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Progestins and risk of breast cancer. Much ado about nothing?

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Summary

In vivo and in vitro studies; epidemiological and statistical publications concerning a possible carcinogenic effect of progesterone – and progestins – raise a question mark. Too many contradictions exist in experimental and clinical studies. A very small increased risk of breast cancer with estrogen therapy has been shown; however bias are often present.

It is not clearly demonstrated that the adjunction of progestin to estrogen significantly increases the risk, nor that it brings about a protective effect. Some recent works seem to indicate that continuous estroprogestin replacement therapy might be deleterious, while a sequential regimen would be not. This is to be confirmed. The results of the Women's Health Initiative Study have been analyzed. The data and conclusions of the medical literature should be interpreted very cautiously.

Key words: Progestins; Breast cancer.

Introduction

When Butenandt discovered the crystallized constituant of progesterone in 1934, could he have imagined what Pandera's Box was open for the future?

Successively adulated and vilipended, progesterone has induced many hard debates. In this article, the aim was to study the present problem of its action on mammary tissue, a privileged target, concerning carcinogenesis.

The problem is clearly set: Have progesterone (P) and progestins (PS) an incidence on the relative risk (RR) of breast cancer (BC): And if yes, would the effect be protective or deleterious?

It must be said that P and various PS have their own characteristics and that it would be dangerous to considerer that all of them have the same action.

It should be noted that estrogens, and in particular estradiol (E2), have a proliferative effect on mammary tissue, increasing tubular and lobular mitoses.

As far as P is concerned, there are two opposed positions: some claim that P has an inhibiting, antiproliferative effect; others assert, on the contrary, that E2 + P have a synergic effect on mammary epithelial proliferation [13, 24].

In vivo studies

Potten [27] studied the influence of age and menstrual cycle on the proliferative activity of intralobular epithelium of the normal breast, using tritiated thymidin; the action was higher at *21.5 days and lower at *7.5 days.

In the same way, Anderson *et al.* [1] observed a peak in proliferative activity at the level of mitoses in the second part of the cycle. The most important variations of this activity were noted in young and nulliparous women. The effect may be due to P alone or to a synergic E2 + P activity, and also to paracrine mechanisms.

On the contrary, Barrat [3] studied the mitotic activity following ten days of treatment with either placebo or with E2 or P in various benign breast diseases. After P treatment, mitotic activity was lower than after E2 or placebo.

Changes in estradiol and progesterone receptors (ERs, PRs) have been analyzed in normal mammary tissue [34]. ERs are detected more frequently in samples obtained in the follicular (68%) than in the luteal (32%)

phase. However, the rate of women with detectable PRs was the same during the two phases (80%). Thus, evolution of PRs is different in mammary and endometrial tissues.

Finally, a Manchester School (Christie Hospital) studied normal human mammary tissue put under the skin of nude mice. E2 and P were injected in doses similar to what is observed during the female menstrual cycle. The conclusion was clear: E2 had a proliferative effect, but the addition of P had no particular, positive or negative effect [16], confirming the hypothesis that P has a neutral effect on a primed E2 mammary target.

We will study further the problem of P and PS and apoptosis.

In vitro studies

Welsch *et al.* [37] has shown, in samples of human mammary tissues in cultures from 2 to 8 days, that the administration of P only had no effect. P decreased induced E2 proliferation when given together.

Gompel [12] found similar results with epithelial cells in culture.

However, Longman [20] has shown that estrogen and estro-progestin associations stimulated mammary cells and particularly malignant cells, while PS had no effect on normal cells and clearly stimulated cancerous cells.

Vignon *et al.* [36] however demonstrated in T47D cells which are PR rich that there was an antagonistic effect of PS versus E2 on cellular proliferation and protein synthesis; Botella's [5] research showed similar results.

It is mandatory to quote recent researches which have been performed on a molecular scale.

Musgrove [22] has shown on cultures of tissue using human cancerous mammary cells, that on one side estrogens were mitogenic while on the other side PS had a biphasic action; transitory acceleration during the G1 phase, before a specific stop-induced PS, in the same G1 phase. The modifications are induced by the D1 cyclin protein (with an increase of kinase activity and phosphorylation).

In spite of the indisputable interest, results of studies in vitro cannot be assimilable without any regard to observations in vivo: cultures of cells and tissues are artificial, mediums and concentrations of substances are special and various, etc.

It should be noted that:

– the action of P alone, the action of estrogens + P on mammary tissue, and a sequential or continuous administration of E2 + P do not have the same results;

– the effects of steroid hormones are different in normal, benign, or malignant mammary tissue.

Thus, we can ascertain that there is a great relativity as far as P and PG are concerned in mammary tissue.

What are the data provided *by epidemiology*?

Hormonal contraception

We are not aware of any study charging PG concerning the BC risk in women with a benign breast disease. As for progestin-only contraception, Skegg [33] showed in a case-control study where women had received an oral pill, levonorgestrel 0.03 mg or norethisterone 0.35 mg, that the RR was not increased: 1.1 (0.73-1.5). However he observed an increased risk for contraception started before 34 years old and taken during the ten years preceding the BC. The risk however decreased if PS had been used more than ten years before.

Paul *et al.* [23] observed similar results with injectable progestins (medroxyprogesterone depot): risk increased in women 25-34 years old who used contraception for more than six years.

Nevertheless, everyone remembers the famous story of the wretched Beagle bitches [35] and the condamnation of chlormadinone and MPA by the Food and Drug Administration. However, one cannot assimilate human beings and experimental results. These beagles frequently had spontaneous mammary tumours, and metabolism of progestins is quite different in women and beagles.

On the other hand, Selman *et al.* [32] found that MPA induced a stimulation of secretion of GH and IGF1 in bitches.

Pike *et als.* demonstration [25], according to which estroprogestative compounds that were rich in P increased the RR of BC cannot be accepted. His classification concerning the power of progestins was established on an out of date test: the delay to menstruation.

Let us quote the paper of Plu-Bureau *et al.* [26] on women with benign breast disease. The authors found a protective effect for 19 nortestosterone derivatives, but not for pregnane or norpregnane compounds: however, this was not a randomized study and this point removes a great part of its credibility.

Hormonal replacement therapy in menopause (HRT)

The following subject has to be discussed: Does the addition of PG to estrogens induce an increase of RR of BC?

Let us first quote two studies:

– one concerns the macacus cynomolgus monkey (macaca fascicularis) which shows that animals receiving premarin + MPA (medroxyprogesterone acetate) had mammary hyperplasia more frequently than those receiving placebo or premarin alone [6];

– the other one concerns the results of mammary biopsies in postmenopausal women receiving estradiol or estradiol + MPA or no treatment at all.

An increase of mammary epithelial density was also found for E2 + MPA [15].

A deduction is that ducto-lobular activity is higher with the estro-progestin association, but does that fact have a significant meaning concerning a possible incidence on BC?

Berkvist *et al.* [4] found an increase of RR of BC when progestins were added to estrogens, but his results were at the limit of significance because the number of cases was very small and because it appeared that the duration of administration of the progestin was too short in each cycle.

On the contrary, Gambrell [11] found a protective effect due to progestins, but his methodology was criticized.

Colditz *et al.* [7] did show a significant difference concerning BC risk with estrogen alone and estrogen + progestins [1, 41].

Colditz and Rosner performed a more complete study from the Nurses' Health Study with a follow-up of 16 years (980,000 women) which was more valuable: E2 alone RR = 1.11 vs E2 + PG = 1.58 [8].

Recent publications have been disturbing:

Schairer *et al.* [31] published the results of a cohort study followed from 1980 to 1995 concerning 46,355 menopausal women. When one carefully examines the curves, in spite of the growing slopes, they appear to have a weak value: The confidence intervals (CIs) are large and most often include unity as far as estrogens or estrogens + progestins are concerned.

For the last regimen, the gradient is more marked but CIs are very large. The reason is that only 4% of patients received the association, thus the value is questionable. Moreover the dosage and menstrual duration of PG are not mentioned.

The publication of Magnusson *et al.* [21] leads to similar conclusions. The authors tried to distinguish pregnane and nortestosterone compounds: only the latter ones were responsible for an increase of the RR of BC. However this was a case control study with possible bias.

In these two last studies, the weight parameter was underlined: An increase of RR occurred especially in lean women: measurement was by the Body Mass Index or Quetelet's index with limits, which can be fixed arbitrarily.

In fact, in the cited studies, bias are possible and may have induced false results.

– Bias towards recruitment of patients for HRT: Why does the great majority receive estrogens only and a small proportion estrogens + progestins?

– Mainly there are bias of follow-up: patients receiving HRT are indisputably more carefully followed. They have more clinical examinations, more mammograms, more breast biopsies, etc. Colditz *et als.* attempt to help with *equalization* does not change the reality of the problem.

– Other parameters may be of paramount importance, e.g. alcohol [14].

Thus, until 2001 it appeared mandatory that a prospective randomized study be undertaken and published.

Another problem is to clarify if the association of E2 + PS has to be given sequentially (reproducing the hormonal profile of the menstrual cycle) or simultaneously without interruption or with a periodic interruption (treatment of 25-26 days/month).

According to various works, mammary density, evaluated by mammograms, is higher for E2 + PS than for estrogens alone. This is in agreement with experimental data cited at the beginning of this article. It would also seem that a periodic interruption is preferable to continuous administration.

The work of Foidart's team [10] concerning apoptises of mammary cells occurring 24 to 48 hours after stopping nomegestrol acetate is in agreement, since apoptosis plays an antagonistic role vs cellular proliferation, allowing a favorable physiological balance.

However, the real effect of a cycle with a few days interruption of HRT is not known and no statistical study assesses its value.

Conversely, Ross *et al.* [29] published a study which seems to demonstrate that the RR of BC would be higher for sequential than for continuous treatments, but the results are at the limit of significance.

Finally, mammary density is presently a field of interest. Confirming the first work of Wolfe [38] it clearly appears that a dense constitution of mammary glands exhibits an increased risk of BC compared to a weakly dense one.

Nevertheless, one must distinguish constitutional and induced HRT density. Indisputably, HRT increases mammary density in a non negligible rate of cases; but iatrogenic density is not similar to genetic density. The increase occurs rapidly and also disappears rapidly, two or three weeks after stopping HRT.

It should be noted that water retention and hypervascularisation play a paramount role [2]. It can be said that, in spite of what has been claimed, HRT mammary density cannot be accepted as an index of risk of BC.

Now, let us mention the specificity of various progestins.

The power of a progestative compound is proportional to the intensity of its binding to progesterone receptors.

But other ways have been discovered:

– Jordan *et al.* [17] using MCF7 cells, showed that some progestins, products of nortestosterone, stimulated proliferation by the pathway of estrogen receptors.

– Poulin *et al.* [28] using ZR751 cells, showed that progestins could act in the way of androgen receptors.

– Coletta *et al.* [9] showed that gestodene induced a very important increase of secretion of TGFβ in T47D cells.

It is known that TGFβ is a powerful inhibitor of epithelial cell growth.

– P. Selman *et al.* [32] showed that MPA induced a stimulation in secretion of GH and IGF1 in the female dog (cf *supra*).

Finally, the recent discovery of two isoforms, A and B, of progesterone receptors should be discussed.

It is not impossible, though not demonstrated today that, like for estradiol receptors α and β, this duality may play a role in the endocrine mechanism of mammary cancerogenesis.

Recently a study was published in JAMA (2002, 288, 321-33): This WHI study (Women's Health Initiative) is entitled: "Risks and benefits of estrogen plus progestin in healthy postmenopausal women". Principal results from the Women's Health Initiative – randomized controlled study. It has induced several problems and questions among scientists and the medical world, strongly amplified by the media.

What are the data?

16,608 non-hysterectomized menopausal women (50-79 years old) were randomized.

2 arms	8,506	Premarin 0.625 mg/day MPA 2.5 mg/day	one pill a day
	8,102	Placebo	

The "bomb" was the premature stop of this trial at 5.2 years of study (in fact, it was scheduled for 8.5 years).

Herein, the analysis will be focused on BC: 290 cases of invasive BC were registered with RR = 1.26 (1.00-1.89). The increase in HRT patients was 26%. In fact, in absolute values, the increase is "very small" (G. Colditz *et al.*): +8 yrs./10,000 women.

It confirms the results of Schairer *et al.* [31] and those of Magnusson *et al.* [21], but the WHI study was a double-blind randomized study.

However, there are two questions:

1) How was the randomization performed? - 8,506 for the HRT vs 8,102 for the placebo (thus a difference of 404 cases = 2.5%).

2) The nominal risk was significant; the adjusted risk was not significant. Moreover, the following reservations should be expressed:

1) This work only concerns the formulation of premarin 0.625 mg + MPA 2.5 mg/day. It is not known what could be the results of

– other dosages (lower doses for example);

– the administration of a natural estrogen (estradiol) and another progestin (progesterone, norpregnane).

2) Even if the results were significant, it seems that they were "limited". C.I.: from 1 to 1.59 – from an individual point of view the risk is weak.

3) What is particular is the early arrival of BC during HRT, i.e. ≤ 4 years. In these conditions, one could logically *incriminate a promotion effect*, revealing an occult tumoral lesion.

4) However, no increase of in situ cancers was observed.

5) The increase is particularly clear for women who received HRT *before* entering the randomized study.

6) Can "healthy women" be considered?

– 1/3 of them were obsese - BMI;

– many were hypertensive and received specific blood pressure treatment;

– the mean age was 63 years old - thus, older than in French HRT women.

7) Last, but not least, only for patients given estrogen (hysterectomized women) was there no apparent increase, and the study continues for them.

8) It should be noted that other studies on iatrogenic mammary proliferation have concerned, as a matter of fact, the premarin + MPA association:

– the Söderquist team in cynomolgus monkeys [34];

– the Hofseth team in menopausal women (cf *supra*) [15].

Conclusions

It is admitted that, if there is an increase of RR of BC with estrogenotherapy, the risk is really very small [30] and numerous bias lead to some doubt.

In France, it is mandatory to associate progestins with estrogens to prevent endometrial hyperplasia and the risk of endometrial cancer. However, hysterectomized women can receive estrogens alone, without progestins.

The WHI randomized study confirms the previous published results.

The hypothesis of a selective action of progestins concerning the histological type of BC has been raised.

According to a Seattle team [19], the increase of BC would be due essentially to the increase of invasive lobular carcinoma (10-fold less frequent than invasive canalar carcinoma). However the number of cases is small and the results are at the limit of significance.

Thus, considering the very numerous publications which often are contradictory, considering the diametrically opposite theories and the possible bias; considering that results are frequently at the limit of significance; considering that one must not confuse the percentages and the absolute values as Santen *et al.* clearly explained [30], we believe that one must take a critical and cautious position. Therefore, with a question mark, the title of the famous comedy by Shakespeare is quoted "Much ado about nothing" (?).

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