

American Society for Reproductive Medicine Menopausal MEDICINE

Volume 14, Number 1, 2006

FOR CLINICIANS WHO PROVIDE CARE FOR WOMEN

The Role of Progestogens in Breast Cancer: Experimental vs. Clinical Data

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The role of progestogen addition to estrogen therapy in the postmenopause has come into scrutiny since the results of the WHI estrogen only arm have been published as compared to the WHI combined arm (estrogen plus progestin) (1,2). In contrast to the WHI combined arm, in the estrogen only arm no increase but rather a reduction of breast cancer risk was present, which was significant for patients with more than 80% adherence to study medication. These results indicate a negative effect of progestogens concerning breast cancer risk. However, the question remains, the combination of estrogens with syn-

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thetic progestins, as well as with natural progesterone may elicit the same increased risk.

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

Experimental Data

For a long time, the prevailing opinion was that the addition of progestogens to estrogen replacement therapy could reduce breast cancer risk. This was attributed to in vitro data, where progestogen addition clearly reduced 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; and 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 antiproliferative effect. However, higher concentrations may

FROM THE EDITOR

David F. Archer, M.D.

The interactions between hormones and target tissue have been of significant interest for many years. Nothing is more relevant than the role of exogenous estrogen and/or a progestogen and the incidence of breast cancer in postmenopausal women. There is voluminous literature of the hormonal effects on the female breast, both in vitro and in vivo, and in numerous animal studies, not to mention the human. Recent attention has focused on the role of exogenous hormones in postmenopausal women as risk factors for breast cancer. This is certainly due to the Women's Health Initiative, which found an increased hazard ratio for invasive breast cancer in the estrogen plus progestin arm, but a decreased hazard ratio for breast cancer in the estrogen-only arm. A possible reason for this discrepancy could be the progestin.

Drs. A. O. Mueck and H. Seeger address the role of progestins in breast cancer, to clarify the in vitro effects compared to the clinical effects. Whether or not there is a direct link between the use of a progestin and the induction of a breast neoplasm remains elusive. What is apparent is that there are multiple questions remaining regarding the progestin and the overall outcome of both in vitro and in vivo

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A Newsletter of the
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The ASRM is pleased to acknowledge the generous contribution of Wyeth Pharmaceuticals toward the publication of this newsletter.

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be required in vitro in short-time tests in which the reaction threshold can only be achieved with supra-physiological 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. Most data indicate comparable results; however, only a few experiments have been done with a higher number of different progestins in the same cell model. Therefore, we will focus here only on our own experiments, in which we have compared six synthetic progestins and progesterone in the same cell model (Table 1). 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 has not been incorporated into in vitro experiments to date.

Normal Breast Epithelial Cells
MCF10A, a human, non-tumorigenic, estrogen and progesterone receptor-negative breast epithelial

cell line was used for these experiments (3). 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 to 1 μ M. 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.

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. In combination with growth factors, the ratio was reduced significantly compared to the growth factor alone by MPA and CMA (i.e., favoring an additional proliferative effect). MPA produced a four-fold reduction in the ratio in comparison to growth factors alone at 100 nM and 1 μ M ($p < 0.05$), and CMA had a significant effect at 1 μ M only, reducing the ratio 3-fold. P, NET, LNG, DNG, GSD and KDG had no significant effect on the growth factor-induced stimulation of MCF10A.

Cancerous Breast Epithelial Cells
HCC1500, a human estrogen and progesterone receptor-positive primary breast cancer cell line was used. For stimulation of the cells estradiol alone, a growth factor mixture alone, and a combination of both was used. 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 at both concentrations compared to

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. (+ = increase; - = decrease of the ratio; Ø = no effect as compared to the stimulans alone)

Progestin	Normal cells	Cancerous cells		
	Growth factors	Growth factors	Estradiol	Growth factors + Estradiol
Progesterone	Ø	+	+	+
MPA	--	++	++	++
CMA	--	++	++	++
Norethisterone	Ø	--	++	++
Levonorgestrel	Ø	--	++	++
Desogestrel	Ø	--	Ø	++
Gestodene	Ø	-	++	++
Dienogest	Ø	--	+	Ø

growth factors alone ($p < 0.05$), the greatest effect being at 100 nM, with a doubling of the ratio, i.e., an inhibitory effect. CMA also caused a significant increase in the ratio, with the greatest effect seen at 1 µM, yielding over a 2-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, 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 progestogens CMA, MPA, NET, LNG, DNG, GSD and P significantly increased the ratio 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 progestogen 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 favoring 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 progestogens 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 progestogen 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 progestogens 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 (4), different, perhaps contrary progestogen 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.

Clinical Data

The most important epidemiological studies since 1999, investigating the effect of progestogen 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 of MPA (by far the most used progestogen in HRT) compared to other progestogens.

In most studies using MPA as progestin (upper part of the table), the risk was significantly increased. In the WHI trial, to date, 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 of 5.6 years (13).

In the lower part of the table studies are summarized, which were mainly conducted in Europe where mostly progestins other than MPA have been used. As with MPA, also with other progestins, an increased breast cancer risk 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 differentiation between further progestogens, perhaps with the exception of the natural progesterone. Often, details on the dosage and duration, and even 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 more strongly increase breast cancer risk. 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 four to five years.

In terms of the difficulty to differentiate between the various progestogens in analyzing epidemiological studies, we like to give an example with the study that investi-

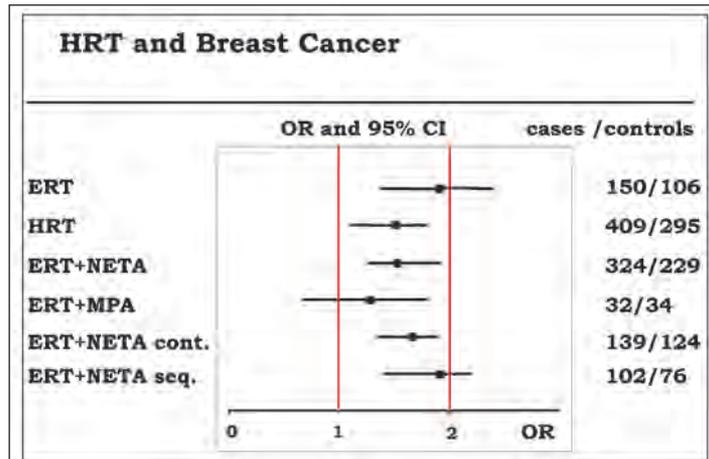


Figure 1: Influence of hormone replacement therapy on breast cancer risk in postmenopausal women according to Ref. 16. (ERT: estrogen replacement therapy=estrogen-only therapy; HRT: hormone replacement therapy=combined estrogen plus progestin therapy; NETA: norethisterone acetate; MPA: medroxyprogesterone acetate; OR: odds ratio)

gated the effect of ERT and HRT comparing MPA or NETA (16). 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. As is shown in Figure 1, a significant breast cancer risk increase was found for NETA users, but not for MPA users. However, case numbers 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 five 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 (23).

Of special note are two cohort studies (18,22) using micronized progesterone for the 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 (18). In the second cohort study (22), including 54,548 women, an increase of breast cancer risk with oral synthetic progestins (1.4, 95% CI, 1.2-1.7), but not with progesterone (0.9; 95% CI 0.7-1.2) was found (22). However, the mean duration of HRT use was only 2.8 years. This study had a follow-up of 2 years including about 10,000 additional postmenopausal women. In this extended study, the relative risks of the first evaluation have been confirmed which indicates that progesterone added to transdermal estradiol may be safer than oral synthetic progestins added to transdermal or oral estrogens (24).

A reason for these special findings could be that progesterone metabolism may be different from that of synthetic progestins. One study demonstrated that progesterone metabolism in normal breast tissues favors metabolites, which may have anti-carcinogenic properties (25). However, they also suggested that there might be metabolites enhancing

Table 2: Epidemiological studies on breast cancer risk during estrogen plus progestin therapy						
First author, year	duration	Relative risk or odds ratio (95% CI)		cont.	MPA	Others
		Estrogen/Progestin	seq.			
Schairer, 2000 ⁵	< 4 years > 4 years		1.1 (0.8-1.7) 1.5 (1.0-2.4)		+	-
Ross, 2000 ⁶	5 years	1.24 (1.07-1.45)	1.38 (1.13-2.68)	1.09 (0.88-1.30)	+	-
Chen, 2002 ⁷	> 5 years	1.49 (1.29-1.74)			+	-
Newcomb, 2002 ⁸	> 5 years	1.58 (1.16-2.15)			+	-
Weiss, 2002 ⁹	> 5 years	1.37 (1.06-1.77)	1.00 (0.69-1.46)	1.54 (1.10-2.17)	+	-
Porch, 2002 ¹⁰	< 5 years > 5 years	1.11 (0.81-1.52) 1.76 (1.29-2.39)			+	-
HERS, 2002 ¹¹	6.8 years	1.27 (0.84-1.94)			+	-
WHI, 2002 ¹	> 6 years	1.26 (1.00-1.59)			+	-
Li, 2003 ¹²	> 15 years	2.0 (1.3-3.3)	2.9 (1.3-6.6)	1.8 (1.0-3.3)	+	-
WHI, 2003 ¹³	5.6 years	1.24 (1.01-1.54)			+	-
Lee, 2006 ¹⁴	Current users	1.29 (1.23-1.35)			+	-
Persson, 1999 ¹⁵	1-6 years > 6 years	1.4 (0.9-2.3) 1.7 (1.1-2.6)			-	+
Magnusson, 1999 ¹⁶	Ever	1.63 (1.37-1.94)	1.48 (1.08-2.04)	1.41 (1.09-1.83)	-	+
MWS, 2003 ¹⁷	Current user	2.0 (1.88-2.12)			-	+
De Lignieres, 2002 ¹⁸	> 5 years			0.98 (0.65-1.5)	-	+
Olsson, 2003 ¹⁹	> 4 years		2.23 (0.90-5.56)	4.60 (2.38-8.84)	-	+
Jernström, 2003 ²⁰	5 years			3.3 (1.9-5.6)	-	+
Stahlberg, 2004 ²¹	6 years	2.7 (1.96-3.73)			-	+
Fournier, 2005 ²²	5.8 years	1.4 (1.2-1.7) (synthetic progestins) 0.9 (0.7-1.2) (progesterone)			-	+

breast cell proliferation; and since progesterone metabolism is very different between individuals, 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, not yet investigated nor published. Now, in an abstract, initial information is available. In one of the largest population-based case/control studies, from the U.K.

General Practice Research Database (2.4 million women), it was shown that an increase breast cancer risk was found only for oral combined preparations, but not for combi-patches, i.e. complete transdermal estrogen/progestin administration (26).

Conclusion

Experimental data comparing various progestogens in the same in vitro model present strong evidence that there may be differences

between the various progestogens with regard to breast cancer risk. Especially of concern may be the differentiation between primary and secondary risks, 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, to date, cannot discriminate between the various progestins, mostly due to too small or incomparable patient numbers in the subgroups with the various progestins. However, there is evidence that natural progesterone, and possibly also the transdermal usage of synthetic progestins, may avoid an increased risk; however this must be proven in further clinical trials.

The authors revealed no potential conflicts of interest.

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Tibolone and the Breast

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At menopause, the natural decline of sexual steroids, progesterone, androgens and estrogens synthesized by the ovaries affects all tissues that have the capacity to respond to these hormones. Estrogens are responsible for producing the main menopausal symptoms, such as vasomotor instability, i.e., hot flashes and others – urogenital atrophy, osteopenia/osteoporosis. The restoration of all estrogenic activity in every tissue that has the capacity to respond to it is a characteristic of the pharmacological effects of estrogens. Some tissues even respond to this hormone when it is not necessary or desirable to have such response, i.e., the breast and the endometrium. Current investigation at both pre-clinical and clinical levels is in providing selective estrogenic activity to only certain tissues. The therapeutic armamentarium available at the moment has not completely solved these problems. This is due to the limitation of steroid hormones to provide tissue specific activity which may result in undesirable clinical effects. Both undesirable clinical effects and the limited tissue activity make it necessary to add complementary drugs or other compounds, such as a progestagen to estrogens. This is why new therapeutic agents are emerging and being tested in clinical trials with the anticipation of closing the existing gap between the totality of symptoms and the unwanted response on breast or endometrium. These new drugs also add new side effects, i.e., dry mouth with antidepressants. Thus, the present chal-

lenge in menopausal treatment is to discover the magic bullet.

The effectiveness and side effects of estrogen and progestogen (E+P), and specifically conjugated equine estrogens and medroxyprogesterone acetate (CEE/MPA), have been challenged after the publication of the results of the large, randomized clinical trials, HERS and WHI. This is due to the finding that in these randomized, placebo controlled clinical trials there were unfulfilled expectations, such as no cardiovascular protection or the untoward associated effects such as breast cancer. Heart and breast health remain two of the most important issues when evaluating new therapeutic modalities in symptomatic, climacteric women.

Tibolone

One of the emerging drugs, already registered in more than 80 countries worldwide, but not approved by the FDA in the United States, is tibolone. The worldwide experience accrued to date includes more than 4 million women years of use. Knowing how to manage tibolone, what to expect with its use, and its potential role in the treatment of climacteric patients is becoming very important. As of 2002, tibolone has been recognized as a STEAR (Selective Tissue Estrogenic Activity Regulator), differentiating it from other types of Selective Receptor Modulators (SRMs), such as SERMs, SPRMs, SARMs, and SPARMs (1). The family of SRMs having estrogenic modulation are called SERMs. They differentiate themselves from estrogens in their broader range of action, including a variation from complete agonistic actions at the estrogen receptor (present in estrogens) to complete antagonism (not present in estrogens). STEARs add to this spectrum of different pre-receptor activity in specific tissues, depending on enzyme activation or deacti-

vation (enzymatic regulation) and enzyme activities of tibolone or its metabolites (selective tissue metabolism). This will determine the unique differential clinical effects at different body sites of the postmenopausal woman that should be affected to relieving menopausal symptoms and have a positive action on bone mineral density, mood and libido with no effects on breast and endometrial tissue.

Tibolone Metabolism

After oral administration, tibolone is rapidly converted by the intestine and the liver, mainly into two estrogenic metabolites (3α - and 3β -OH-metabolites), which are responsible for its estrogenic effects on bone, vaginal and menopausal complaints. A third metabolite, the $\Delta 4$ -isomer, has progestogenic and androgenic activities, and is formed in the liver, intestine, and locally in the endometrium (preventing endometrial stimulation) (2).

Menopause and the Breast

As reproductive function, specifically the need for lactation, has already ceased at menopause, the breast is in its involution stage, and most of the glandular tissue is replaced by connective, and then by adipose tissue, which gives a more translucent image on mammograms. Menopause, a hypergonadotrophic hypogonadal stage, is an ever-increasing natural phenomenon in our societies, accruing due to the ever-growing population of menopausal women. The recency, in evolutionary time, of the extended age expectancy of modern women (77 years in 1950) has not overcome the logical natural process of malignant mutations, a product of the great number of extra replications of the breast stem cells, which exceeds the capacity of the mechanism of restoring the telomeres damaged in

this life-long division process. Mammals in a natural environment generally have a lifespan that ends shortly after they are past or on the verge of the end of their reproductive life. Humans have long passed this period with the advancement of modernism. Women will live a third of their lives in a menopausal status (3) with a very low amount of natural estrogen and progesterone circulating. This status probably has not been planned by nature. Also, the addition of a growing nulliparous population and the postponement of parity to women over the age of 30 accrue a population of genetically unprotected women. There has not been enough time for natural selection of the better equipped mutants to resist the mitotic disease. This might be part of the explanation of why two-thirds of breast cancers are diagnosed after menopause.

Why, at this age, is there a concern with the breast? Postmenopausal women are frequently diagnosed with benign breast symptoms, but often fear a real, but relatively uncommon, oncology risk. Depending on the menopausal product used to control symptoms, there could be a minor increase in the range of two-fold in the worst case scenario for breast cancer, according to some observational studies. The conflicting results between the breast, menopausal status and hormonal therapy (HT) are that breast symptoms could be spontaneous or HT induced and the breast cancer risk that is associated with HT. The deficient luteal phase and anovulatory cycles in the premenopausal woman can exacerbate premenstrual breast symptoms. After menopause, the fibro-glandular atrophy, plus the adipose substitution, can cause an increase in weight of the breast, which can result in musculoskeletal discomfort. HT can induce mastalgia and nodularity in

variable proportions in postmenopausal women.

The phenotype or configuration of the breast is probably more important in the occidental world than its real role set by nature – lactation. The breast has come to be a very visible structure with ornamental connotations far away from its biological function. This highly

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valued view is supported by the negative perception of the body that women have after breast cancer surgery as shown by Ganz (4) where the category "unhappy with body" is always selected in more than 75% of these cases independent of age and more than 85% in the younger age (25-34) individuals.

The way breast tissue responds to internal and external factors, such as hormones and growth factors, is specifically related to its biological characteristics or internal biology, as dictated by genes. These genes are expressed locally, and determine the metabolic pathways that are functioning from a biochemical standpoint at a specific moment in time.

What Can Tibolone Offer the Human Breast?

The accrued experience with tibolone in preclinical and clinical studies is very promising for symptomatic treatment, effects on other organ systems, and specifically in relation to breast tissue homeostasis. We are still awaiting the results of a large, blinded, randomized clinical trial that can confirm that tibolone is a safe drug relative to the menopausal breast.

The role of local synthesis of estrogens in breast tumors is something already shown by several authors (5), as is the importance of steroid sulfatase (STS) and estrogen sulfotransferase (EST) in maintaining the intracrine homeostasis of the breast. Androstenedione is the substrate for the aromatase enzyme that locally, irreversibly forms estrone. Estrone can be converted even further into the more potent estrogen estradiol by the type I 17 β -HSD enzyme and reconverted to the less potent estrogen estrone by the type II 17 β -HSD isoenzyme. The normally present sulfotransferase enzyme can convert estrone into an inactive product, estrone sulfate; but the enzyme sulfatase, also present in breast tissue, can deconjugate estrone sulfate to estrone. So, the sulfatase-sulfotransferase system plays an important role in regulating the amount of free estrogens in breast tissue. It has been shown that tibolone can interfere with this system by inhibiting the formation of estradiol from estrone sulfate via inhibition of sulfatase. Tibolone and its $\Delta 4$ metabolite can induce synthesis of type II 17 β HSD, diminishing the presence of estradiol locally (6).

Breast Cell Homeostasis with Tibolone

The balance between mitotic rate and apoptotic rate is very important

in preserving the cellular homeostasis in the breast. The conversion of estradiol in the direction of the biologically inactive estrone sulfate can explain the lower expression of cellular activity found in tibolone users. Reduced cellular activity was present when mammary biopsies were performed in one-year symptomatic menopausal users and compared with their own basal biopsies (7). This study showed that the proliferation marker Ki67 has a decrease in its expression from the basal mean of 6.4 to 4.2 after one year of tibolone use. An opposite effect was found with the oral combination of CEE+MPA, which had an increase from 6.0 at basal biopsies to 10.7 in the Ki67 index after one year of use. Apoptosis being a mode of regulation of the number of cells in the breast, and avoidance of the proliferation of a mutated cell was also measured in the same study. There were divergent results a decrease of Bcl-2, an apoptosis marker, from 31.9 to 24.4 in tibolone users and an increase from 31.1 to 37.6, in CEE+MPA users. It is also interesting to note that in this same study not all women under the same treatment had the same direction of response to Ki67 and Bcl-2, which shows that each woman is different, with a different capacity to respond to different agents. A decrease in Ki67 expression was found with tibolone use in 80% of cases, while 13.3% were increased and 6.7% had no change. Comparable results were found for Bcl-2.

Women who used the combined therapy (CEE+MPA) showed a very small number of decreased values for Ki67, only 5.3% of cases, while the proportion that increased was very high, 78.9% of the cases, and 15.8% of them experienced no change. The CEE+MPA effect on the apoptosis marker Bcl-2, found the number of cases with a decrease was

26.3%, while 47.0% increased, and 26.7% had no change. This shows that it is very difficult to predict the response of an individual subject to a specific hormonal treatment.

Breast Density with Tibolone

There are several reasons to suspect that the increase in breast density reported with conventional HT may not be identical to the high breast density associated with an increased risk of breast cancer (8). This issue, hormone-induced breast density, and its clinical significance have been extensively reviewed in the Summer/Fall 2005 issue of *Menopausal Medicine* by the participating authors and there is no need to do it again. But it is worthy to reinforce that tibolone has a completely divergent effect as compared with estrogens or estrogen plus progestin combinations. The first report of our group, presented at the Copenhagen FIGO meeting in 1997, and then published (9), showed that tibolone resulted in a 3.3% increase in mammogram density after one year of use.

This result being similar to the controls. These findings were different from HT-containing preparations, either estrogen, alone, or combined with progestins, with increased mammographic density ranging from 26.7% to 66.7%. The study of breast biopsies already cited (7) is consistent with other publications on the topic. The mammographic findings with tibolone is in concordance with our previous trial that tibolone has a very different effect on breast density than estrogens or its combination with progestins used as HT in postmenopausal women.

Our last study shows that only 5.5% of cases had a higher mammographic density after one year than at baseline with tibolone treatment, compared with 57.9% in the case of

combined HT of CEE+MPA. Both groups also showed a differential decrement in breast density of 50% and 36.8%, with the rest (44.5% and 5.3%, respectively) remaining unchanged. There is a tendency for HT to increase mammographic density.

Our data again demonstrates the differential response of individual patients to particular therapies. These findings could be the result of the differential response in cell homeostasis that each HT preparation achieves.

Breast Sensitivity (Mastalgia) with Tibolone

This is a very relevant issue when using HT because it can be the reason for discontinuing treatment. Several clinical studies have shown that tibolone has a much lower rate of breast pain associated with its use than HT combinations of estrogen plus progestin or estrogen alone. In 1995, Ginsburg et al (10) showed that transdermal estrogen resulted in a 20% incidence of breast pain that decreased to 1.4% when the women were switched to tibolone. Other reports have shown similar effects, as Lundström et al (11) found 2.4% of tibolone users with breast tenderness and 17.1% in CEE+MPA users, and when compared to E2+NETA, it was 3.6% for tibolone and 33.3% for E2+NETA. Palomba et al (12) showed no differences for breast tenderness and mastalgia between tibolone and placebo users after six and 12 months.

Non-Clinical Experiences with Tibolone

All clinical results published in recent years have a good correlation with all the basic science and pre-clinical studies done with tibolone. Studies performed in rats, monkeys, and human normal and tumor breast cells show the same type of response as in breast cell homeosta-

sis. These data offer great hope that tibolone can play a major role in the future treatment of menopausal women worldwide.

Tibolone and Breast Cancer Risk

Based on preclinical data, it is anticipated that tibolone will not adversely affect the breast. The preclinical data in breast cancer cells, MCF-7 cells types MCF-7(A) and MCF-7(H) and T47D cells types T47D-A T47D-S, found based on comparatively measured DNA ($\mu\text{g/ml}$) concentrations, that cell growth is between half and one-fifth that of tibolone compared to estradiol (13). Also, the animal model of breast cancer induction with DMBA has been used to monitor growth inhibition of various compounds (13). Tibolone had an equivalent inhibitory response to tamoxifen in this model, and showed no interaction between them. One study has shown that this effect was not reversed by flutamide, so it is not an androgenic mediated effect (14).

The preclinical research with tibolone also shows that it inhibits the conversion of estrone sulfate to estradiol in MCF-7 breast cancer cells in a dose response manner (15). However, there is a different effect of the different metabolites with a lower effect of $\Delta 4$ -tibolone (16), and that tibolone and its metabolites inhibits the sulfatase activity (E1S \rightarrow E2) in T47D cells, also in a dose response manner (15). Further, it was demonstrated that tibolone and its metabolites had a tissue-specific inhibition of sulfatase activity in the breast, but not in the bone (17). The effect of tibolone on apoptosis in normal breast epithelial cells was three times higher than with estradiol by flow cytometry (18). Tibolone's effect on proliferation of normal epithelial cells in human breast tissue was smaller compared to estradiol (19) and on Bcl-2 expression,

using western blots, three to four times lower than with estradiol in the same tissue (19).

Recurrence of Breast Cancer After Use of HT

All observational studies have a high amount of biases, such as selection bias, healthy user effect, etc., and are usually short-term observations with a limited number

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of subjects. It was necessary, in order to determine the association between breast cancer and HT, to design prospective, randomized clinical trials. Four of them are well known: the Stockholm clinical trial began in 1996 (20), the HABITS in 1997 (21), WISDOM (UK) Trial in 2000 (22), and the LIBERATE trial in 2002. These studies were planned to recruit 1,000, 1,300, 2,000 and 2,600 subjects, respectively, with previous surgery treatment for breast cancer. The first three of the trials tested different combinations of estrogens + progestins versus no HT; and none of them enrolled the planned number of participants. By the time they were stopped, Stockholm had 362 (36.2%), the HABITS

442 (34.0%) and the WISDOM Trial 178 (5.9%) enrolled participants.

The reason for canceling the studies was, as in the case of the HABITS trial, due to an increased relative risk of 3.3 (95% CI, 1.5-7.4) in recurrent breast cancer between treated and untreated. As the Stockholm trial had been joined with the HABITS, it was also stopped by the Data Safety Monitoring Board (DSMB), although it had a RR of only 0.82 (95% CI, 0.35-1.9). However, the combined RR for both trials was 1.8 (95% CI, 1.03-3.1). As a consequence of the cancellation of the Stockholm trial, the U.K. trial was also stopped, as it was thought that it would be very difficult to reach the projected number of 3,000 participants needed when, at that time, it had recruited only 178 participants after almost three years of enrollment.

The LIBERATE trial compares, in a double-blinded fashion, two different doses of tibolone -1.25 and 2.5 mg- versus a placebo randomly distributed in postmenopausal symptomatic subjects with prior breast cancer surgery in the previous five years at 245 sites in 32 European, Asian, and Latin American countries. It has recruited 3,149 participants with a mean age of 52.6 years, whose breast cancer ranged from stage I to IIIB. It has undergone five DSMB reviews, the last on Sept. 28, 2005. The recommendation of the DSMB was to continue the trial. Great hopes are being placed on this trial because it can give us many answers to the questions that have been raised after the WHI combined studies (2002-2004) and the U.K. Million Women Study (2003). The incidence of breast cancer in the Million Women Study found a very high risk population for breast cancer (overall recurrence expected of 15%). The first clue to the outcome of the LIBERATE trial of tibolone

will be at the time of the report of the first primary analysis planned for mid-2007 (23).

A very complete review of tibolone characteristics, considering all different sites that this STEAR acts upon, is in the report by the International Consensus Group (24).

Conclusion

The response to HT is highly variable between individual women, and depends on a variety of factors including the type and dosage of HT, the sensitivity of the individual, demographic characteristics and biotype. Tibolone is a tissue-specific steroid with estrogenic, progestagenic and androgenic activity. In vitro studies show that tibolone inhibits the production of estradiol in breast cells, inhibits proliferation, and increases apoptosis.

In animal studies, tibolone inhibits the growth of DMBA-induced mammary tumors. In contrast to estrogens, tibolone does not induce breast tenderness and does not increase mammographic density. These data show that tibolone has an effect on the breast that is different from that of estrogens.

Tibolone may be a future alternative for treating symptomatic postmenopausal women who survive breast cancer treatment.

The author revealed the following potential conflict of interest: External Medical Advisor for Organon-Chile.

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From the Editor continued from page 1 studies. The current paper using various progestins identifies differences in molecular biology in normal and neoplastic breast cell lines, in vitro. These data should be interpreted with caution because of the limited amount of information that is available on progestins and the risk of breast cancer in women.

An alternative to what we know as standard estrogen plus progestin therapy has been the use of tibolone (Livial) in postmenopausal women. This product has been available in Europe for over 10 years with extensive use in postmenopausal woman. One of the concerns is the role of tibolone and the risk of breast cancer.

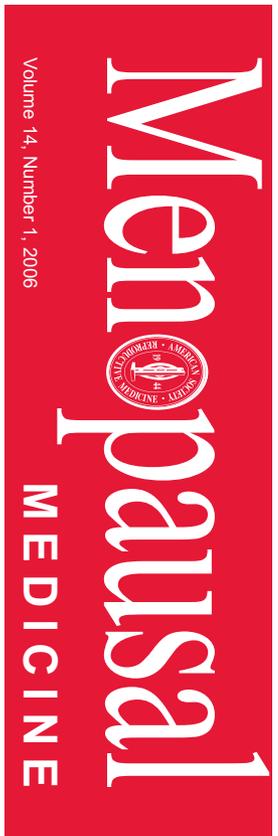
Dr. P. Lavin addresses this issue using his group's research on the female breast, with a very comprehensive review of the current literature. The U.S. Food and Drug Administra-

tion has recently issued a non-approval letter to Organon, Inc., the sponsor of tibolone for its use in postmenopausal women. The reasons for this non-approval letter are not readily apparent and are known only to the Food and Drug Administration and the pharmaceutical sponsor.

Suffice it to say that there was a significant reaction to the news that tibolone will not be marketed in the United States, since many had expected tibolone to achieve regulatory approval. The current non-approval status has resulted in frustration among those who have been closely allied with the clinical development of this product.

Suffice it to say that a full evaluation of tibolone's safety will not be possible for the scientific community until the results of several large clinical trials sponsored by Organon are completed and published. The current

information in this article should be taken in the context that tibolone is available internationally. The data presented are relevant to our readers even though tibolone is not available for U.S. consumers.



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