Clinical Development of Estrogen Modulators for Breast Cancer Chemoprevention in Premenopausal vs. Postmenopausal Women

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Abstract   Tamoxifen has proven to be beneficial in the chemoprevention of breast cancer in women at increased risk for the disease. Other compounds that mediate the estrogen pathway remain to be tested for clinical efficacy. The mechanism of action, efficacy, and dose response of the estrogen modulators is determined by the hormonal milieu of the host which should be considered in the early clinical trials for dose range finding studies and surrogate endpoint biomarker (SEB) evaluation. This review presents the hormonal effects to consider in the clinical testing of an agent in premenopausal vs. postmenopausal cohorts. Recommended SEBs that may be evaluated in Phase I/II clinical trials of estrogen modulators for breast cancer chemoprevention are presented. J. Cell. Biochem. Suppl. 34:103–114, 2000. © 2000 Wiley-Liss, Inc.

Hormonal approaches to breast cancer have been successful in both the treatment and prevention of the disease. Studies in breast cancer epidemiology and laboratory in vitro and in vivo investigations have suggested a role for estrogen in the initiation, promotion and spread of breast cancer. In the treatment setting, estrogen modification with oophorectomy or tamoxifen has had a significant impact on the survival of women diagnosed with breast cancer [Early Breast Cancer Trialists Group, 1992]. The chemopreventive properties of tamoxifen were first demonstrated by the reduction of second primaries in a meta-analysis of breast cancer survivors who had taken the drug for five years [Fisher et al., 1989; Rutqvist et al., 1991]. Based on this analysis, tamoxifen became the lead compounds in breast cancer chemoprevention studies. A large breast cancer prevention trial recently completed in the United States showed a 49% risk reduction for invasive breast cancer among subjects who took tamoxifen; the benefit was seen in both pre- and postmenopausal age groups [Fisher et al., 1998]. These results are encouraging, and other compounds that affect estrogen are currently under evaluation to determine whether they might have clinical advantages over tamoxifen. Clinical testing of estrogen modulators has shown that the hormonal milieu is a critical determinant of their mechanism of action, efficacy, and dose response. This review will present some of the estrogen-mediated effects to be considered in testing estrogen modulators for cancer prevention in premenopausal and postmenopausal women.

The clinical effects of estrogen have been extensively studied in postmenopausal women. In this population, decreased estrogen is associated with risk for osteoporosis, cardiovascular disease, and vasomotor symptoms. Emerging evidence suggests that neuropsychiatric function may also be impacted [Grady et al., 1992; Henderson et al., 1994]. In contrast, the effect of estrogen modulation in premenopausal women is largely unknown, although the impact in this younger cohort is of great interest because cyclic epithelial cell proliferation and turnover relevant to breast cancer initiation is

primarily a premenopausal phenomenon. This is potentially a time when chemopreventive interventions might have the greatest impact. Furthermore, genetic testing is identifying high-risk individuals at a young age for whom a chemopreventive intervention would be of great value. Studies are needed to define the overall health impact of estrogen pathway disruption in hosts with intact ovarian function. Breast cancer chemopreventive agents that modulate the estrogen pathway will need to address the clinical risks and benefits relative to menopausal status.

Estrogen Modulators as Chemopreventive Agents

Estrogen modulators may act as chemopreventive agents by disrupting estrogen production, receptor binding, or receptor activation. The agents presented as examples of estrogen modulators with chemopreventive potential are selective estrogen receptor modulators (SERMs), aromatase inhibitors, phytoestrogens, and indole-3-carbinol.

Selective Estrogen Receptor Modulators (SERMs)

The primary actions of SERMs are mediated through an interaction with the estrogen receptor (ER). Like other steroid nuclear receptors, ER is a ligand-activated transcription factor [reviewed in Katzenellenbogen, 1996]. The mechanism of tissue selectivity for agonist and antagonist activity is related to the transcriptional activation functional domains of the ER, called AF-1 and AF-2. Transactivation of AF-1 is hormone-independent, whereas AF-2 is hormone-dependent. The response to partial agonists in different cell types relates to the degree to which the response is mediated by AF-2 activation. Additional tissue specificity is conferred by DNA response elements distinct from the estrogen response element (ERE). One such unique site is the raloxifene response element (RRE). Activation of the RRE by benzothiophenes activates transcription of the TGF-β3 gene, an important regulator of bone remodeling. RRE activation may contribute to raloxifene's bone preserving effects [Yang et al., 1996]. AP-1 activity may further determine agonist/antagonist properties of the SERMs. For example, estrogen and tamoxifen stimulate uterine proliferation and activate AP-1 in uterine tissue. In contrast, raloxifene has no uterotrophic activity and does not activate AP-1 [Parker, 1995; Webb et al., 1995].

SERMs are formulated to elicit either estrogenic or antiestrogenic responses in different tissues, ideally antagonism in breast and uterine tissue and agonism in bone and the cardiovascular system [Baker and Jaffe, 1996; Grese and Dodge, 1996; Kauffman and Bryant, 1996]. The two major classes of synthetic SERMs include nonsteroidal triphenylethylenes (e.g., tamoxifen, toremifene, droloxifene, idoxifene) and the benzothiophenes (e.g., raloxifene, LY353381·HCl) [reviewed in Gradishar and Jordan, 1997].

Aromatase Inhibitors

Aromatase inhibitors modulate estrogen through inhibition of steroid aromatase, an enzyme which selectively catalyzes conversion of testosterone to estradiol or androstenedione to estrone, the final and rate-limiting step in estrogen synthesis [Cole and Robinson, 1990]. They are indicated for breast cancer treatment in postmenopausal women who have failed tamoxifen treatment. Aromatase is expressed in several tissues in women, including breast, ovary, adipose, liver, and muscle. In postmenopausal women, adipose tissue is the major source of circulating estrogens through aromatization of adrenal androstenedione [Santen, 1986; Simpson et al., 1994]. Within the breast, adipose tissue is the major extratumoral source of aromatase, although aromatase is detected in normal breast epithelial cells [Esteban et al., 1992]. It has been reported that aromatase activity is higher in adipose tissue from breast cancer patients than those with benign breast disease [Miller and Mullen, 1993]. Also, aromatase activity has been detected in both breast carcinoma and stromal spindle cells of breast tumors [Berstein et al., 1996; Esteban et al., 1992; Santen et al., 1994]. If aromatase expression correlates with local estrogen production, then adipose, breast epithelial, and breast carcinoma tissues may contribute to a high estrogen production within the breast.

Nonsteroidal aromatase inhibitors include aminoglutethimide, rogletimide, fadrozole, anastrozole (Arimidex®), letrozole, and vorozole [reviewed in Kelloff et al., 1998]. Of these, vorozole has potent chemopreventive activity in carcinogen-induced animal models of breast cancer. Steroidal irreversible inhibitors include formestane (4-hydroxyandrostenedione), exemestane, and atamestane. Exemestane is in early clinical breast cancer prevention trials.

Phytoestrogens: Isoflavones

Soybeans and more than 90 other plants have been shown to have estrogenic activity [Reinli and Block, 1996]. The primary phytoestrogens in soybeans are the isoflavones: genistein, daidzein, and glycitein and their glucoside conjugates genistin, daidzin, and glycitin. Preclinical data suggest that phytoestrogens are chemopreventive in breast cancer models. For example, N-methyl-N-nitrosourea (MNU)-treated mice fed a soy-enriched diet had decreased multiplicities and increased latencies of mammary gland tumors. Although several active components of soy can account for this effect, one component, genistein, has demonstrated efficacy in this animal model [Baggott et al., 1990; Barnes et al., 1990, 1994; Hawrylewicz et al., 1991].

At the cellular level, genistein competes with estradiol for binding to estrogen receptors [Shutt and Cox, 1972; Verdeal et al., 1980] and may stimulate estrogen responses, though less effectively than estradiol [Bickoff et al., 1962; Cheng et al., 1953; Folman and Pope, 1966; Markiewicz et al., 1993]. The in vitro effect of genistein is biphasic; low concentrations stimulate cell growth and estrogen-dependent gene expression, whereas higher concentrations inhibit cell growth [Wang et al., 1996]. Estrogen antagonistic effects of genistein may be mediated through the induction of sex hormone binding globulin (SHBG) [Mousavi and Adlercreutz, 1993], inhibition of aromatase [Campbell and Kurzer, 1993] and 17β-hydroxysteroid oxidoreductase activities [Makela et al., 1994, 1995], and impairment of the CNS or pituitary response to gonadotropins [Faber and Hughes, 1991; Kaldas and Hughes, 1989; reviewed in Adlercreutz and Mazur, 1997].

Estrogen Metabolism Modifiers: Indole-3-Carbinol

Epidemiological studies have shown that consumption of cruciferous vegetables such as broccoli, cauliflower, cabbage, and brussel sprouts is associated with decreased risk for cancer in humans [Young and Wolf, 1988]. One naturally occurring component of cruciferous vegetables is indole-3-carbinol (I3C). More specifically, I3C is an autolysis product of glucobrassicin that may reduce breast cancer incidence through modulation of cytochrome P450-dependent estradiol metabolism. Estradiol is metabolized via two competing pathways, 2-hydroxylation and 16α-hydroxylation. The products of the respective pathways have opposing effects. 2-Hydroxyestrone functions as an estrogen receptor antagonist, while 16α-hydroxyestrone covalently binds the estrogen receptor, decreases its degradation, and has estrogenic effects similar to estradiol. Increased estradiol 16α-hydroxylation has been associated with an increased risk for breast cancer in women [Schneider et al., 1982], and 16α-hydroxyestrone has been reported to be genotoxic to mammary cells [Swaneck and Fishman, 1991]. While attempts to directly decrease estradiol 16α-hydroxylation have been unsuccessful, enhancement of the alternate 2-hydroxylation pathway has resulted in a corresponding reduction of 16α-hydroxylation [Bradlow et al., 1994]. In rodents, I3C increases estradiol 2-hydroxylation under the same experimental conditions where it reduces mammary [Bradlow et al., 1991] and endometrial [Kojima et al., 1994] tumors. In humans, I3C has been shown to enhance estradiol 2-hydroxylation [Bradlow et al., 1994; Michnovicz et al., 1997]. Additionally, induction of phase II enzymes by I3C may increase estrogen conjugation and excretion [Guyton, 1991], and metabolites of I3C, such as indolo[3,2-b]carbazole (ICZ), may exhibit direct antiestrogenic activity by down-regulation of ER [Liu et al., 1994].

Breast Epithelium Proliferation and Apoptosis and the Menstrual Cycle

When cancer incidence is the primary prevention outcome, both cost and subject recruitment can be extremely limiting. However, cancer prevention trials may be conducted more expeditiously when intermediate biomarkers serve as surrogate endpoints (SEBs) for cancer incidence, thereby reducing trial size and duration. SEBs for breast cancer include markers that have been identified in breast epithelium, such as proliferation and apoptosis.

Since estrogen is a breast mitogen, estrogen modulators primarily exert protective effects by inhibiting epithelial cell proliferation and may induce apoptosis. A reduction in epithelial proliferation may be reflected by decreased breast density on mammograms or other imaging mode [Boyd et al., 1995a,b; Warner et al., 1992]. Additionally, cellular markers can be evaluated by tissue sampling with fine needle aspirates or core biopsies [Fabian et al., 1994; Zujewski et al., 1997]. However, since hormonal fluctua-

tions affect the breast epithelium, menstrual cycle phase should be taken into consideration for any SEB evaluation. Concentrations of estradiol, the most potent naturally occurring estrogen, varies up to 10-fold within the menstrual cycle with peaks in the follicular and luteal phases, the latter peak coinciding with a rise in progesterone. Estrone, an estrogen derived primarily from peripheral conversion of androstenedione, increases two-fold midcycle, coincident with an increase in androgen production [Speroff et al., 1994]. Several studies have evaluated physiologic variations in breast proliferation by menstrual phase. The greatest degree of proliferation is found in the luteal phase, which overlaps with elevations in estradiol and progesterone. The range of detectable proliferation varies with staining procedure and method of evaluation. In a study of healthy volunteers, repeat breast aspirates were stained for Ki-67/MIB-1, an antibody expressed during G_1, S, G_2, and mitosis. In the aspirates from premenopausal women, 2.3% of cells stained positive in the luteal phase compared with 1.1% of cells from the follicular phase, and proliferation was positively correlated with progesterone levels [Soderqvist et al., 1997]. A five-fold variation in proliferation between phases was found using an antibody specific for S phase, Ki-S5, in specimens obtained from women undergoing reduction mammoplasties. Higher overall proliferation rates were observed among women with family histories of breast cancer in first- and second-degree relatives [Olsson et al., 1996]. A 10-fold variation in breast epithelium proliferation was reported by thymidine labeling index studies in women with natural menstrual cycles and those on oral contraceptives [Going et al., 1988; Potten et al., 1988].

Apoptotic cells may be evaluated by morphologic features of nuclear condensation and DNA fragmentation or cellular staining for nicked DNA (TUNEL assay). In a study of surgical breast specimens where apoptotic cells were identified based on morphology and quantified as a portion of cells per lobule, a greater percentage of cells in the lobule were apoptotic at the end of the menstrual cycle, i.e., days 24–28 [Ferguson and Anderson, 1981]. Although other studies have evaluated apoptosis in normal breast tissue both by nicked DNA staining and morphology, they did not take into account the phase of the menstrual cycle [Allan et al., 1992; Hassan and Walker, 1998].

The expression of *bcl-2* has been used as a indirect correlate of apoptosis. Because *bcl-2* function is related to the cell's ability to overcome or delay apoptosis, *bcl-2* staining may inversely correlate with apoptotic activity [Hengartner and Horvitz, 1994]. Two studies have examined the expression of *bcl-2* over time in relation to the menstrual cycle. Sabourin et al. [1994] studied reduction mammoplasty specimens (n = 50) from premenopausal women, including data collected on menstrual phase acquired by questionnaire and body temperature recordings. Specimens obtained throughout the follicular phase had a progressive increase in acini and ductal cellular staining, peaking in tissues obtained from day 13 to day 17 of the menstrual cycle. The percentage of cells staining positively varied from 40–90% to <15% [Sabourin et al., 1994]. A late follicular phase peak in *bcl-2* staining was confirmed in a study of benign breast biopsies. The *bcl-2* staining was three- to four-fold greater in the ducts and lobules of specimens obtained in the follicular phase compared with those from the luteal phase [Ferrieres et al., 1997].

Estrogen Modulators, the Ovary, and Ovarian-Pituitary Axis

In the clinical development of an estrogen modulator for younger women, the effect of the agent on ovarian function, the ovarian-pituitary axis, and ovarian safety must be considered. The concerns include preservation of future fertility and safety from ovarian cancer risk, especially in those women with a genetic predisposition for both breast and ovarian cancer.

The risk of ovarian cancer from using estrogen modulators is largely speculative. Experience with tamoxifen in both breast and ovarian cancer treatment has been reassuring [Cook et al., 1995; Hatch et al., 1991], and clinical evidence for an ovarian cancer risk from tamoxifen is derived only from case reports [Cohen et al., 1996]. Another tamoxifen-associated ovarian pathology is the formation of ovarian cysts [Lindahl et al., 1997; Powles et al., 1994; Shushan et al., 1996]. Although the cysts associated with tamoxifen have been benign, they present challenges in making intervention decisions for women enrolled in clinical trials.

The etiology of ovarian cancer is poorly understood. Reduced risk of ovarian cancer is attributed to a decrease in lifetime ovulatory cycles, and factors of risk-reduction include multipar-

ity and oral contraceptive use [Cramer et al., 1983; Hildreth et al., 1981]. Our understanding of ovarian cancer pathogenesis is hindered by the lack of animal models that mimic human disease. Ovarian cancer is thought to originate when an initiated surface epithelial cell undergoes repetitive cell turnover, a process required for ovarian expansion to accommodate a maturing follicle [Godwin et al., 1993]. Local hormonal elements, including mitogenic gonadotropins, may act as initiators of carcinogenesis [Capen et al., 1995; Simon et al., 1983]. The age distribution of ovarian cancer, with a higher occurrence in postmenopausal women, has been attributed to increased gonadotropin levels with age, but epidemiologic data have not been conclusive [Helzlsouer et al., 1995]. Because our understanding of this disease is incomplete, development of the ideal estrogen modulator whose pharmacodynamic profile eliminates ovarian cancer risk remains a challenge. Premenopausal women in clinical trials of estrogen modulators should have hormonal levels monitored and ovarian ultrasound imaging. Some of the known effects of estrogen modulators on ovarian function are described below.

Evidence suggests that SERMs will cause an initial increase in ovarian steroidogenesis independent of gonadotropins. For some women on tamoxifen, an FSH and LH cycle may persist and ovulation may occur. In women who retain menses while on tamoxifen, serum estrogen and estradiol concentrations may be three- to five-fold greater than untreated controls. The increase occurs at the level of the ovary and is not associated with a rise in LH or FSH; levels of SHBG, prolactin, and progesterone do not increase out of the normal range [Jordan et al., 1991]. Results of several studies have shown depressed LH and FSH levels and increased SHBG levels with tamoxifen, toremifene, idoxifene, and droloxifene; these changes may be secondary to the increase in ovarian steroidogenesis [Coombes et al., 1995; Rauschning and Pritchard, 1994; reviewed in Gradishar and Jordan, 1997]. In primates, raloxifene disrupted cyclic ovarian activity and increased ovarian steroidogenesis within 90 days, concurrent with rising estradiol levels. A reversible impairment in fertility has been shown for raloxifene in rodents [Buelke-Sam et al., 1998]. However, in premenopausal women, raloxifene neither prevented ovulation nor changed the general pattern of LH and FSH, but it did affect the magnitude of the gonadotropin response [Baker et al., 1998].

The aromatase inhibitors do not consistently affect serum estrogen levels and have not been shown to increase gonadotropin levels. Vorozole is one of the few aromatase inhibitors reported to influence estrogen levels in premenopausal women; a single dose significantly reduced plasma estradiol levels 60% after 8 h [De Coster et al., 1990]. In contrast, formestane did not affect estrogen levels in premenopausal breast cancer patients even at the maximum tolerated dose [Dowsett et al., 1992]. The incomplete inhibition of ovarian estrogen production by aromatase inhibitors is not attributed to an early secondary rise in gonadotropins [Dowsett et al., 1992].

Premenopausal women on soy-enriched diets have shown effects on the hypothalamic-pituitary-gonadal axis resulting in variations in menstrual cycle length, suppressed midcycle gonadotropin surges, and changes in estradiol and progesterone concentrations [Cassidy et al., 1994, 1995, 1996; Lu et al., 1996a,b, 1997]. In controlled clinical studies, consumption of the isoflavones genistein and daidzein have been shown to lengthen menstrual cycles, especially the follicular phase [Cassidy et al., 1994; Lu et al., 1996a], suppress midcycle FSH and LH surges [Cassidy et al., 1994], and decrease or delay the peak progesterone concentrations [Cassidy et al., 1995; Lu et al., 1996a,b]. Serum estradiol has been shown to decrease in most of these studies [Lu et al., 1996a,b, 1997]; however, some increases have been found [Cassidy et al., 1994].

The Cardiovascular and Bone Benefits of Estrogen Modulators to Postmenopausal Women

The potential for mortality reduction from cardiovascular disease and osteoporosis and relief from the vasomotor symptoms of menopause has led approximately 20% of U.S. women to take hormone replacement therapy (HRT) [McNagny, 1997]. The clinical development of an estrogen modulator for breast cancer prevention in postmenopausal women will identify and compare its beneficial effects to those offered by HRT.

Cardiovascular Benefits

Among the many effects of estrogen on the cardiovascular system are an increase in the hepatic metabolism of lipids and lipoproteins.

Specifically, syntheses of apolipoprotein B (apo B) receptor, apolipoprotein A-1 (apo A-1), and very low density lipoprotein cholesterol (VLDL) are enhanced by estrogen. Increased apo B receptor accelerates the catabolism of apo B complexed to LDL, resulting in a decrease in LDL. The increase in apo A-1 and VLDL results in increased high density lipoprotein (HDL) and triglyceride levels, respectively [Love et al., 1977]. Other estrogen-mediated effects on the cardiovascular system are direct effects on the arterial smooth muscle wall, LDL-cholesterol oxidation, platelet aggregation, and insulin sensitivity [McGrath et al., 1998; Nasr and Breckwoldt, 1998]. Of the lipid indices, elevated LDL and decreased HDL have been associated with an increased risk for cardiovascular disease. A favorable effect on each of these indices reduces that risk [Brewer, 1989].

The effect of tamoxifen on the serum lipids and lipoproteins of postmenopausal women has been studied in women with and without breast cancer, and the effects are consistent in both groups. A decline in total cholesterol from 10% to 13% and LDL cholesterol from 17% to 23% is found within three to six months of beginning tamoxifen therapy. Although the decrease may be evident as early as three months, it may not plateau for at least six months [Love et al., 1991; Thangaraju et al., 1994]. Unlike estrogen therapy, tamoxifen does not affect total HDL cholesterol levels. However, when HDL cholesterol subfractions were evaluated, HDL_2 increased by 47% while HDL_3 and total HDL remained unchanged. Tamoxifen use for nine months causes a striking decrease in total serum lipoproteins of up to 60%. Opposite trends are seen when the two apolipoproteins are assayed. Apo B declines by 12%, while apo A-1 increases 11% to 16% [Elisaf et al., 1996; Morales et al., 1996]. Analysis of confounding factors for these lipid and lipoprotein changes indicates no relationship between age, smoking, or alcohol use [Bertelli et al., 1988]. In a randomized, placebo-controlled trial in which postmenopausal women without breast cancer received two years of treatment, tamoxifen changed the biochemical indices of lipids and lipoproteins to the same extent as in breast cancer patients. Changes in all parameters were noted at six months and remained stable at 24 months [Grey et al., 1995].

Ingestion of soybean proteins, from which the isoflavones are derived, reduces serum levels of total and LDL cholesterol [e.g., Carroll, 1991; Wagner et al., 1997]. The proposed mechanisms for this effect include enhancement of hepatic metabolism and effects on other hormones such as thyroid, insulin, and glucagon. A decreased rate of atherosclerotic plaque formation has also been associated with isoflavone intake. This effect may result from inhibition of LDL oxidation, platelet aggregation, and endothelial proliferation [Adlercreutz and Mazur, 1997; Parker, 1995; Wilcox and Blumenthal, 1995; Wiseman, 1996; Wiseman and O'Reilly, 1997].

A beneficial effect of tamoxifen on cardiovascular mortality has been shown in several randomized trials for breast cancer treatment [Constantino et al., 1997; Early Breast Cancer Trialsists Group, 1992; McDonald et al., 1995; Ragaz and Coldman, 1998; Rutqvist and Mattsson, 1993]. However, the NSABP P-1 prevention trial in high-risk women unaffected by cancer did not demonstrate a reduction in cardiovascular events [Fisher et al., 1998]. It is important to evaluate the relevance of lipid and lipoprotein measurements as SEBs for cardiovascular mortality in light of the recently completed HERS study that showed HRT lacked benefit in secondary prevention of cardiovascular disease. Although the women who received HRT had a 14% reduction in LDL cholesterol and an 8% increase in HDL cholesterol after four years of follow-up, cardiovascular outcomes were not reduced [Hulley et al., 1998]. These data confound the value of lipid measurements as SEBs as well as the role of HRT. Further clarification of HRT effects will come from results of The Women's Health Initiative, which includes younger women and women who receive unopposed estrogen [Women's Health, Initiative Study, Group, 1998].

Benefits to Bone Health

Osteoporosis is the occurrence of fractures as a result of a low bone mass. Bone loss in the spine after menopause occurs at a rate of 2–3% per year and may be prevented with estrogen replacement therapy [Lindsay and Cosman, 1997]. From the available data, the estrogen modulators under development all have the potential to prevent further bone loss. Furthermore, tamoxifen has demonstrated the ability to prevent bone fractures [Fisher et al., 1998].

Bone mineral density (BMD) and serum or urinary markers of bone turnover may be mea-

sured as SEBs to test the efficacy of an agent for osteoporosis prevention. Modalities that may be used to measure bone mass include single- and dual-photon absorptiometry, dual X-ray absorptiometry, and quantitative computed tomography [Dambacher et al., 1998; Jergas and Genant, 1997; Mole et al., 1998]. The superiority of one technique over another is not obvious from the literature [Grampp et al., 1997]. Serum markers of bone turnover include indices of bone resorption and formation. Markers of bone resorption include collagen degradation products measured in the urine (hydroxyproline, *N*-telopeptide, *C*-telopeptides, pyridinolone, and deoxypryidinolone) and measures of osteoclast activity (urinary calcium and serum acid phosphatase) [Minisola et al., 1998; Ross and Knowlton, 1998]. Alkaline phosphatase and osteocalcin are markers of bone formation and reflect osteoblastic activity; however, the latter may reflect either formation or resorption. Indices of bone resorption tend to respond within one to three months of an intervention, while those of bone formation lag behind [Christenson, 1997]. The urinary telopeptide markers, *N*-telopeptide and *C*-telopeptide, may be the most specific and sensitive markers of systemic osteoclast activity [Eyre, 1997].

THE NEED FOR MEASUREMENT OF BONE AND CARDIOVASCULAR ENDPOINTS OF ESTROGEN MODULATORS IN PREMENOPAUSAL WOMEN

Lipids

The effect of estrogen modulators on plasma lipids and lipoproteins in premenopausal women is less striking than in the postmenopausal cohort, which may be attributed to lower baseline levels. Tamoxifen treatment of premenopausal women decreases total lipids, total cholesterol, free cholesterol, free fatty acids, and LDL, and increases HDL, and triglycerides. All lipid and lipoprotein effects of tamoxifen are induced within three months of starting treatment, but do not peak until six months or more [Ilanchezhian et al., 1995]. A pronounced transient decrease in LDL-to-HDL cholesterol ratio occurs during the first six months of tamoxifen therapy, which may be secondary to an early hyperestrogenemia phase [Dnistrian et al., 1993]. Preclinically, raloxifene causes a significant reduction in serum cholesterol in ovary-intact rats, but, in contrast to ovariectomized rats, a clear dose-response effect is not

seen [Kauffman et al., 1997]. Soy proteins have significantly reduced total cholesterol levels throughout the menstrual cycle in premenopausal women; less significant reductions in triglycerides were seen [Cassidy et al., 1994, 1995; Petrakis et al., 1996].

Bone

In premenopausal women, the benefits or risks of an estrogen modulator may be more difficult to prove since bone mass is stable during this phase. Ideally, an estrogen modulator in this cohort would have little effect on bone remodeling. Presently, only limited data are available. Powles et al. [1996] found a 1.4% per year decline of vertebral bone mass in premenopausal women who received tamoxifen for three years. In contrast, vertebral bone mass in the placebo control group increased by 1.2% per year [Powles et al., 1996]. The effect of other estrogen modulators on bone turnover will be evaluated in future clinical trials. Preclinically, a negative effect of tamoxifen on dynamic measurements of cortical bone formation has been found in ovary-intact rats, in contrast to the preventive effect seen with ovariectomized rats. Such models may prove to be useful predictors of clinical behavior of estrogen modulators in bone [Sibonga et al., 1996]. For example, LY353381·HCl treatment protected ovariectomized rats from bone loss, and, in contrast to tamoxifen, no significant effect on bone mineral density was found in the ovary-intact rats [Rowley et al., 1997]. The isoflavones genistein and daidzein also have been studied in the rat model; bone loss is prevented by treatment with isoflavones in ovariactomized rats, but its effect in ovary-intact rats was not evaluated [Ishida et al., 1998].

The Uterine Effects of Estrogen Modulators

An increased risk for endometrial cancer with tamoxifen use was found in the NSABP P-1 trial with a relative risk ratio of 2.53 overall. Although an equivalent number of endometrial cancer cases occurred in the placebo group (pre- and postmenopausal), the risk associated with tamoxifen was only seen in the postmenopausal group [Fisher et al., 1998]. Newer generations of SERMs are especially promising because they lack uterotrophic activity and may even be estrogen antagonists in uterine tissue [Kauffmann and Bryant, 1996]. A favorable pharmacodynamic profile on uterine tissue is most

relevant for postmenopausal women, because the disease is more prevalent in this cohort [Burke et al., 1997].

CONCLUSION

The development of estrogen modulators for breast cancer chemoprevention is complex because of the myriad physiologic functions affected by estrogen. This review has addressed some of the concerns which can be monitored in a clinical trial. In the premenopausal cohort, the effect of the agent on the ovary and ovarian-pituitary axis should be monitored; one should proceed with caution in high-risk groups. Since

premenopausal women have levels of estrogen that protect against bone loss and cardiovascular damage, the potential agent should not adversely affect these parameters. In the postmenopausal cohort, any intervention initiated to prevent breast cancer should be considered relative to the risks and benefits of HRT [Gibaldi, 1997; Parker et al., 1997; Skafar et al., 1997]. Successful interventions with estrogen modulators must improve the aggregate risk-benefit profile seen with HRT. Measuring SEBs in clinical trials may be useful in predicting safety relative to osteoporosis and cardiovascular disease (Table I). Other concerns that have not been addressed, and for which reliable SEBs are not known, are effects on neuropsychiatric function and risk of thromboembolism, which is seen in both tamoxifen treatment and prevention settings and is related to estrogen agonist activity, since it also occurs with HRT [Fisher et al., 1998; Hulley et al., 1998; McDonald et al., 1995; Pritchard et al., 1996]. Although depressed antithrombin III levels have been associated with risk of thromboembolism, a consistent correlation has not been found [Sismondi et al., 1994]. As the field advances, the reliability of current SEBs will improve, and testing modalities will be developed to address the overall health of women on estrogen modulators for breast cancer chemoprevention.

TABLE I. Recommended SEBs for Phase I/II Clinical Trials of An Estrogen Modulator

Site	Method	Surrogate endpoint biomarkers
Breast	FNA or core biopsy	proliferation (Ki-67/MIB-1)
		apoptosis (TUNEL, *bcl*-2)
Bone	Nuclear scan	bone mineral density (BMD)
		single- and dual-photon absorptiometry, dual X-ray absorptiometry, or quantitative computed tomography
	Urine	hydroxyproline
		N-telopeptide
		C-telopeptides
		pyridinolone
		deoxypryidinolone
		calcium
	Blood	acid phosphatase
		alkaline phosphatase
		osteocalcin
Cardiovascular System	Blood	cholesterol
		HDL
		triglyceride
		LDL
		VLDL
		coagulation profile (PT, PTT, fibrinogen)
Ovary	Blood	FSH
		LH
		estradiol
		progesterone
		SHBG
	Imaging	Transvaginal Vaginal Ultrasound (TVUS)
Uterus	Imaging	TVUS
	Endometrial sampling	histology

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