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The Combination of a Novel Selective Estrogen Receptor Modulator with an Estrogen Protects the Mammary Gland and Uterus in a Rodent Model: The Future of Postmenopausal Women's Health?

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The Women's Health Initiative Study and other reports have created major uncertainty among postmenopausal women and physicians concerning hormone replacement therapy. We have thus investigated the possibility of replacing the progestin in hormone replacement therapy by a novel selective estrogen receptor (ER) modulator having potent and pure antiestrogenic activity in the mammary gland and uterus. As measured by changes in histology and Cdc47 labeling in the rat model, the present study shows that the stimulatory effect of estradiol in the mammary gland and uterus is efficiently blocked by simultaneous administration of the novel selective ER modulator EM-652, but bone mineral density is preserved and serum cholesterol is decreased. After the administration of 14C-labeled EM-652, we observed that there is no detectable radioactivity in the brain. Moreover, ERα immunoreactivity remained constant in the hypothalamus after EM-652 treatment, whereas ERα became almost undetectable in the mammary gland and uterus. The present data show the poor or absent access of EM-652 to the brain, whereas the effects of estrogens are efficiently neutralized in the mammary gland and uterus. Such data support the exciting possibility of a novel approach that could meet most of the needs of women's health at menopause, namely control of hot flushes and prevention of breast, uterine, and ovarian cancer as well as osteoporosis and potentially helping brain function and preventing Alzheimer's disease with no identifiable risk or negative effect. (*Endocrinology* 144: 4700–4706, 2003)

THERE IS NO medical problem with a higher negative impact on women's health than menopause. In fact, the rapid decrease in circulating 17β-estradiol (E_2) at menopause, coupled with the demonstrated beneficial effects of exogenous estrogens on menopausal symptoms (1, 2) and bone resorption (3–6), has focused most of the efforts on various forms of estrogens as well as to combinations of estrogen and progestin as hormone replacement therapy. The progestin should avoid the risk of endometrial cancer induced by estrogens administered alone. This classical approach, however, has recently been seriously questioned or even abandoned by a large proportion of women after new data indicating that the combination of Premarin and Provera (Prempro) causes a 26% increase in the incidence of breast cancer at 5.2 yr of follow-up with a potential negative impact on cardiovascular events (4). Moreover, the same study has shown that following Prempro, breast cancer was more difficult to detect due to an increase in the percentage of women with abnormal mammograms, thus resulting in cancer being diagnosed at a more advanced stage, compared with the placebo group (7).

The selective estrogen receptor (ER) modulator (SERM) EM-652 is a benzopyran derivative originally developed for the prevention and treatment of breast cancer (8–14). This orally active antiestrogen is the only SERM known to exert pure antiestrogenic activity in the mammary gland and endometrium in the rat, monkey, and mouse (13–15) as well as in human breast and uterine carcinoma cells *in vitro* (8, 11, 12) and *in vivo* in nude mice (10, 15). In fact, EM-652 is the compound having the highest affinity of all known compounds for the ER (8, 14, 16, 16a). It exerts pure antiestrogenic activity on both ERα and ERβ (13). It is of interest to mention that despite its pure antiestrogenic activity and ability to completely block estrogenic action in the mammary gland and endometrium, EM-652 inhibits bone loss and decreases serum cholesterol after ovariectomy in the rat, the effect being achieved at a 5- to 10-fold lower concentration or dose than raloxifene (17).

Because E_2 is well recognized to exert beneficial effects on vasomotor symptoms or hot flushes and there are additional potential benefits of estrogens on cognition, memory, and Alzheimer's disease (18–22), a potentially ideal regimen for women's health at menopause could be the use of an estrogen in combination with EM-652, a pure antiestrogen having no or minimal access to the brain. Using this approach, the estrogen would remain free to control hot flushes, the main reason women consult their physician at menopause, with the potential added benefits on cognitive functions, memory, and Alzheimer's disease, whereas the risks of breast and uterine cancer would be eliminated by the antiestrogen that would simultaneously prevent bone loss and fractures and improve the lipid profile.

Abbreviations: E_2, 17β-Estradiol; ER, estrogen receptor; OVX, ovariectomized; SERM, selective ER modulator; WHI, Women's Health Initiative.

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The present study was performed to validate this promising novel approach using well-recognized parameters of estrogen action in the rat mammary gland and uterus. The data show that the stimulatory effects of estradiol in the uterus and mammary gland are very efficiently neutralized by the coadministration of EM-652. The effect is explained by the particular high affinity of EM-652 for the ER (8, 14, 16, 16a), thus neutralizing the effect of the estrogen in the mammary gland and uterus but not in the brain.

Materials and Methods

Animals and treatment

Ten- to 12-wk-old female Sprague Dawley rats [Crl:CD(SD)Br] (Charles River Laboratory, St. Constant, Canada) weighing 215–265 g at time of ovariectomy were used. The animals were housed individually in an environmentally controlled room (temperature 22 ± 3 C; humidity 50 ± 20%; 12-h light,12-h dark cycles, lights on at 0715 h). The animals were allowed free access to tap water and a certified rodent feed [Lab Diet 5002 (pellet), Ralston Purina, St. Louis, MO]. The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care and the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with the Canadian Council on Animal Care Guide for Care and Use of Experimental Animals.

Three similar experiments were performed. In the first one, the animals were randomly distributed into 11 groups of 14 animals as follows: group 1, intact control; group 2, ovariectomized (OVX) control; group 3, OVX + E_2 (1 mg/kg); group 4, OVX + EM-652 (1 mg/kg); and groups 5–11, OVX + E_2 (1 mg/kg) + EM-652 (0.0001, 0.001, 0.01, 0.1, 1, 10, or 100 mg/kg, respectively). On the first day of the study, the animals of the appropriate groups were bilaterally ovariectomized under isoflurane anesthesia. The tested compounds were then given once daily by oral gavage as a suspension in 0.4% methylcellulose (0.5 ml/rat) from d 2 to wk 35 of the study. Animals of groups 1 and 2 received the vehicle alone during the same time period.

In the second experiment, the duration of treatment was 14 d and the daily dose of E_2 was 2 mg/kg; the lowest dose of EM-652 was 0.01 mg/kg and the highest dose was 10 mg/kg. On d 15 of the study, four animals per group were perfused with 10% buffered formalin, and tissues were processed for histological examination. The other animals were killed by exsanguination at the abdominal aorta under isoflurane anesthesia. The uterus was removed, stripped of adhering fat, and weighed.

In a third similar experiment, five groups of 14 animals were used. The duration of the treatment was 6 months, and the daily dose of E_2 was 1 mg/kg and the EM-652 dose was 2.5 mg/kg.

Immunohistochemistry

Immunostaining was performed using an SP kit (Zymed, San Francisco, CA). Paraffin sections of 4 μm were deparaffinized in toluene and rehydrated through ethanol. Endogenous peroxidase activity was eliminated by preincubation with 3% H_2O_2 in methanol for 20 min. A microwave antigen retrieval technique using citrate buffer was used (24). After cooling the slides, nonspecific binding sites were blocked by incubation with 10% goat serum for 30 min. Sections were then incubated with a mouse anti-Cdc47 antibody (NeoMarkers, Lab Vision Corp., Fremont, CA) diluted 1:300 or with a monoclonal anti-ERα (Oncogene, Boston, MA) diluted 1:150 for 1 h at room temperature, washed in PBS buffer, and incubated with biotinylated antimouse secondary antibody for 10 min and thereafter with streptavidin-peroxidase for another 10 min. Diaminobenzidine was used as the chromogen to visualize the biotin/streptavidin-peroxidase complex, under microscope monitoring. Counterstaining was performed using no. 2 Gill's hematoxylin for 1 min. In the negative controls, the primary antibody was replaced by nonimmune mouse IgG (Vector Laboratories, Burlingame, CA) at a concentration similar to that of the primary antibody.

For the brain, after 4% paraformaldehyde perfusion, 20 μm frozen sections were immunostained with a rabbit anti-ERα antibody diluted 1:1000 (MC-20, Santa Cruz Biotechnology, Santa Cruz, CA) according to the same method mentioned above.

ER assay

Uterine tissue was homogenized in 10 volumes of buffer A [25 mM Tris-HCl, 1.5 mM EDTA, 10 mM α-monothioglycerol, 10% glycerol, 10 mM sodium molybdate (pH 7.4)], and the homogenates were centrifuged at 105,000 $\times$ g for 60 min. The steroid-binding assay was performed with freshly prepared cytosol. $[^3H]E_2$ binding was measured using the dextran-coated charcoal absorption technique as previously described (16a, 25).

Tissue distribution of radioactivity after oral administration of [^{14}C] EM-652 in the rat

A single oral dose of [^{14}C] EM-652 (lot no. SA-332–16) was administered at the dose of 13 mg/kg in 0.4% aqueous methylcellulose (2 ml/kg). Radioactivity was then measured in the blood and tissues at various time intervals ranging from 4–168 h. The study was performed under good laboratory practice conditions.

Statistical analyses

Data are expressed as the means ± SEM. Statistical significance was determined according to the multiple-range test of Kramer (26).

Results

Inhibition of the stimulatory effect of E_2 in the uterus

The 84% reduction in uterine weight observed 35 wk after ovariectomy was completely reversed by daily oral administration of E_2 at the 1-mg/kg dose, whereas increasing doses of EM-652 led to a progressive inhibition of the stimulatory effect of E_2 (Fig. 1). In fact, a 39% inhibition of the effect of E_2 was already observed at the low 0.01-mg/kg dose of the antiestrogen, and 64, 86, and 91% inhibitions were observed at the 0.1-, 1-, and 10-mg/kg doses, respectively.

Because both E_2 and EM-652 are known to prevent bone loss after ovariectomy (17), an important question is whether the combination of both compounds will maintain the beneficial effects on bone mineral density. As can be clearly seen in Fig. 2, although E_2 and EM-652 used alone have compa-

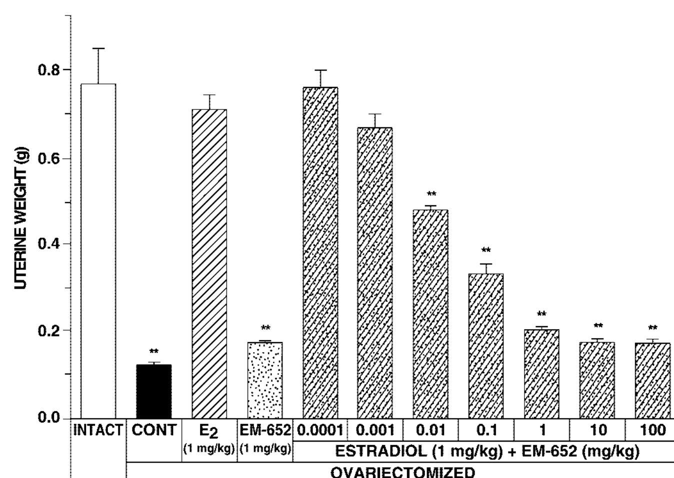

FIG. 1. Effect of increasing daily doses of EM-652 for 35 wk on estradiol-stimulated uterine weight. Animals were ovariectomized and received either E_2 alone at the daily oral dose of 1 mg/kg or the same dose of E_2 in association with increasing daily doses of EM-652 ranging from 0.0001–100 mg/kg. For comparison, a group of animals were left intact and one group of OVX animals received EM-652 alone at the dose of 1 mg/kg. Data are presented as means ± SEM. *, $P < 0.05$; **, $P < 0.01$ experimental *vs.* the group of OVX animals treated with E_2.

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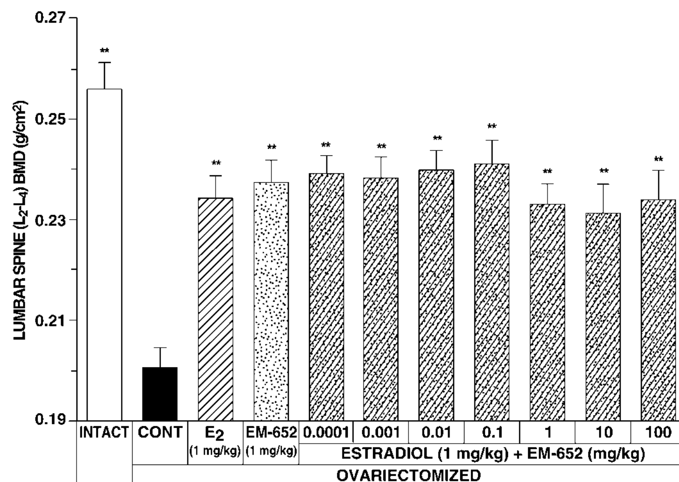

FIG. 2. Effect of increasing doses of EM-652 in combination with E_2 (1 mg/kg) on lumbar spine bone mineral density (BMD). The experimental conditions are as described in the legend of Fig. 1. **, $P < 0.01$ experimental *vs.* control OVX.

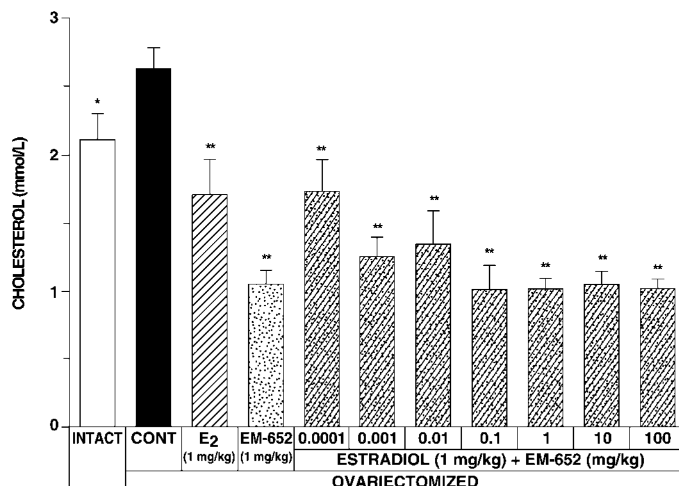

FIG. 3. Effect of increasing doses of EM-652 in combination with E_2 (1 mg/kg) on serum cholesterol. The experimental conditions are as described in the legend of Fig. 1. *, $P < 0.05$; **, $P < 0.01$, experimental *vs.* control OVX.

rable protective effects on bone mineral density, the combination of E_2 (1 mg/kg) with a large range of doses of EM-652 led to bone mineral density values indistinguishable from E_2 or EM-652 alone. On the other hand, the inhibitory effect of E_2 on serum cholesterol (17) is not only maintained but also further increased when daily doses of EM-652 of 0.001 mg/kg or higher are used in combination with E_2 (Fig. 3), thus suggesting that EM-652, at a very low dose (0.001 mg/kg or higher), predominates over E_2 in the regulation of serum cholesterol levels.

To obtain more information on the interactions between the estrogen and EM-652, we next examined the changes in Cdc47 labeling (27) as well as the levels of ERα in the endometrium and mammary gland after 2 wk of treatment with a fixed amount of E_2 and increasing doses of EM-652.

The preventative action of EM-652 on the stimulatory effect of E_2 on the endometrial epithelial cells is well illustrated in Fig. 4. In fact, when the Cdc47 was used as a marker of cell proliferation (Fig. 4, A–E), almost all the nuclei of the epithelial cells lining the lumen and glands were labeled in the intact animals (Fig. 4A), thus indicating cycling cells. The absence of estrogen in OVX animals caused a marked inhibition of cell proliferation, thus resulting in the absence of Cdc47 labeling in almost all nuclei (Fig. 4B). E_2 treatment, on the other hand, restored cell proliferation (Fig. 4C), whereas addition of the low dose of 0.01 mg/kg EM-652 to E_2 markedly inhibited Cdc47 labeling (Fig. 4D). On the other hand, when the animals received a dose of 1 mg/kg of EM-652, an almost complete inhibition of labeling, similar to that in OVX animals, was observed (Fig. 4E).

When ERα labeling was examined in the same groups of animals (Fig. 4, F–J), the nuclei of all the endometrial epithelial cells of intact, OVX and E_2-treated OVX animals were labeled (Fig. 4, F–H). A significant decrease in ERα labeling was already observed at the 0.01-mg/kg dose of EM-652 (Fig. 4I), whereas a near-complete absence of labeling was seen at the 1-mg/kg dose of the SERM (Fig. 4J). The inhibition of ERα levels observed by immunocytochemistry is clearly confirmed by direct measurements of $[^3H]E_2$ binding in uterine cytosol (Fig. 5). In fact, $[^3H]E_2$ binding is already 52% inhibited at the 0.03-mg/kg dose of EM-652, whereas an 86% inhibition is achieved at 0.1 mg/kg. Higher doses of the SERM led to even greater inhibition of ERα.

Inhibition of the stimulatory effect of estrogen in the mammary gland

Effects comparable with those found in the uterus were observed in the mammary gland: although E_2 stimulated the development of the acini (Fig. 6H) to a level at least as advanced as that seen in intact animals (Fig. 6F), the addition

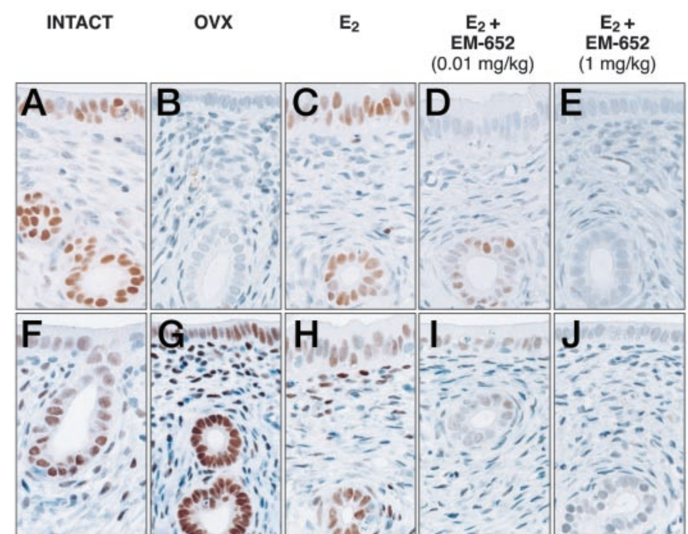

FIG. 4. Effect of daily oral treatment of OVX animals for 2 wk with E_2 (2 mg/kg) alone (C and H) or with estradiol in combination with 0.01 mg/kg (D and I) or 1.0 mg/kg (E and J) of EM-652 on Cdc47 (A–E) and ERα (F–J) labeling in the uterus. Intact (A and F) and OVX (B and G) animals are presented as controls (×600). In all the photographs, the epithelial cells lining the endometrial lumen are seen in the *upper part*, whereas the glands are located in the *middle* or the *lower part* of photographs.

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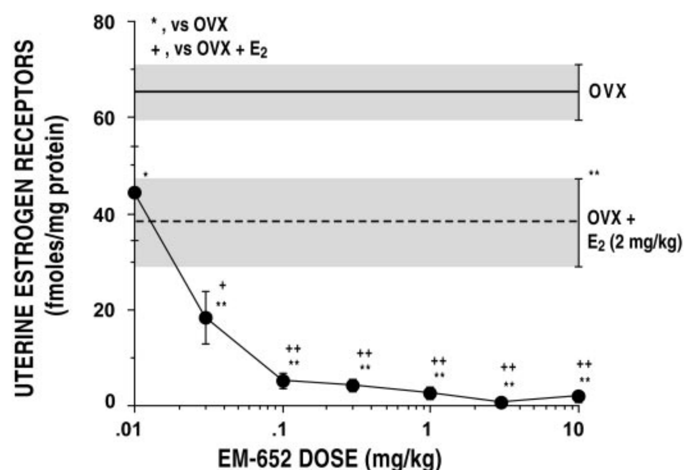

FIG. 5. Effect of increasing doses of EM-652 for 2 wk on [^{3}H]E_2 binding in the rat uterus. The experimental conditions are as described in the legend of Fig. 4. *, $P < 0.05$; **, $P < 0.01$, experimental *vs.* control OVX.

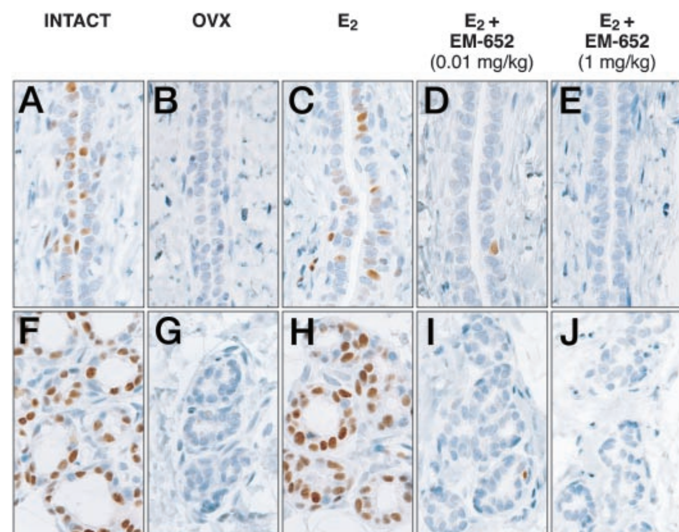

FIG. 6. Effect of daily oral treatment of OVX animals for 2 wk with estradiol (2 mg/kg) alone (C and H) or in combination with 0.01 mg/kg (D and I) or 1 mg/kg (E and J) of EM-652 on Cdc47 labeling in mammary gland ducts (A–E) and acini (F–J). Intact (A and F) and OVX (B and G) animals are presented as controls. (×600).

of the antiestrogen at the dose of 0.01 mg/kg to E_2 reduced the acini to an atrophic pattern (Fig. 6I) not different from that seen after ovariectomy (Fig. 6G). It is of interest to mention that the level of atrophy and development of the mammary gland acini and ducts are closely correlated with cell proliferation estimated by Cdc47 labeling. Thus, the atrophic acini of OVX animals were associated with an almost complete absence of Cdc47 labeling (Fig. 6G), whereas the vast majority of cell nuclei in the developed acini of intact (Fig. 6F) or OVX animals treated with E_2 (Fig. 6H) were labeled. When the low dose (0.01 mg/kg) of EM-652 was combined with E_2, few labeled nuclei were observed in the atrophic acini (Fig. 6I), and an almost complete absence of labeling was achieved at the 1.0-mg/kg dose of EM-652. A similar Cdc47 labeling pattern was observed in the ducts (Fig. 6, A–E).

As illustrated in Fig. 7, ERα labeling in both the ducts and

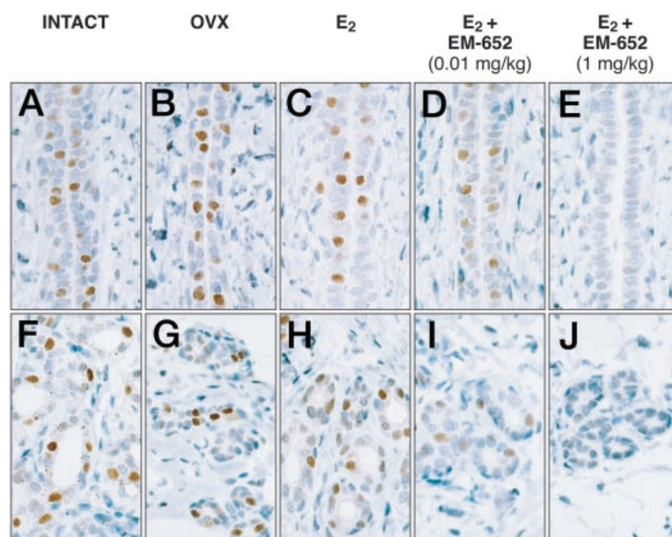

FIG. 7. Effect of daily oral treatment of OVX animals for 2 wk with E_2 (2 mg/kg) alone or in combination with EM-652 on ERα labeling in the mammary gland ducts (A–E) and acini (F–J). The experimental conditions are as described in the legend to Fig. 6 (×600).

acini was slightly decreased when EM-652 at the dose of 0.01 mg/kg was added to E_2 (Fig. 7, D and I), but an almost complete disappearance of ERα labeling was observed at the 1.0-mg/kg dose of the SERM (Fig. 7, E and J).

Although the above-described data clearly show the ability of a low dose of EM-652 to neutralize the effect of E_2 in the mammary gland and uterus, it was important to assess the access of EM-652 in the brain. After single oral administration of [^{14}C] EM-652 (13 mg/kg), most tissues of the rat reached maximal concentrations of radioactivity 6 h post dose. At that time interval, radioactivity content in the brain was below the limit of detection or less than 0.019 pg equivalent of [^{14}C] EM-652/g tissue, but values of 1.40, 1.11, 1.08, and 0.531 pg equivalent of [^{14}C] EM-652/g tissue were measured in the lung, adrenal, pituitary gland, and kidney, respectively. Such data indicate that at least 50-fold lower concentration of [^{14}C] EM-652-derived radioactivity reaches the brain, compared with the peripheral tissues.

As illustrated in Fig. 8, no apparent change in the immunoreactivity of ERα was observed in the hypothalamic area after treatment with EM-652, but the same treatment led to an almost complete disappearance of ERα immunoreactivity in the uterus (Figs. 4J and 5) and in both the ducts and acini of the mammary gland (Fig. 7, E and J).

Discussion

The present data clearly demonstrate that a low dose of EM-652, a novel SERM possessing pure and highly potent antiestrogenic activity in the mammary gland and uterus, neutralizes the stimulatory effect of E_2 in these two tissues of main interest for women at menopause. The histological findings are well supported by Cdc47 labeling, a reliable marker of cell proliferation. Such data clearly support the hypothesis that the concomitant administration of EM-652 in postmenopausal women receiving estrogen for the relief of vasomotor symptoms, nervousness, sleeplessness, cognition,

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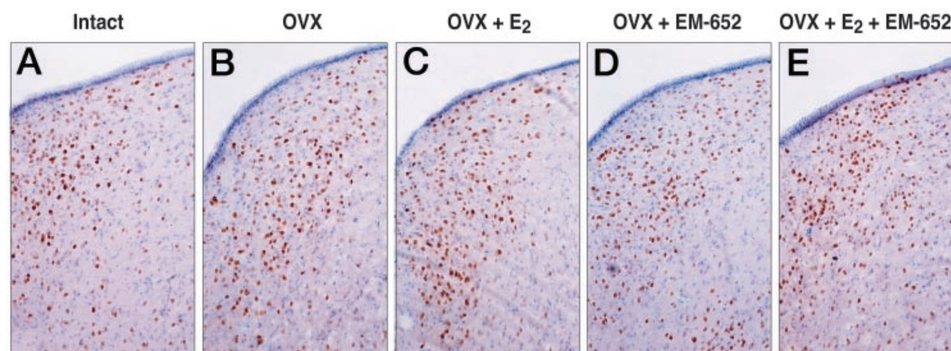

FIG. 8. Frozen sections of the basomedial region of the hypothalamus immunostained with an antibody to ERα. Intact animals (A), OVX animals (B), OVX animals that received a daily oral dose of E_2 (1 mg/kg) for 6 months (C), a daily oral dose of EM-652 (2.5 mg/kg) for 6 months (D), or a combination of E_2 + EM-652 for the same duration of treatment (E). The ependymal cells lining the third ventricle are seen in the *upper part* of the photographs. The ERα immunoreactivity is exclusively localized in the nuclei and a similar labeling pattern is seen in all the different groups (×150).

and prevention of memory loss and Alzheimer's disease should block the stimulatory effect of the estrogen on the breast and endometrium, thus preventing breast and endometrial cancer.

The Women's Health Initiative (WHI) study is the first randomized, controlled trial to show in a prospective study that an estrogen plus progestin increases the risk of breast cancer in postmenopausal women, the increased risk being 26% at 5.2 yr of follow-up (4). It should be mentioned that although progestins are known to be effective in protecting the endometrium against the stimulatory effect of estrogens, convincing preclinical and clinical data have indicated for some time that progestins have a negative impact on breast cancer (28–30). In fact, progesterone has been shown to stimulate the proliferation of murine mammary epithelium, this effect being additive to that of estrogens (31). Moreover, some studies have reported that human mammary epithelial proliferation is increased during the luteal (high progesterone) phase of the menstrual cycle (16a, 27–29). Finally, the recent results of the WHI study have shown that the combination of estrogen plus progestin increased the incidence of breast cancer, which was more difficult to detect because of an increase in the percentage of women with abnormal mammograms (7). The cancer was then diagnosed at a more advanced stage, compared with the placebo group (7).

The WHI data, in fact, are consistent with pooled epidemiological observations, which indicated a 15% increased breast cancer risk in women who received estrogen plus progestin for less than 5 yr and a 53% increased risk for longer-term use (32). In support of the WHI study, many epidemiological studies have indicated that women who take estrogen plus progestin are at greater risk of breast cancer than women who take estrogen alone (33–36). On the other hand, in the Heart and Estrogen/progestin Replacement Study, a statistically nonsignificant 27% increase in breast cancer risk was observed at 6.8 yr of follow-up in the group of women who took estrogen plus progestin (37). In fact, the WHI study continues for the hysterectomized women taking estrogen alone (4). This fear of breast cancer is added to uterine bleeding, breast tenderness, and fluid retention known to be associated with hormone replacement therapy (3, 4).

EM-652 has already shown characteristics superior to those of all other known SERMs. This compound is the most potent to inhibit mammary gland and endometrial estrogen-stimulated growth (10–15, 17, 38–41). In phase II trials, EM-652 has shown a significant rate of positive responses in women with breast cancer who had failed under tamoxifen (14), thus indicating the superiority of this compound over tamoxifen in women with breast cancer.

Although, so far, EM-652 has been studied only in women who had failed tamoxifen, two other SERMs, namely tamoxifen and raloxifene, have been shown, in a preventive setting, to reduce the incidence of breast cancer in women (42, 43). Contrary to EM-652, tamoxifen and raloxifene, however, possess some intrinsic estrogenic activity in the mammary gland and uterus: in fact, both have been shown to exert some stimulatory effect on the proliferation of human breast cancer cells *in vitro* in the absence of estrogen (12). Moreover, both compounds cause a significant stimulation of estrogen-sensitive alkaline phosphatase activity in human endometrial carcinoma cells (11) and stimulate the rat uterus (17, 44). In agreement with preclinical data, tamoxifen has been found to increase the risk of advanced aggressive endometrial cancer (45). Moreover, tamoxifen does not decrease the level of ER in breast cancer cells (46–48), whereas the present data show that EM-652 decreases ER levels in both the mammary gland and endometrium. In analogy with the nonsteroidal antiestrogen EM-652, the estradiol-derived antiestrogens ICI 164384 and ICI 182780 increase ER degradation, thus contributing to the inhibition of estrogen action (49).

EM-652 is thus the only known SERM having no estrogenic activity in the mammary gland and uterus in the absence of estrogen. Moreover, this compound has been shown to completely block the estrogenic stimulatory effect of tamoxifen and raloxifene in human breast cancer xenografts and endometrial carcinoma Ishikawa cells (10, 11). An important consequence of the pure and potent antiestrogenic activity of EM-652 is that this compound permits a more complete inhibition of growth of human breast cancer tumors *in vivo* in nude mice (15), thus suggesting the possibility of a more rapid and more complete inhibition of breast cancer development and growth and a reduced likelihood of development of resistance to treatment.

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By far, the most frequent reason women consult their physician at menopause is the occurrence of hot flushes, nervousness, and sleeplessness, a problem efficiently treated by estrogen replacement therapy. Because the site responsible for hot flushes is believed to be the brain and EM-652 has poor access to the brain, it is expected that the administration of estrogens to postmenopausal women will control hot flushes and potentially help cognitive functions and memory as well as prevent Alzheimer's disease without interference by the antiestrogen. On the other hand, as well demonstrated in the present study, EM-652 should eliminate the negative effects of estrogens in tissues situated outside the brain, especially the risk of breast and uterine cancer.

A widely used preparation for oral estrogen replacement therapy is conjugated equine estrogens. These preparations are a combination of up to 10 different estrogenic compounds (50). In a similar experiment using OVX female rats after the administration of a daily oral dose of Premarin (0.5 mg/kg) alone or in combination with EM-652 (2.5 mg/kg) for 9 months, we found that although ERα immunoreactivity was strong in the OVX and intact control groups, it was decreased in the uterus and mammary glands of the two experimental groups who received EM-652 (data not shown). When the ERα immunoreactivity was examined in the hypothalamus, the strong labeling was not decreased by EM-652, thus showing results similar to Fig. 8.

The proposed novel approach not only eliminates the negative effects of estrogens on the breast, uterus, and ovary but also eliminates the need to use a progestin to protect against endometrial proliferation, thus avoiding the recently demonstrated stimulatory effect of progestins on breast cancer (4, 7, 28–30, 33–36, 51, 52). EM-652, due to its high affinity for the ER, should also avoid the risk of ovarian cancer recently reported to be associated with long-term use of estrogens (53–55). In fact, two recent studies have indicated that the use of estrogen replacement therapy for 10 yr or more increased the risk of ovarian cancer (53, 55). Moreover, a recent Swedish study (54) has reported that estrogen used alone and estrogen plus progestin used sequentially may be associated with an increased risk of ovarian cancer, whereas no such risk was associated with the continuous use of the combination pill.

Because of its highly potent and pure antiestrogenic activity, EM-652 should not only eliminate the risk of breast, uterine, and ovarian cancer associated with estrogen use (4, 49–51), but it should also reduce the spontaneous incidence of these cancers, which are diagnosed in 13.3% (breast cancer), 2.7% (endometrial cancer), and 1.7% (ovarian cancer) of women during their lifetime.

The present data support the exciting possibility of a novel strategy that could well provide an appropriate answer to a series of important needs identified in postmenopausal women. In fact, if the present data obtained in the rat model become a reality in women, this tissue-specific hormone replacement therapy could well meet the most important needs of women at menopause, namely control of hot flushes; potential improvement of cognitive functions and memory (56); decrease in the risk of Alzheimer's disease; and, most importantly, preventing breast cancer, uterine cancer, ovarian cancer, and bone loss.

Acknowledgments

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