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INHIBITORY EFFECT OF THE NOVEL ANTI-ESTROGEN EM-800 AND MEDROXYPROGESTERONE ACETATE ON ESTRONE-STIMULATED GROWTH OF DIMETHYLBENZ[*a*]ANTHRACENE-INDUCED MAMMARY CARCINOMA IN RATS

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The novel anti-estrogen EM-800 and medroxyprogesterone acetate (MPA) inhibit estrone (E_1)-stimulated growth of dimethylbenz[*a*]anthracene (DMBA)-induced mammary tumors in a rat model. After 65 days, ovariectomy (OVX) decreased total tumor area to 9.6 ± 3.9% of initial size, while E_1 (1.0 µg, s.c., twice daily) stimulated tumor growth to 225 ± 40.9% of initial size. Daily oral administration of 2.5 mg/kg body weight of EM-800 completely abolished E_1-stimulated tumor growth. A low daily dose of EM-800 (0.25 mg/kg body weight) or MPA (1 mg, s.c., twice daily) used alone partially reversed the stimulatory effect of E_1 on the growth of DMBA-induced tumors. The combination of both compounds, however, caused a more potent inhibitory effect than each compound used alone. A high dose of EM-800 completely or almost completely inhibited the E_1-stimulated vaginal and uterine weights, respectively. The same dose of EM-800 completely reversed the inhibitory effect of E_1 on serum luteinizing hormone levels. Uterine, vaginal and tumoral estrogen and progesterone receptor levels were reduced markedly following treatment with EM-800. Our data show that the combination of the pure anti-estrogen EM-800 with the androgenic compound MPA achieves greater inhibition of the growth of DMBA-induced mammary carcinoma than that achieved by each compound used alone. ***Int. J. Cancer*** **73:580–586, 1997.**

Estrogens play a predominant role in breast cancer development and growth (McGuire *et al.,* 1975; Poulin and Labrie, 1986). Thus, special attention has focused on the mechanisms of action of estrogens and on the development of blockers of estrogen biosynthesis and action at the target cell level since the existing ablative procedures, either surgical or medical, do not permit complete elimination of estrogens (Miller, 1987; Wells and Santen, 1984).

Since the first step in the action of estrogens in target tissues is binding to the estrogen receptor, a logical approach for the treatment of estrogen-sensitive breast cancer is the use of anti-estrogens, or compounds that block the interaction of estrogens with their specific receptor. In fact, inhibition of estrogen action with the anti-estrogen tamoxifen is the standard therapy for breast cancer, at all stages of the disease (Furr and Jordan, 1984; Swedish Breast Cancer Cooperative Group, 1996). Unfortunately, tamoxifen possesses some estrogenic activity, a characteristic that potentially limits its efficacy in breast cancer therapy in women (Poulin *et al.,* 1989*b;* Wakeling *et al.,* 1991).

In support of the clinical data showing an inhibitory effect of androgens on breast cancer, we have demonstrated the direct anti-proliferative effect of androgens on the growth of hormone-sensitive ZR-75-1 human breast cancer cells *in vitro* (de Launoit *et al.,* 1991; Poulin *et al.,* 1988; Simard *et al.,* 1990, 1992, 1989) under both basal and estrogen-induced incubation conditions as well as *in vivo* using the same human breast cancer cell line in nude mice (Dauvois *et al.,* 1991). Medroxyprogesterone acetate (MPA) is a synthetic ''progestin'' that binds to the androgen, glucocorticoid as well as to progestin receptors (Labrie *et al.,* 1987; Poulin *et al.,* 1989*a,* 1991). This compound is used frequently in the endocrine therapy of advanced breast cancer in women (Haller and Glick, 1986). MPA has been shown to inhibit the growth of dimethylbenz[*a*]anthracene (DMBA)-induced mammary carcinoma in rats (Dauvois *et al.,* 1989*b*). Moreover, we have observed that the main action of the synthetic progestin MPA on ZR-75-1 cell proliferation is due to its androgen receptor–mediated inhibitory action (Poulin *et al.,* 1989*a,* 1991).

Mammary carcinoma induced by DMBA in rats is a widely used model for studies on the role of estrogens in breast cancer (Asselin *et al.,* 1977; Dauvois *et al.,* 1989*a*). The development and growth of these tumors are particularly sensitive to the stimulatory action of estrogens and prolactin (PRL) (Asselin *et al.,* 1977; Welsch, 1985). The present study aims at characterizing the effect of the novel anti-estrogen EM-800 on the growth of mammary carcinoma induced by DMBA in rats. Since estrone (E_1) is the most important circulating estrogen in post-menopausal women (Labrie, 1991; Siiteri and MacDonald, 1973), we have used it as a stimulus for tumor growth in order to have access to an *in vivo* model of post-menopausal breast cancer in women (Li *et al.,* 1994).

The benefits of combined treatment with an androgen and an anti-estrogen have been reported in advanced breast cancer (see Ingle *et al.,* 1991 and references therein). In the present study, in addition to investigating the effect of the pure anti-estrogen EM-800 on the E_1-stimulated growth of DMBA-induced mammary carcinoma, we have examined the anti-tumoral activity of the combination of EM-800 with the androgenic MPA at submaximal doses.

MATERIAL AND METHODS

Animals

Young female Sprague-Dawley rats [Crl:CD(SD)Br] were obtained from Charles River (St. Constant, Québec, Canada) and housed 2 per cage in a 12 hr light and 12 hr darkness (lights on at 07:15 AM)– and temperature (22 ± 2°C)–controlled environment. Animals received Purina (Lasalle, Canada) rodent chow and tap water *ad libitum.* Animal studies were conducted in a Canadian Council on Animal Care (CCAC)–approved facility in agreement with the CCAC *Guide for Care and Use of Experimental Animals.*

Chemicals

Estrone, MPA and DMBA were purchased from Sigma (St. Louis, MO). The novel anti-estrogen EM-800 ((+)-7-pivaloyloxy-3-(4′-pivaloyloxyphenyl)-4-methyl-2-(4′′-(2′′′- piperidinoethoxy-)phenyl)-2H-benzopyran) was synthesized in the medicinal chemistry division of our laboratory (Gauthier *et al.,* 1997; Simard *et al.,* 1997).

Induction of mammary tumors by DMBA

Mammary tumors were induced by a single intra-gastric administration of 20 mg of DMBA in 1 ml of corn oil at 50–52 days of age. Three to 4 months later, tumor site, size and number were recorded. Animals bearing tumors with a size of 0.8–1.5 cm in

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diameter were selected and divided into homogeneous groups with respect to tumor size and number.

Treatment

All rats were bilaterally ovariectomized under isoflurane-induced anesthesia and randomly assigned to the following groups of 12 animals: 1, ovariectomy (OVX); 2, control or OVX + E_1 (1 µg, s.c., twice daily); 3, OVX + E_1 + EM-800 (0.25 mg/kg body weight, p.o., once daily); 4, OVX + E_1 + EM-800 (2.5 mg/kg body weight, p.o., once daily); 5, OVX + E_1 + MPA (1 mg, s.c., twice daily); and 6, OVX + E_1 + MPA (1 mg) + EM-800 (0.25 mg/kg body weight). All compounds were prepared in a 4% ethanol, 4% polyethyl glycerol-600, 1% gelatin and 0.9% NaCl suspension. Control animals received the same volume of vehicle.

Measurement of response

The 2 largest perpendicular diameters of each tumor were measured with calipers to estimate tumor size (Asselin *et al.*, 1977). The total tumor area of each rat on the day of OVX was taken as 100%. Throughout the treatment, tumors were measured at 3–7 day intervals. A change in tumor size was expressed as a percentage of initial tumor size. Categories of response of tumors were evaluated as follows: ''progression'' refers to an increase in tumor size of more than 25% in relation to initial tumor size; likewise, ''partial response'' is a decrease of more than 50%, while ''complete response'' is disappearance of tumors; ''stable response'' corresponds to tumors which decreased in size by less than 50% or increased in size by less than 25% (Li *et al.*, 1995).

Animals were killed by cervical dislocation following 65-day treatment. Blood was collected and serum samples were kept at −80°C until assayed. Tumoral, uterine and vaginal tissues were removed immediately, freed from connective and adipose tissue, weighed, frozen in liquid nitrogen and stored at −80°C until assayed.

Steroid receptor assays

Preparation of cytosol. Tumoral, uterine and vaginal tissues were homogenized with a PT-10 homogenizer (Brinkman, Mississauga, Canada) at a setting of 8 for 10 sec in 20 vol. of buffer A (25 mM Tris-HCL, 1.5 mM EDTA, disodium salt, 10 mM α-monothioglycerol, 10% glycerol and 1.5 mM sodium molybdate, pH 7.4). Homogenates were centrifuged at 105,000 *g* for 60 min at 4°C. Steroid binding assays were performed with freshly prepared cytosol. Protein concentrations were determined by the method of Bradford (1976) using BSA as standard.

Progesterone and estrogen receptor assays. 6,7 [^{3}H]-17,21-Dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R5020) (87 Ci/mmol), [^{3}H]-estradiol (115 Ci/mmol) and unlabeled R5020 were purchased from NEN (Boston, MA) while diethylstilbestrol (DES) was purchased from Sigma. Bound [^{3}H]-R5020 and [^{3}H]-estradiol were measured using the dextran-coated charcoal absorption technique (Asselin *et al.*, 1977; Asselin and Labrie, 1978). In brief, 200 µl of cytosol were incubated with 100 µl of [^{3}H]-R5020 (200,000 cpm, final concentration 8 nM) and dexamethasone (final concentration 120 nM) or 100 µl of [^{3}H]-E_2 (90,000 cpm, final concentration 3 nM) in the presence or absence of a 100-fold excess of unlabeled R5020 overnight at 4°C or a 100-fold excess of DES for 3 hr at room temperature. Unbound steroids were separated by incubation for 15 min at 4°C with 300 µl of 0.5% norit A, 0.05% dextran T-70 (DCC) in buffer B (1.5 mM EDTA, disodium salt, 10 mM α-monothioglycerol and 10 mM Tris-HCL, pH 7.4) and centrifugation at 3,000 *g* for 15 min. Aliquots of supernatant (300 µl) were measured for radioactivity with 10 ml of scintillation fluid.

Radioimmunoassay

Serum luteinizing hormone (LH) levels were measured by double-antibody radioimmunoassay (RIA) using rat hormones (LH-I-6 for iodination and rat LH-RP-2 as standard) and rabbit anti r-LH-S-8 anti-serum, generously supplied by the National Pituitary Program (Baltimore, MD).

Statistical analysis

All data are presented as means ± SEM. Statistical significance was calculated according to the test of Duncan-Kramer (Kramer, 1956).

RESULTS

Inhibition of DMBA-induced tumor growth

After 65 days of treatment with E_1, the average tumor area reached 225 ± 40.9% of initial tumor size, while in the OVX group (in the absence of estrogens), the tumors continuously regressed to only 9.6 ± 3.9% of initial tumor size ($p < 0.01$). Treatment with MPA reversed by 84.1% the stimulatory effect of E_1 and decreased tumor area to 44 ± 9.6% of initial size ($p < 0.01$). However, treatment with EM-800 reversed by 82.9% the stimulatory effect of E_1 at the low dose of 0.25 mg/kg body weight, while the stimulatory effect of E_1 was abolished at the high dose of EM-800, a value of 10.4 ± 3.5% of initial size being measured ($p < 0.01$). Combination of the low-dose EM-800 (0.25 mg/kg body weight) and MPA further inhibited E_1-stimulated tumor growth to 16.2 ± 5.7%, a more potent inhibitory effect than that achieved with each compound used alone ($p < 0.05$ *vs.* MPA alone but non-significant *vs.* low EM-800 dose alone; Fig. 1).

Figure 2 illustrates the distribution of categories of response to the various treatments. Fifty percent and 42% of tumors showed complete and partial responses, respectively, during the 65-day observation period in OVX animals, while only 8% of tumors remained stable and no tumor progressed. In contrast, in the

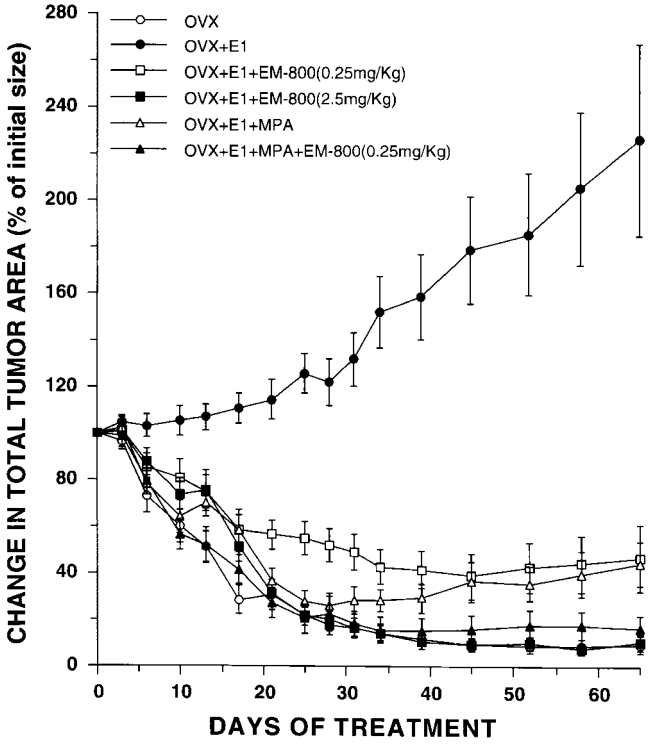

FIGURE 1 – Effect of 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or MPA (1 mg s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA on the E_1 (1.0 µg, s.c., twice daily)–stimulated growth of DMBA-induced mammary carcinoma in ovariectomized rats. The change in tumor size is expressed as % of initial tumor size. Data are expressed as means ± SEM.

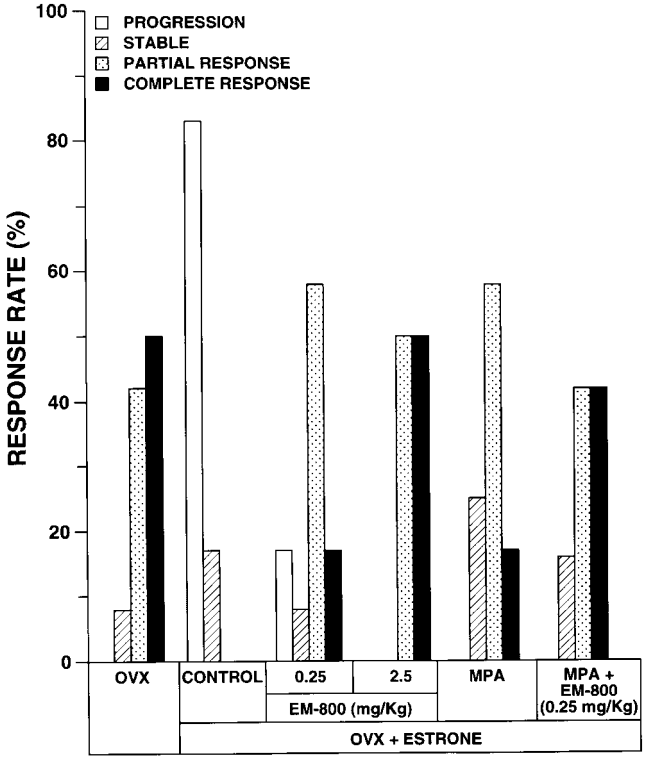

FIGURE 2 – Categories of response of DMBA-induced mammary carcinoma to 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or 1 mg MPA (s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA in E_1-supplemented OVX rats. Categories of response are described in ''Material and Methods''.

animals treated daily with 1 μg E_1, 83% of tumors progressed and the remaining 17% of tumors did not show significant changes (stable response). Treatment with the low dose of EM-800 led to 58% and 17% of partial and complete responses, respectively, while 17% and 8% of tumors were recorded as progression and unchanged, respectively. The high dose of EM-800, however, completely antagonized the stimulatory effect of estrone on the growth of mammary carcinomas induced by DMBA and resulted in 50% of tumors showing complete response and 50% showing partial response. After treatment with 1 mg MPA, 58% of tumors showed partial response, while 17% had disappeared (complete response) and the remaining 25% remained stable. In the group of animals treated with both MPA and the low dose of EM-800, 42% of tumors showed a complete response and another 42% showed a partial response, while 16% remained stable.

Effect on uterine, vaginal and adrenal weights

As shown in Figure 3*a*, E_1 stimulated uterine weight to 390 ± 11 mg ($p < 0.01$) from a value of 138 ± 7 mg in OVX animals. EM-800, however, inhibited E_1-stimulated uterine weight in a dose-related fashion. At the doses of 0.25 and 2.5 mg/kg body weight, EM-800 reversed by 71% and 89% the stimulatory effect of estrone, respectively, thus decreasing uterine weight to 211 ± 8 mg ($p < 0.01$) and 167 ± 7 mg ($p < 0.01$) in the corresponding groups. In the presence of E_1, MPA resulted in a further increase in uterine weight to 624 ± 25 mg ($p < 0.01$), a value 60% higher than that observed with E_1 alone. However, this additional increase in uterine weight caused by the combination E_1–MPA was partially reversed to 475 ± 34 mg ($p < 0.05$) by the simultaneous administration of 0.25 mg/kg body weight of EM-800.

Comparable results were obtained on vaginal weight (Fig. 3*b*), where an 89% increase, from 172 ± 5 mg to 325 ± 9 mg ($p < 0.01$), was induced by E_1 replacement in OVX rats. Low-dose EM-800 (0.25 mg/kg body weight) led to a 91% blockade of the E_1-stimulated increase, to a value of 186 ± 5 mg ($p < 0.01$), while the higher dose of EM-800 (2.5 mg/kg body weight) completely reversed the stimulatory effect of estrone, to 166 ± 4 mg ($p < 0.01$), a value even lower than that achieved by OVX or the absence of estrogens. In E_1-supplemented animals, MPA further increased vaginal weight to 462 ± 28 mg ($p < 0.01$), this additional increase being almost completely inhibited by the addition of 0.25 mg/kg body weight of EM-800, to 346 ± 22 mg.

Daily administration of E_1 slightly, but significantly, increased adrenal weight, from 64 ± 2 mg in OVX animals to 74 ± 4 mg ($p < 0.05$; data not shown). EM-800, however, had no effect on the E_1-induced stimulation on this parameter, while MPA not only reversed the stimulatory effect of E_1 but caused an important inhibition of adrenal weight, to 20 ± 3 mg ($p < 0.01$). This effect of MPA was not affected by co-administration of EM-800.

Effect on serum LH levels

It can be seen in Figure 4 that E_1 replacement in OVX rats led to 53% decrease in serum LH, from 8.4 ± 0.6 to 4.0 ± 0.6 ng/ml ($p < 0.01$). Increasing doses of EM-800 in E_1-treated rats caused a dose-dependent increase in serum LH levels. At the doses used, EM-800 reversed the inhibitory effect of E_1 on serum LH levels, from 4.0 ± 0.6 to 6.2 ± 0.6 ng/ml (74% of the value of OVX, $p < 0.01$) and 8.8 ± 0.8 ng/ml (105% of the value of OVX, $p < 0.01$), respectively. In contrast, MPA decreased serum LH levels to undetectable levels ($p < 0.01$). The effect of MPA was not affected by simultaneous treatment with EM-800.

Effect on uterine, vaginal and tumoral steroid receptor levels

Supplementation of OVX rats with E_1 decreased uterine [^{3}H]-E_2 binding, from 230 ± 35.7 to 94.9 ± 8.1 fmol/mg protein ($p < 0.01$) (Fig. 5*a*), while no significant effect was observed on vaginal estrogen receptor (ER) levels (Fig. 5*b*). However, increased ER levels, from 7.5 ± 1.6 to 14.8 ± 2.9 fmol/mg protein ($p < 0.05$), were measured in the DMBA-induced mammary tumors (Fig. 5*c*). In addition, E_1 supplementation increased uterine progesterone receptor (PR) levels from 142 ± 5.4 to 238 ± 18.2 fmol/mg protein ($p < 0.01$) (Fig. 6*a*) and vaginal PR levels from 23.0 ± 10.9 to 253 ± 35.3 fmol/mg protein ($p < 0.01$) (Fig. 6*b*) as well as tumoral PR levels from 8.5 ± 3.8 to 61.0 ± 10.0 fmol/mg protein ($p < 0.01$) (Fig. 6*c*). Treatment with EM-800 significantly inhibited uterine, vaginal and tumoral ER levels in a dose-related fashion (Fig. 5). In agreement with the decrease of ER concentrations, uterine, vaginal and tumoral PR contents also were decreased by the same treatment with EM-800 (Fig. 6). Treatment with MPA, at the dose used, decreased ER levels only in the tumoral tissue, while it had no significant effect in the uterus or vagina (Fig. 5). However, it significantly reduced PR levels in all 3 tissues examined (Fig. 6). The combination of low-dose EM-800 (0.25 mg/kg body weight) and MPA inhibited ER (Fig. 5) and PR (Fig. 6) levels in the above-mentioned 3 tissues.

DISCUSSION

Since E_1 is the predominant circulating estrogen precursor in post-menopausal women, E_1 supplementation in OVX rats bearing DMBA-induced mammary tumors provides a model which mimics the conditions of post-menopausal breast cancer (Li *et al.,* 1994). Using this model, our present data show that the novel anti-estrogen EM-800 and MPA both exert potent inhibitory effects on the E_1-stimulated growth of DMBA-induced mammary carcinomas. Most importantly, the combination of the 2 compounds at submaximal doses induced a greater inhibition than those achieved by each compound used alone, thus reaching 96.9% inhibition of the E_1-stimulated growth of mammary carcinoma induced by DMBA. Moreover, EM-800 alone, at the higher dose of 2.5 mg/kg

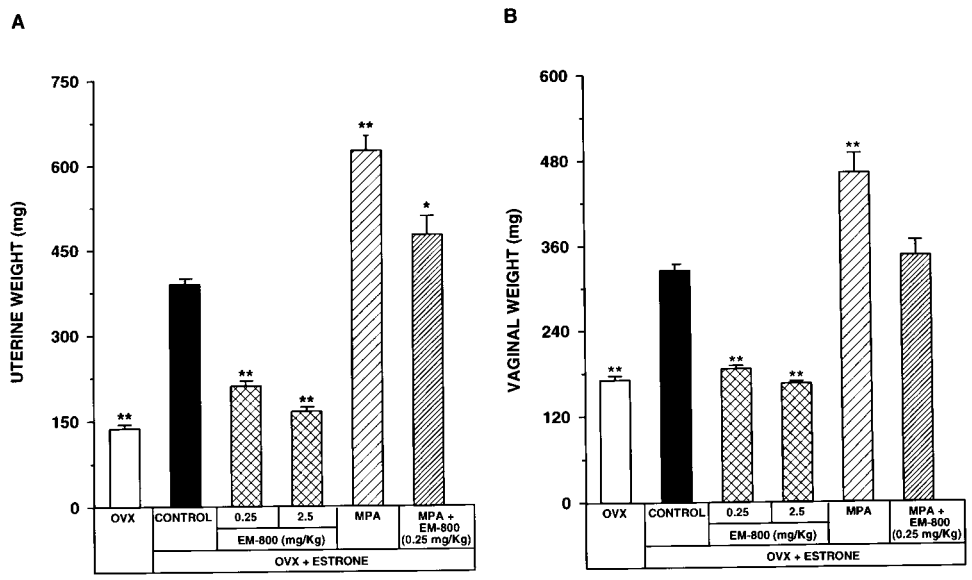

FIGURE 3 – Effect of 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or MPA (1 mg s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA on uterine weight (*a*) and vaginal weight (*b*) in ovariectomized rats supplemented with E_1 (1 µg, s.c., twice daily). $*p < 0.05$, $**p < 0.01$ *vs.* OVX + E_1 control.

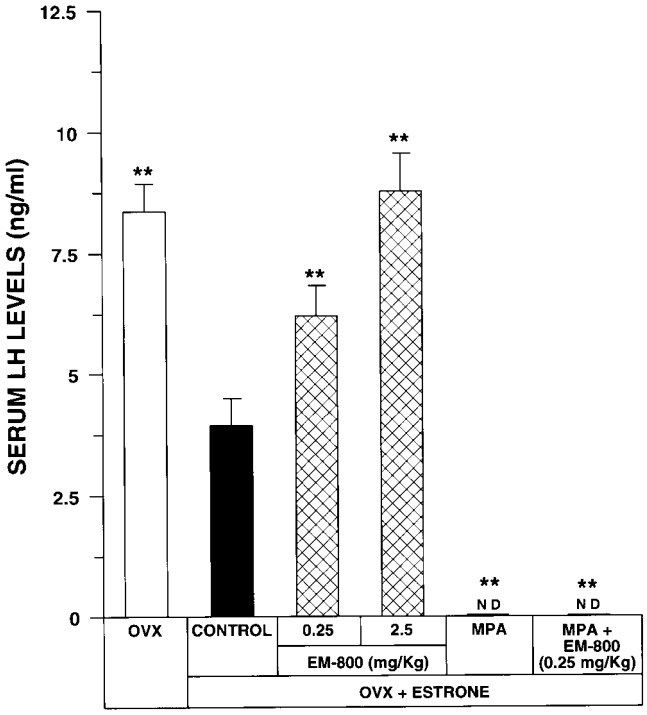

FIGURE 4 – Effect of 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or MPA (1 mg s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA on serum LH levels in ovariectomized rats supplemented with E_1 (1 µg, s.c., twice daily). $**p < 0.01$ *vs.* OVX + E_1 control.

body weight, abolished the stimulatory effect of E_1 on the growth of these tumors, thus demonstrating the particularly high efficacy of this novel anti-estrogen.

In addition, our data show that EM-800 decreases ER levels in the 3 estrogen-sensitive tissues studied, the uterus, vagina and DMBA-induced tumor. To exclude the possibility that the down-regulation observed in the present study was due to the masking effect of EM-800 on the availability of ER sites to radiolabeled E_2, we measured ER content using the estrogen receptor enzyme immunoassay (ER-EIA; Abbott kit, Montreal, Canada). The results obtained with the ER-EIA paralleled those described above for the radioligand assay (data not shown), thus confirming the observation that EM-800 markedly decreases ER protein as well as binding levels in the tissues examined.

PR is a well-recognized estrogen-responsive parameter (Horwitz and McGuire, 1978). The present observation that the new anti-estrogen EM-800 causes a marked inhibition of PR levels in the uterus, vagina as well as DMBA-induced tumors illustrates the potent anti-estrogenic activity of EM-800. On the contrary, tamoxifen, a non-steroidal anti-estrogen with intrinsic estrogenic activity, stimulates PR levels in the rat uterus (Jordan *et al.,* 1980) as well as in DMBA-induced tumors (Boccardo *et al.,* 1987). The steroidal anti-estrogen ICI 164,384, a compound considered as being a full antagonist in most models studied, acts as an estrogen agonist on PR levels in the uterus and vagina of the guinea pig (Pasqualini *et al.,* 1990) and a partial agonist in Ishikawa human endometrial carcinoma cells (Jamil *et al.,* 1991). However, ICI 164,384 shows no stimulatory activity on PR expression in breast cancer cells (Wakeling and Bowler, 1988) and in the immature rat uterus (May *et al.,* 1989). It has been suggested that the down-regulation of ER by anti-estrogens is due to the increased turnover of the ER protein (Dauvois *et al.,* 1992). Since anti-estrogens possessing partial estrogenic activity have little effect on ER turnover (Eckert *et al.,* 1984), it appears that the ability of anti-estrogens to increase the degradation of the ER protein is a characteristic of pure anti-estrogens. Whether the down-regulation of ER by EM-800 as demonstrated in the present study is due to the increased turnover of the ER protein remains to be investigated.

The pure anti-estrogenic activity of EM-800 can be estimated by its effect on LH secretion at the hypothalamo–pituitary level. Estrogens inhibit the secretion of luteinizing hormone–releasing hormone (LH-RH), thus resulting in decreases in LH and follicle-stimulating hormone (FSH) secretion (Ferland *et al.,* 1976). The present study shows that treatment with E_1 decreases serum LH levels in OVX rats, while treatment with EM-800 completely

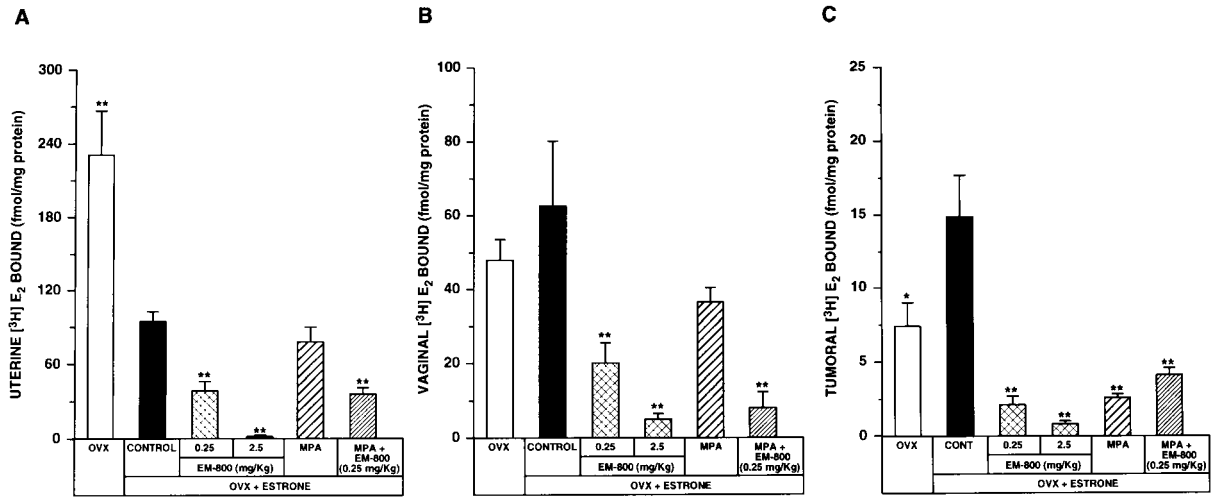

FIGURE 5 – Effect of 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or MPA (1 mg s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA on uterine (*a*), vaginal (*b*) and tumoral (*c*) estrogen receptor levels in ovariectomized rats supplemented with E_1 (1 µg, s.c., twice daily). $*p < 0.05$, $**p < 0.01$ *vs.* OVX + E_1 control.

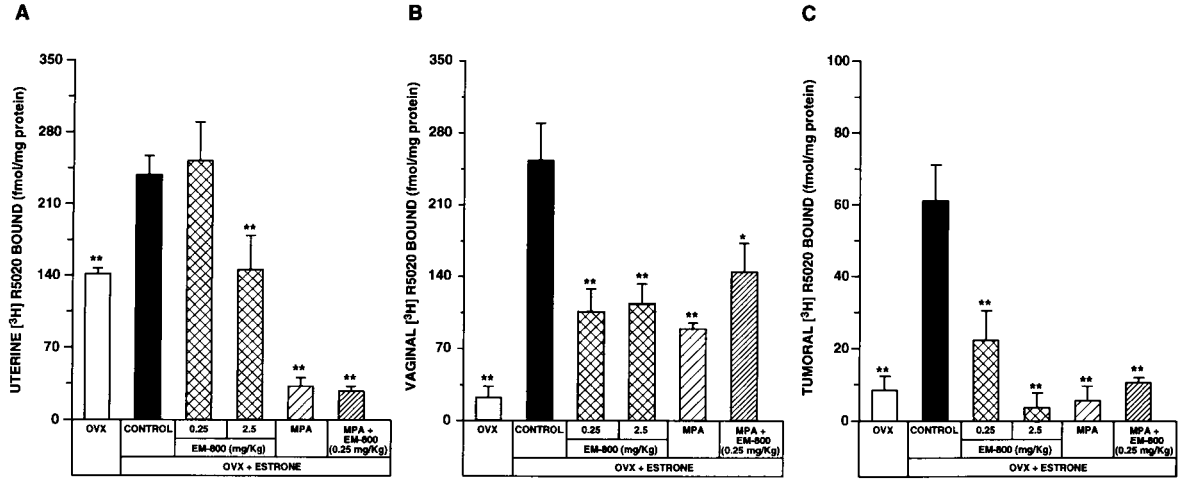

FIGURE 6 – Effect of 65-day treatment with the anti-estrogen EM-800 at doses of 0.25 and 2.5 mg/kg body weight (orally, once daily) or MPA (1 mg s.c., twice daily) or the combination of EM-800 (0.25 mg/kg body weight) and MPA on uterine (*a*), vaginal (*b*) and tumoral (*c*) progesterone receptor levels in ovariectomized rats supplemented with E_1 (1 µg, s.c., twice daily). $*p < 0.05$, $**p < 0.01$ *vs.* OVX + E_1 control.

reverses the inhibitory effect of E_1. In fact, EM-800 induces an OVX-like increase in serum LH levels, thus clearly demonstrating the pure anti-estrogenic activity of EM-800 at the hypothalamo–pituitary level in rats.

MPA, however, due to its intrinsic androgenic activity (Labrie *et al.*, 1988, 1987), leads to undetectable serum LH levels. The effect of EM-800 on the hypothalamo–hypophyseal axis observed in our study is unique since no other anti-estrogens have been reported to share this property. Tamoxifen and its derivatives, such as toremifene and droloxifene, inhibit serum LH and FSH levels in post-menopausal women, thus illustrating their estrogenic activity (Kivinen and Maenpaa, 1990). ICI 182,780, a steroidal anti-estrogen with more potent anti-estrogenic activity than ICI 164,384 (Wakeling *et al.*, 1991), exerts no effect on gonadotropin secretion (Dukes *et al.*, 1992), suggesting its lack of anti-estrogenic activity at the hypothalamo–pituitary level, at least in monkeys.

Since MPA is a synthetic progestin that binds to the androgen, glucocorticoid as well as progestin receptors (Labrie *et al.*, 1987; Poulin *et al.*, 1989*a*, 1991), the increase in uterine and vaginal weights may be caused by interaction with the androgen receptor, while the decrease in adrenal weight is best attributed to its glucocorticoid activity. The decrease in serum LH induced by MPA can be attributed to the androgenic activity. As mentioned earlier, MPA has been shown to exert a direct inhibition of the proliferation of ZR-75-1 human breast cancer cells *in vitro* (Poulin *et al.*, 1989*a*). An androgen receptor–mediated direct anti-proliferation mechanism (Labrie *et al.*, 1993; Li *et al.*, 1992) as well as an inhibition of the intracellular formation of the active estrogen estradiol by inhibiting 17β-hydroxysteroid dehydrogenase (17β-HSD) have been suggested to explain the anti-tumoral effect of MPA in DMBA-induced rat mammary carcinoma (Li *et al.*, 1995).

The additive anti-tumoral effect of an anti-estrogen and an androgen has been demonstrated in DMBA-induced rat mammary carcinoma (Dauvois *et al.*, 1991; Li *et al.*, 1995) as well as in breast cancer patients (Ingle *et al.*, 1991; Tormey *et al.*, 1983). The present data show that the anti-estrogen EM-800 and the androgenic compound MPA exert complementary anti-tumoral effects in rat mammary carcinoma induced by DMBA. It is likely that the

additive inhibitory effect is achieved through different receptor-mediated mechanisms, EM-800 exerting its inhibitory effect *via* ER-mediated events and MPA *via* androgen receptor–mediated mechanisms.

In summary, our results show the pure anti-estrogenic effect of EM-800 as well as its potent inhibitory effect on the E_1-stimulated growth of DMBA-induced mammary carcinoma in rats. In addition, our data show that the combination of EM-800 and MPA at submaximal doses achieves a more potent anti-tumoral effect than that achieved by each compound used alone.

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