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Review

Are the progestins responsible for breast cancer risk during hormone therapy in the postmenopause? Experimental vs. clinical data

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Abstract

Evidence is increasing suggesting that adding progestins to estrogen replacement therapy may be more harmful then beneficial, however it is debatable whether all progestins act equally on breast epithelial cells.

Experimental data with the comparison of various progestins in the same *in vitro* model present a rather high evidence that there may be differences between the various progestins regarding breast cancer risk. Especially of concern may be to differentiate between primary and secondary risk i.e. between benign and malignant breast epithelial cells.

The epidemiological studies and especially the Women's Health Initiative (WHI) trial, so far the only prospective placebo-controlled interventional study, demonstrate an increased risk under combined estrogen/progestin therapy, but they have the limitations that they up to now cannot discriminate between the various progestins mostly due to too small or not comparable patient numbers in the subgroups with the various progestins. However, there is evidence that the natural progesterone, possibly also the transdermal usage of synthetic progestins, may avoid an increased risk, but this must be proven in further clinical trials.

Keywords: Progestins; Breast cancer risk; Experimental data; Clinical data

Contents

The role of progestin addition to estrogen therapy in the postmenopause has come into scrutiny since the results of the WHI mono arm are published as compared to the WHI combined arm [1,2]. The WHI trial used the combination of conjugated equine estrogens plus medroxyprogesterone acetate (MPA). In contrast to the WHI combined arm, in the estrogen only arm no increase but rather a reduction of breast cancer risk was evaluated, which was significant for patients with more than 80% adherence to study medication. This result indicates a negative effect of progestins concerning breast cancer risk. However, the question remains still open, in as far the combination of estrogens with synthetic progestins as well as with natural progesterone may elicit the same increased risk. Thus, there remain many questions on the extrapolation of the WHI results to all synthetic progestins and to natural progesterone.

In the present short review, the available experimental and clinical data regarding progestin addition and breast cancer risk is summarized.

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1. Experimental data

For a long time, the prevailing opinion was that the addition of progestins to estrogen replacement therapy could reduce the breast cancer risk. This was attributed to *in vitro* data, where progestin addition could clearly reduce the proliferation of breast cancer cells.

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*. Limitations of *in vitro* study might be the high concentrations needed for an effective anti-proliferative effect. 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.

Thus *in vitro* experiments, although only conducted for a short time and with high pharmacological concentrations, can simulate special *in vivo* conditions. But comparisons should always be done in the same model, since cell culture conditions can have a strong influence on the results. However, *in vitro* experiments clearly cannot replace clinical studies, but they are very useful to evaluate mechanisms and to explore possible differences between substances (when tested in the same model), which then should be proved in clinical trials.

There are numerous experimental data available on the effect of progestins on the proliferation of normal and cancerous breast epithelial cells (e.g. [3–6]). Most data led to comparable results, however, only few experiments have been done with a higher number of progestins in the same cell model. Therefore we will focus here only on own experiments, in which we have compared six synthetic progestins and progesterone in the same cell model (Table 1) [7]. We investigated effects on proliferation as well as on apoptosis. In addition, a possible influence of the stroma was considered by including the most important stromal growth factors in the same model. To our knowledge, this probably important stromal influence was not incorporated in *in vitro* experiments so far.

1.1. Normal breast epithelial cells

MCF10A, a human, non-tumorigenic, estrogen and progesterone receptor-negative breast epithelial cell line was used for these experiments [8]. Progesterone (P), chlormadinone acetate (CMA), norethisterone (NET), medroxyprogesterone acetate (MPA), gestodene (GSD), 3-ketodesogestrel (KDG) and dienogest (DNG) were tested at the concentration range of 1 nM–1 μM. For stimulation of the MCF-10A cells, a mixture of growth factors was used. As outcome proliferation and apoptosis were measured and the ratio of apoptosis to proliferation was compared. Proliferation is quantified by measuring light emitted during the bioluminescence reaction of luciferene in the presence of ATP and luciferase. Apoptosis was measured by the Cell Death Assay, which is based on the quantitative sandwich-enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones. Photometric enzyme immunoassay quantitatively determines cytoplasmic histone-associated DNA fragments after induced cell death.

The combination of the stroma-derived growth factors epithelial growth factor (EGF), basic-fibroblastic growth factor (FGF) and insulin-like growth factor-I (IGF-I) alone confirmed a proliferative response compared to the assay medium-only control.

These growth factors were chosen, since they have been shown to be most effective in terms of breast epithelial cell proliferation [9].

In combination with growth factors, the ratio was reduced significantly compared to the growth factor alone by MPA and CMA (i.e., favouring an additional proliferative effect). MPA produced a four-fold reduction in the ratio in comparison to growth factors alone at 100 nM and 1 μM ($p < 0.05$), CMA had a significant effect at 1 μM only, reducing the ratio three-fold. P, NET, LNG, DNG, GSD and KDG had no significant effect on the growth factor-induced stimulation of MCF10A.

1.2. Cancerous breast epithelial cells

HCC1500, a human estrogen and progesterone receptor-positive primary breast cancer cell line was used [10]. For stimulation of the cells estradiol alone, a growth factor mixture alone as well as a combination of both was used.

Table 1
Effect of various progestins on the ratio of apoptosis to proliferation in normal cancerous breast epithelial cells in the presence of stroma-derived growth factors or estradiol as stimulans

Progestin	Normal cells (growth factors)	Cancerous cells		
		Growth factors	Estradiol	Growth factors + estradiol
Progesterone	Ø	+	+	+
Medroxyprogesterone acetate	–	++	++	++
Chlormadinone acetate	–	++	++	++
Norethisterone	Ø	–	++	++
Levonorgestrel	Ø	–	++	++
3-Keto-desogestrel	Ø	–	Ø	++
Gestodene	Ø	–	++	++
Dienogest	Ø	–	+	Ø

+, increase; −, decrease of the ratio; Ø, no effect as compared to the stimulans alone.

The combination of the growth factors EGF, FGF and IGF-I alone confirmed a proliferative response compared to the assay medium-only control. MPA in combination with growth factors caused a significant increase in the ratio of apoptosis to proliferation at both concentrations compared to growth factors alone ($p < 0.05$), the greatest effect being at 100 nM, with a doubling of this ratio, i.e., an inhibitory effect. CMA also caused a significant increase in this ratio, with the greatest effect seen at 1 μM, yielding over a two-fold ratio increase. Conversely, NET, LNG, and DNG at both concentrations and GSD and KDG at 1 μM led to a significant reduction in the ratio of apoptosis to proliferation, enhancing the initial proliferative effect induced by the growth factors. P had no significant effect at either concentration.

The results of the combination of the steroids and E2 on the estrogen-receptor positive (ER+) HCC1500 cells showed that the progestins CMA, MPA, NET, LNG, DNG, GSD and P significantly increased the ratio of apoptosis to proliferation towards an anti-proliferative effect to varying degrees compared to E2 alone, with MPA having the greatest effect, followed by NET. KDG had no significant effect at either concentration. No progestin used was able to further enhance the stimulatory effect of E2 on HCC1500 cells, and all but KDG actually inhibited this effect.

The results of combining the steroids with the combination of growth factors (EGF, FGF and IGF-I) and E2 on HCC1500 cells revealed that MPA, GSD, CMA and NET all increased the ratio favouring an anti-proliferative effect compared to the proliferative effect of growth factors and E2 alone. P, LNG, DNG and KDG had no significant effect at either concentration.

In summary, these results indicate that progestins are different in their ability to induce proliferation or inhibit the growth of benign or malignant human breast epithelial cells dependently or independently of the effects of stromal growth factors and E2. Thus, on the basis of experimental data the choice of progestin for hormone therapy may be important in terms of influencing a possible breast cancer risk.

A further important result from our experimental research seems to be the fact that the influence of the progestins can differ largely between normal and cancerous breast epithelial cells. This would have clinical relevance for the use of HRT after breast cancer, which is of course contraindicated in routine therapy. But as even in the normal population women express malignant cells, shown by post mortem analyses [11], different, may be contrary progestins effects in benign or malignant cells may have relevance for the primary breast cancer risk of postmenopausal women treated with HRT. Therefore, this field should be further investigated.

2. Clinical data

The most important epidemiological studies since 1999 investigating the effect of progestin addition to estrogen replacement therapy in terms of the primary risk of breast cancer are summarized in Table 2, depicting relative risks or odds ratios with 95% confidence intervals for sequential as well as continuous combined therapy, duration of hormone treatment and use

Table 2
Epidemiological studies on breast cancer risk during estrogen plus progestin therapy

Reference	Duration	Relative risk or odds ratio (95% CI)					
		Estrogen/progestin	Seq.	Cont.	MPA	Others	
Schairer et al. [12]	<4 Years, >4 years		1.1 (0.8–1.7), 1.5 (1.0–2.4)		+	–	
Ross et al. [13]	5 Years	1.24 (1.07–1.45)	1.38 (1.13–2.68)	1.09 (0.88–1.30)	+	–	
Chen et al. [14]	>5 Years	1.49 (1.29–1.74)			+	–	
Newcomb et al. [15]	>5 Years	1.58 (1.16–2.15)			+	–	
Weiss et al. [16]	>5 Years	1.37 (1.06–1.77)	1.00 (0.69–1.46)	1.54 (1.10–2.17)	+	–	
Porch et al. [17]	<5 Years, >5 years	1.11 (0.81–1.52), 1.76 (1.29–2.39)			+	–	
HERS [18]	6.8 Years	1.27 (0.84–1.94)			+	–	
WHI [1]	>6 Years	1.26 (1.00–1.59)			+	–	
Li et al. [19]	>15 Years	2.0 (1.3–3.3)	2.9 (1.3–6.6)	1.8 (1.0–3.3)	+	–	
WHI [2]	5.6 Years	1.24 (1.01–1.54)			+	–	
Lee et al. [21]	Current users	1.29 (1.23–1.35)			+	–	
Persson et al. [22]	1–6 Years, >6 years	1.4 (0.9–2.3), 1.7 (1.1–2.6)			–	+	
Magnusson et al. [23]	Ever	1.63 (1.37–1.94)	1.48 (1.08–2.04)	1.41 (1.09–1.83)	–	+	
MWS [24]	Current user	2.0 (1.88–2.12)			–	+	
De Lignieres et al. [25]	>5 Years			0.98 (0.65–1.5)	–	+	
Olsson et al. [26]	>4 Years		2.23 (0.90–5.56)	4.60 (2.38–8.84)	–	+	
Jernström et al. [27]	5 Years			3.3 (1.9–5.6)	–	+	
Stahlberg et al. [28]	6 Years	2.7 (1.96–3.73)			–	+	
Fournier et al. [29]	7 Years	1.69 (1.5–1.91), (synthetic progestins), 1.0 (0.83–1.22), (progesterone), 1.16 (0.94–1.43), (dydrogesterone)			–	+	

of MPA (by far the most used progestin in HRT) compared to other progestins [12–29].

In most studies using MPA as progestin (upper part of the table), the risk was significantly increased. In the WHI trial, up to now the only prospective, randomized interventional study, the final calculation showed a significant risk increase with an odds ratio of 1.24 (CI 1.01–1.54) for a duration treatment of 5.6 years [20].

In the lower part of the table, studies are summarized, which were mainly conducted in Europe where mostly other progestins than MPA have been used. As with MPA also with other progestins, an increased breast cancer risks was seen. Overall no relevant differences between the relative risk calculations for other progestins compared to MPA were observed. The available data also do not allow to differentiate between further progestins. Often details on the dosage and duration, and even of the type of the applied progestin are missing.

Comparing the studies, which differentiate between sequential and continuous combined hormone therapy, no conclusive data were found. However, it seems that continuous combined hormone therapy may increase breast cancer risk more stronger. As can be seen in Table 2, the breast cancer risk was enhanced by relative risks or odds ratios between 1.11 and 2.7 but often with wide confidence intervals. According to these trials, it is proven that the combination of estrogen with progestin increases breast cancer risk when applied for 4–5 years.

In terms of the difficulty to differentiate between the various progestins in analyzing epidemiological studies we like to give an example with the study of Magnusson et al. [23], who investigated the effect of ERT and HRT comparing MPA or NETA. In this population-based case–control study, 3.345 women with breast cancer in the hormone group and 3.454 women with breast cancer in the control group were included. The final statistical calculation was done with 663 and 495 cases. A significant breast cancer risk increase was found for NETA users but not for MPA users. However, case number for HRT using MPA was only a tenth of that of NETA users and in the treatment group using ERT plus MPA for more than 5 years only five cases were included in the statistical calculation. Stratifying for sequential and continuous combined estrogen/NETA therapy did not reveal any significant differences.

The largest (but by no means the best) observational study with risk assessments during HRT is the Million Women Study (MWS), a non-randomized population-based cross-sectional study (with prospective control of therapy for 1% of the recruited patients evaluating breast cancer risk). Different progestins have been evaluated (MPA, norethisterone, norgestrel, levonorgestrel), and no significant differences have been found. However, the MWS had major methodological flaws, which should be considered when referring to this study [30]. The most obvious limitation in the design of the study is that the exposure data were collected at the time the women were recruited to the study rather than when the cancer was diagnosed (or the study terminated). Recruitment could have been up to 6 years prior to the diagnosis of cancer (or the end of the study). So many women can have changed types, doses and regimens during and before enrolment in the study. In addition, women who attend for routine mammography may not be representative of the population at large.

Of special remark are two cohort studies [25,29] using micronized progesterone for combination with estrogens, which showed no increase in breast cancer risk when combining transdermal (patches) or percutaneous (gels) estradiol therapy with progesterone.

In the first study, including 3.175 French women, with transdermal estradiol combined with micronized progesterone no significant effect on the risk of breast cancer after a mean duration of 9 years of HRT was observed [25]. In the second cohort study [29] including 80.377 women, an increase of breast cancer risk with oral synthetic progestins (1.69, 95% CI 1.50–1.91), but not with progesterone (1.0; 95% CI 0.83–1.22) and dydrogesterone (1.16; 95%CI 0.94–1.43) was found. The mean duration of HRT use was 7 years. This study had a mean follow-up of 8.1 years.

A reason for these special findings could be that progesterone metabolism may be different to that of synthetic progestins. Wiebe et al. [31] demonstrated that progesterone metabolism in normal breast tissues favours metabolites which may have anticarcinogenic properties. However, they also suggested that there might be metabolites enhancing breast cell proliferation and since progesterone metabolism is individually very differently, these findings need further investigation.

Whether there are differences in the risk potential between oral and transdermal progestin replacement (e.g. using combi-patches) was, to our knowledge, as yet not investigated or not published so far. Now in an abstract a first information is available: In one of the largest population-based case/control studies, from the UK General Practice Research Database (2.4 million women), it was shown that an increased breast cancer risk was found only for oral combined preparations but not for combi-patches, i.e. complete transdermal estrogen/progestin administration [32].

3. Conclusion

Experimental data with the comparison of various progestins in the same *in vitro* model present rather high evidence that there may be differences between the various progestins regarding breast cancer risk. Especially of concern may be to differentiate between primary and secondary risk i.e. between benign and malignant breast epithelial cells. This differentiation seems to be important for the progestin MPA. Since even in "clinical healthy" women malignant cells can be expressed, this experimental finding may have relevance and should be further investigated.

The epidemiological studies and especially the WHI trial, so far the only prospective placebo-controlled interventional study, demonstrate an increased risk under combined estrogen/progestin therapy, but they have the limitations that they up to now cannot discriminate between the various progestins mostly due to too small or not comparable patient numbers in the subgroups with the various progestins. However, there is evidence that the natural progesterone, possibly also the transdermal usage of synthetic progestins, may avoid an

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