Hormonal Therapy and Chemoprevention

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■ **Abstract:** Hormone replacement therapy (HRT) can increase the quality as well as the length of life, but a prolonged use can also increase the risk of breast cancer. The combination of HRT and a selective estrogen receptor modulator (SERM) such as tamoxifen may retain the benefits while reducing the risks of either agent. A post hoc analysis of the Italian Tamoxifen Prevention Study showed a borderline significant reduction of breast cancer among women who were on HRT continuously and tamoxifen as compared with continuous HRT users who received placebo. Recent studies suggest that the standard dose of tamoxifen may be reduced to one-quarter (i.e., 10 mg every other day) without loss of its beneficial biological effects. Since the endometrial effect of tamoxifen seems to be both dose and time dependent, a dose reduction could substantially reduce the risk of endometrial cancer while retaining its preventive efficacy. On the other hand, the addition of HRT containing progestins could also minimize the risk of endometrial cancer associated with tamoxifen. Moreover, estrogen should reduce the incidence of vasomotor and urogenital symptoms, which are a major reason for tamoxifen withdrawal in prevention studies. Notably, in the National Surgical Adjuvant Breast Project (NSABP) P-1 trial, women ages 50 or younger had no increased incidence of adverse events, including endometrial cancer and venous thromboembolic events. One possible explanation for the lack of toxicity in premenopause is the presence of adequate circulating estrogen levels which prevent tamoxifen to act as an estrogen agonist at these target tissues. Moreover, data from the current Italian tamoxifen prevention trial indicate that the compliance was substantially higher for de novo and current HRT users as compared to women who never received HRT during the study. The combination of HRT and tamoxifen at low doses could thus reduce the risks and side effects while retaining the benefits of either agent. ■

Key Words: hormone replacement therapy, selective estrogen receptor modulators, tamoxifen

Although the concept of a benefit of hormone replacement therapy (HRT) on life expectancy mostly derives from epidemiologic studies, there is good evidence that the use of HRT increases the quality of life and has the potential to reduce overall mortality (1). HRT is highly effective for the treatment of women with typical menopausal complaints, such as hot flashes, night sweats, insomnia, increased fatigue and irritability, depression, skin changes, and urogenital atrophy (2).

There is a vast literature on the large benefits of estrogens on osteoporosis, cardiovascular disease and genitourinary symptoms (1). Specifically HRT is able to increase bone mineral density (BMD) in the spine and hip of postmenopausal women, the greater increase being noted in those receiving conjugated equine estrogens plus continuous medroxyprogesterone acetate (MPA) (3). These effects reduce the risk of fractures, but decline rapidly after cessation of treatment. Therefore HRT would have to be given for a very long period to women in their 60s and 70s to decrease the risk of hip fracture

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in particular. The length of this period and the minimal effective dose of HRT to prevent fractures in late postmenopause is, however, still uncertain.

Many observational studies have shown that estrogen has protective effects on the cardiovascular system (1,4,5). The atheroprotective effects of estrogen are not only attributable to alterations in serum lipids, but also to direct actions of estrogens on blood vessels (5).

However, clinical studies do not provide satisfactory confirmation of this potential benefit (1,6). Notably the Heart and Estrogen/Progestin Replacement Study (HERS), a secondary prevention trial in women with coronary heart disease (CHD), has shown that such a treatment did not reduce the overall rate of CHD events and did produce an increase in thromboembolic events (7). Of interest, a time-treatment interaction has been noted in this trial, with an increase in venous events in the first year of treatment, followed by a decrease thereafter (8).

Based on this clinical trial, it is not recommended to start HRT in order to obtain secondary prevention of CHD. However, even if a cause-effect relationship is not confirmed, there is strong evidence that HRT lowers the risk of CHD in women without such a history, and it could be appropriate to suggest the use of HRT in these women (2). In addition, unpublished data from the ongoing Women's Health Organization HRT study in mainly healthy women show a slight increase in vascular events in the first 2 years of treatment, followed by a trend toward a decrease (http://www.nci.nih.gov/whi/).

While the benefits of HRT are increasingly appreciated by women, the major obstacle to its widespread use is the fear of breast cancer.

HRT AND BREAST CANCER

The meta-analysis of 51 epidemiologic studies including 52,705 individuals with breast cancer and 108,411 control women accounting for 90% of the worldwide evidence (9) has shown that use of oral HRT is associated with an overall increased risk of breast cancer (RR = 1.14, SE = 0.03, $p = 0.00001$). The risk increased with the duration of HRT (RR = 1.35, 95% CI 1.21–1.49 after an average of 11 years) and progressively decreased after HRT discontinuation, with no excess risk after 5 years from cessation. Of interest, the magnitude of the increased risk (2.3% per year, 95% CI 1.1–3.6%) is comparable with that associated with each year of delayed menopause (i.e., 2.8%, 95% CI 2.1–3.4%), strongly confirming the hypothesis that maintenance of a premenopausal hormonal milieu may account for the reported increased risk in HRT users. Of importance, the increased risk observed in current and recent HRT users was greater for women with lower body mass index (i.e., BMI < 25 kg/m^2).

Although little information was available regarding hormonal type and dose and 80% of these women had used oral estrogen alone, the addition of progestins was associated with a higher relative risk (RR) of breast cancer than estrogen alone. The RR was 1.15 (SE = 0.19) and 1.53 (SE = 0.33) in current or recent users of estroprogestins for less than 5 years and more than 5 years, respectively, compared with a RR of 0.99 (SE = 0.08) and 1.34 (SE = 0.09) for current or recent users of estrogens alone. Finally, cancers in women who had ever used HRT tended to be less advanced clinically than those who had never used it. In this regard, a prospective cohort study on 37,105 HRT users of the Iowa Women's Health Study has shown that exposure to HRT was associated with an increased risk of invasive breast cancer with a favorable histotype, while there was little evidence of association with other invasive ductal or lobular cancers or ductal carcinoma in situ (DCIS) (10). These findings have not been confirmed in other studies (11). Also, a trend to a longer survival in HRT users who developed breast cancer compared to those who had never used it has been observed in some studies (12).

A previous analysis from the Nurses' Health Study (13) showed a moderately elevated risk of breast cancer death among postmenopausal women who were taking oral estrogen or had previously used this therapy for 10 or more years. Notably, a recent update of the cohort shows that the addition of progestins was associated with a 9% (SE = 2.5) increased risk per year as compared with 3.3% increased risk (SE = 0.84) with estrogen alone (14). Likewise, a recent analysis from the Breast Cancer Detection Demonstration Project (BCDDP) based on 2,082 incident breast cancer cases found that the estrogen-progestin regimens were associated with greater increases in breast cancer risk than estrogen alone [8% per year, 95% CI 2–16% compared with 1%, 95% CI 0.2–3% for each year of estrogen alone (11)]. Of importance, the increased risk is largely limited to current or recent users and is directly related to the duration of use. An updated analysis in a Swedish cohort also found greater risks with combined therapy; for 6 or more years of current or recent use, the risk of breast cancer was increased by 70% for combined therapy, but no increase was seen for estrogen alone (15). A case control study recently performed in California among 1,897 postmenopausal case subjects and 1,637 postmenopausal control subjects also showed that combined estroprogestin therapy was associated with a higher risk of breast cancer compared with unopposed estrogen

replacement therapy (16). The overall risk (OR) for 5 years of HRT use was 1.10 (95% CI 1.02–1.18). The risk was higher for continuous HRT users (OR = 1.24, 95% CI 1.07–1.45) than for ERT users (OR = 1.06, 95% CI 0.97–1.15). There was a trend for sequential HRT (i.e., with progestins given for 10 or more days per month) being associated with a higher risk (OR = 1.38, 95% CI 1.13–1.68) than combined continuous HRT (i.e., with progestins given continuously), where the OR was 1.09 (95% CI 0.88–1.35), but this was not statistically significant. Overall these studies provide firm evidence that addition of progestin to estrogen does not reduce the risk of breast cancer and suggest that the risk is actually increased (17).

It must be underlined that all the above data are based solely on studies making use of orally administered estrogens. Since the extensive use of transdermal HRT is relatively recent, no epidemiologic data is available on its association with breast cancer risk. However, the parenteral route of administration, in contrast to the oral route, is associated with the following endocrine effects: a trend toward an increase of circulating insulin-like growth factor (IGF)-I levels, one of the most potent breast mitogens (18), a lower conversion to the weak estrogen estrone (19), and a higher availability of free estrogen levels due to unchanged sex hormone binding globulin (SHBG) levels (19,20). These effects might be associated with an increased risk of breast cancer. While further studies are needed to clarify this issue, new strategies to minimize the risk of breast cancer are needed.

CHEMOPREVENTION TRIALS WITH TAMOXIFEN

Tamoxifen is widely used for palliative endocrine treatment of advanced breast cancer and as adjuvant therapy to control micrometastatic relapse and new primaries in women surgically treated for early breast cancer (reviewed in 21). It has been used in three large cooperative phase III trials for prevention of breast cancer in at-risk women. The results of two of these studies, the Royal Marsden Tamoxifen Chemoprevention Trial and the Italian Tamoxifen Prevention Study, have been published in a preliminary form (22,23) and the third, the National Surgical Adjuvant Breast Project (NSABP) P-1 has been reported in full (24). While the results of the European studies have shown no difference so far (22,23), comparison with the larger U.S. study is not appropriate given the limited statistical power and the different population involved.

The NSABP P-1 study started in 1992, recruited 13,388 women at risk for breast cancer (i.e., more than 60 years old; or age 35–59 with an increased risk of invasive breast cancer, using the Gail algorithm, $\geqslant$1.67% in 5 years; or with a history of lobular carcinoma in situ) who were randomized to tamoxifen 20 mg/day or placebo. This trial gave such positive results that an interim analysis led to the closure of the study and that tamoxifen has been registered in the United States for the reduction of risk in women at increased risk as assessed by the Gail model (http://www.nci.nih.gov/).

The P-1 trial has shown that 20 mg/day of tamoxifen reduced the risk of invasive breast cancer by 49% (two-sided $p < 0.00001$), with a cumulative incidence through 69 months of follow-up of 43.4/1,000 in women in the placebo group and 22/1,000 women in the treatment arm. The decreased risk occurred in women of all age groups; ages 49 years or younger (44%), 50–59 years (51%), and 60 years or older (55%). Risk was also found to be reduced in women who had a history of lobular carcinoma in situ (LCIS) (56%) or atypical hyperplasia (AH) (86%) and those with any category of predicted 5-year risk. Tamoxifen reduced the risk of DCIS and LCIS by 50% (two-sided $p < 0.002$) and the occurrence of estrogen receptor (ER)-positive breast cancer by 69%; it had no effect on ER-negative tumors. Even more importantly, the reduction of LCIS and AH suggests a true preventive effect of tamoxifen. Tamoxifen did not alter the rate of ischemic heart disease, but did produce a 20% reduction in the incidence of osteoporotic bone fracture of the hip, radius (Colles'), and spine.

Compared with the placebo group, however, women age 50 or older receiving tamoxifen had more than a two-fold increased risk of early stage endometrial cancer, a three-fold increased risk of pulmonary embolism, and a significant excess of cataracts (24). Notably, however, women age 50 or younger had no increased incidence of adverse events, including endometrial cancer and venous thromboembolic events. This might suggest that the concomitant presence of adequate circulating hormone levels prevents tamoxifen from acting as an estrogen agonist at these target tissues.

Moreover, the NSABP P-1 study shows that tamoxifen use is associated with an increase in specific vasomotor and gynecologic symptoms (25). However, these symptoms do not significantly affect overall physical and emotional health and no correlation has been shown between tamoxifen use and significant levels of affective distress and/or depression. Specifically, symptoms that are substantially more frequent in women using tamoxifen include vasomotor symptoms (cold sweats,

night sweats, and hot flashes), vaginal discharge, and genital itching. Sexual functioning problems are also reported in a slightly higher proportion in the tamoxifen-treated group (main difference 0.78%). Of importance, depression and weight gain are not increased in frequency in the tamoxifen group (25).

Possibly the most important concern is the increased risk of endometrial cancer. In the NSABP P-1 prevention study, the rate of endometrial cancer was increased in the tamoxifen group (RR = 2.53, 95% CI 1.35–4.97), the increased risk occurring predominantly in women age 50 years or older. In women age 49 or younger, the RR was 1.21, 95% CI 0.41–3.60; in women more than 50 years of age, the RR was 4.01, 95% CI 1.70–10.90. This suggests that the woman's endocrine milieu can influence the pharmacodynamic of tamoxifen at the endometrial level. Specifically progesterone could neutralize tamoxifen's agonistic activity on the endometrium similarly to what was previously observed with estrogen replacement therapy. Moreover, all endometrial cancers observed in the P-1 trial were stage I and no endometrial cancer deaths were reported in the tamoxifen group (24). There is also indirect evidence that the risk of endometrial cancer induced by tamoxifen is both time and dose dependent, the higher RR being observed with daily doses of 40 or 30 mg/day of adjuvant tamoxifen (26). Thus one plausible way to lower this risk is a reduction of the dose (27). In this context, we have demonstrated that a dose reduction of tamoxifen to 10 mg on alternate days is comparable to the conventional dose of 20 mg/day on a broad spectrum of biological markers of drug activity, including IGF-I (28,29).

RATIONALE FOR THE COMBINATION OF HRT AND TAMOXIFEN

In 1992 a double-blind, placebo-controlled, randomized trial of tamoxifen was started in Italy in healthy women who had had a hysterectomy for benign conditions. A total of 5,408 women were randomized to receive tamoxifen 20 mg/day or placebo for 5 years. The main endpoint was the incidence of breast cancer.

The preliminary analysis of the study (23) has shown no difference in breast cancer incidence between the placebo (22 cases) and the tamoxifen (19 cases) arms. At present, the study only had 30% power to detect the anticipated 33% reduction of breast cancer in the tamoxifen arm. However, unplanned subgroup analyses gave interesting clues. A borderline significant reduction of breast cancer was observed among women who were continuous HRT users and received tamoxifen. Compared to the 8 cases of breast cancer occurring among the 390 HRT users who were on placebo, there was 1 case of breast cancer among the 362 HRT users who were receiving tamoxifen (RR = 0.13, 95% CI 0.02–1.02). Of interest, in the Marsden trial (22), women who were already on HRT (mostly by the oral route) when they entered the study showed an increased risk of breast cancer compared with nonusers, while the subjects who started HRT while on trial had a significantly reduced risk. However, no interaction was noted between use of HRT and any tamoxifen effect on breast cancer occurrence. There were 12 cancers in the 523 women on tamoxifen who received HRT at some point during the study (mainly after randomization), compared with 13 cancers in the placebo arm out of 507 users of HRT.

As the combination of tamoxifen and transdermal HRT might reduce the risks and side effects of either agent, their combined effect on several cardiovascular risk factors, including blood cholesterol levels, was tested within the trial (30). Compared to small changes in the placebo group, tamoxifen was associated with changes in total, LDL, and HDL cholesterol of −9%, −14%, and −0.8%, respectively, which were similar in continuous HRT users and those who had never used HRT. In contrast, the decrease induced by tamoxifen of total and LDL cholesterol was blunted by two-thirds in women who started HRT while on tamoxifen. Thus the beneficial effects of tamoxifen on cardiovascular risk factors are unchanged in current HRT users, while they may be attenuated in women who start transdermal HRT while on tamoxifen. Notably, previous studies have also shown that the combination of HRT and tamoxifen does not adversely affect their biological effects, including bone density and clotting factors (31).

From the biological point of view, the increased risk of breast cancer associated with HRT use is linked to an increased expression of estrogen receptors in the breast tissue (32), thus leading to an enhanced sensitivity to the mitogenic effect of estrogen. The addition of a SERM capable of reducing this growth-promoting effect on the breast could therefore be useful for women's health maintenance.

Of importance, in the current Italian tamoxifen trial the compliance was 78% at 3 years (75% at 5 years) for women who never took HRT. For women who took HRT at baseline and during the trial, the compliance was 85% at 3 years and 78% at 5 years, while for women who were not on HRT at baseline but who took HRT at some time during the trial the compliance was

92% at 3 years and 88% at 5 years. The figures for 3 years are based on 2,204, 385, and 433 women, respectively, while those at 5 years are based on 700, 111, and 151, respectively. These unpublished data clearly indicate that compliance may be substantially higher in de novo and current HRT users who take tamoxifen or placebo as compared to women who do not take HRT. Of importance, no evidence of an excess of venous thromboembolic events was noted in the group of women taking HRT and tamoxifen.

Altogether, these findings provide the background for further investigations of the combination of HRT and tamoxifen in order to reduce the risks while retaining the benefit of either agent.

RATIONALE FOR A DOSE REDUCTION OF TAMOXIFEN

The risk of endometrial cancer induced by tamoxifen appears to be dose and time dependent. A trend to a dose-response effect is suggested in the meta-analysis of the three Scandinavian trials of adjuvant tamoxifen, where the RR of endometrial cancer was 5.6 in the Stockholm trial of 40 mg/day and 3.3 and 2.0 in the Danish and South Swedish trial of 30 mg/day of tamoxifen, respectively. In the NSABP B-14 trial using a daily dose of 20 mg/day, the RR of endometrial cancer is approximately two times higher than the general population (33,34). A relationship between length of tamoxifen treatment and endometrial cancer incidence is evident in the meta-analysis of all adjuvant trials of tamoxifen (35).

The effect of three doses of tamoxifen on the change in biomarkers regulated by the estrogen receptor has recently been studied (28). A comparable potency of a lower dose of tamoxifen would provide strong support for assessing the preventive efficacy and the safety of low-dose tamoxifen in a larger trial.

A total of 127 healthy hysterectomized women, ages 35–70 years, were randomly assigned to one of the following four treatment arms: placebo, tamoxifen at 10 mg on alternate days, tamoxifen at 10 mg/day, or tamoxifen at 20 mg/day. Comparison between baseline and measurements at 2 months of the following parameters was performed: total cholesterol (primary endpoint), HDL cholesterol, LDL cholesterol, triglycerides, lipoprotein(a), blood cell count, fibrinogen, antithrombin III osteocalcin, and IGF-I. After adjustment for the baseline values there were reductions in circulating levels of total cholesterol, IGF-I, and most of the other parameters of the same magnitude in all three tamoxifen arms (28).

In order to assess whether the blood concentrations of drug and metabolites could explain the biomarker changes and to verify treatment adherence, the blood concentrations of tamoxifen and its main metabolites were measured. In spite of a high correlation between assigned dose and blood levels, no evidence for a concentration-response relationship was observed on most of the biomarkers, suggesting that an 80% reduction in blood levels from the conventional dose (i.e., from a mean ± SD of 136.0 ± 52.7 ng/ml attained with 20 mg/day to 26.8 ± 15.1 ng/ml attained with 10 mg every other day) may not affect in a substantial way the biological activity of tamoxifen (29).

Therefore a mean tamoxifen concentration of approximately 25 ng/ml was associated with comparable changes in most endpoint biomarkers. Moreover, where a trend to a concentration-response relationship was noted, namely, on platelet count and triglyceride levels, the use of the lowest dose seems to be preferable. The concept of a dose reduction is further supported by the observation that tamoxifen has a very high tissue distribution, ranging from 5 to 60 times its blood concentrations (36,37). Assuming linear pharmacokinetics, which seems to be true in the dose range used in our previous study, the breast tissue level attainable with 10 mg on alternate days still exceeds by several times the growth inhibitory concentration of tamoxifen in breast cancer cell lines, which is approximately 35 ng/ml (38,39). In addition, the concomitant activity of metabolite X, which has a significant growth inhibitory activity in breast cancer cell lines (39), may further contribute to the total drug inhibitory activity. Finally, recent in vivo studies in a spontaneous rat mammary tumor model indicate that a daily dose corresponding to approximately 1 mg/day of tamoxifen has a complete preventive efficacy on mammary tumor formation (40).

Of importance, a recent cross-sectional study conducted in older, nursing home residents in New York State long-term care facilities has shown a significant reduction in bone fracture rate among breast cancer women taking 10 mg/day of tamoxifen (41). During the 1.5-year period for which bone fractures were documented, the fracture rates were 7.6% in 5,196 untreated control women, 3.2% in the 125 women receiving 10 mg/day of tamoxifen, and 6.7% in the 1,248 women receiving 20 mg/day of tamoxifen. The OR for 20 mg/day compared to controls is 0.92 (0.72–1.16), while for 10 mg/day versus controls it is 0.31 (0.11–0.87, $p = 0.025$). The hip fracture rates were 5.0% in 5,196 untreated control women, 2.4% in the 125 women receiving 10 mg/day of

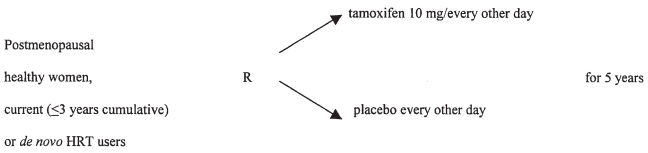

Figure 1. Design of the HoT (Hormones and Tamoxifen) Study.

tamoxifen, and 4.6% in the 1,248 women receiving 20 mg/day of tamoxifen. The OR for 20 mg/day compared to controls is 0.96 (0.72–1.29), while for 10 mg/day versus controls it is 0.31 (0.10–1.02, $p = 0.054$). All these considerations provide strong rationale to assess a lower dose of tamoxifen in a preventive context.

FUTURE STUDIES

On the basis of all the above results, the combination of tamoxifen at low doses and HRT might therefore reduce the risks and side effects while retaining the benefits of either agent. A large trial addressing this issue has been planned. The study is a randomized, double-blind, placebo-controlled phase III trial with the design shown in Figure 1.

The primary objective is to assess if tamoxifen at low doses reduces the incidence of breast cancer in healthy postmenopausal women undergoing or willing to start HRT. The main entry criteria are postmenopausal status (last menstrual period since 6 months and elevated follicle stimulating hormone), compliance with annual mammographic screening, and current (⩽3 years) or de novo HRT use.

The primary endpoint is the incidence of DCIS and invasive breast cancer after 5 years of intervention. The secondary endpoints are the incidence of other noninvasive breast disorders (i.e., LCIS, atypical hyperplasia), endometrial cancer, all other cancers, bone fractures, cardiovascular events, venous thromboembolic events, cataracts, and overall mortality. In a study subgroup, blood concentrations of tamoxifen and N-desmethyltamoxifen will be studied and levels will be correlated with biomarker changes.

Since this is a pragmatic trial, where the main objective is to assess the efficacy of tamoxifen at a low dose in comparison with placebo in HRT users, any type of HRT will be allowed, regardless of type of estrogen or progestin hormones, dose, schedule and type of combination, or route of administration. This rule applies to both current and de novo HRT users. It will be a multicenter trial, and a total of 8,500 subjects is required, 4,250/arm. Five years of treatment are planned, followed by a 5-year follow-up.

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