapy (HRT) after breast cancer diag-

Table 1
The apoptosis, proliferation and apoptotic/proliferation ratios of MCF-7 cells incubated with unopposed 17β-oestradiol (E2) and the combination of 17β-oestradiol and dihydrodydrogesterone

After 144 h	E2 versus controls	E2 + DHD versus controls	E2 + DHD versus E2
Apoptosis	+192%	+367%	+60%
Proliferation			
Mucine 1	+80%	−33%	−50%
Cyclin D1	+80%	+50%	−17%
Apoptotic/proliferation ratio[a]			
Mucine 1	1.6	6.7	4.1
Cyclin D1	1.6	3.1	2.0

[a] A ratio >1 suggests tumour regression; the higher the ratio, the stronger the tumour regression.

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nosis than in women who did not [5]. Continuous combined 17β-oestradiol and dihydrodydrogesterone might reduce relapses of breast cancer (approximately 3–6-fold increase in the ratio of apoptosis/proliferation versus no HRT). Continuous combined 17β-oestradiol and dihydrodydrogesterone also seems to be the treatment of choice in hysterectomised women (approximately 2–4-fold increase in the apoptotic/proliferation ratio versus unopposed 17β-oestradiol).

Although *in vitro* results are not simply transferable, *in vivo* conditions, such as epidemiological studies, also indicated a decreased risk in the development of recurrences in breast cancer survivors using HRT.

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