Synergism between the Anticancer Actions of 2-Methoxyestradiol and Microtubule-Disrupting Agents in Human Breast Cancer

Gui-Zhen Han, Zhi-Jian Liu, Kayoko Shimo, and Bao Ting Zhu

Abstract
2-Methoxyestradiol (2-MeO-E2), a well-known nonpolar endogenous metabolite of 17β-estradiol, has strong antiangiogenic, apoptotic, and anticancerogenic actions in vitro and in vivo at pharmacologic concentrations. We determined in the present study whether 2-MeO-E2 can enhance the anticancer actions of paclitaxel or vinorelbine (two commonly used microtubule-disrupting agents) in several human breast cancer cell lines, including the estrogen receptor–positive MCF-7 and T-47D cells and the receptor-negative MDA-MB-435s and MDA-MB-231 cells. 2-MeO-E2 in combination with paclitaxel or vinorelbine exhibited a synergistic anticancer effect in these human breast cancer cells in vitro, and this synergistic effect was more pronounced when each of the drugs was used at relatively low concentrations. Additional experiments using female athymic BALB/c nu/nu mice showed that p.o. administration of 2-MeO-E2 at 30 mg/kg body weight, once a week for 6 weeks, markedly enhanced the activity of paclitaxel or vinorelbine against the growth of the estrogen receptor–negative MDA-MB-231 human breast cancer xenografts in these animals. In contrast, combination of 2-MeO-E2 with 5-fluorouracil only had a partial additive effect against the growth of these cell lines in culture, and no synergistic effect was observed. Interestingly, when doxorubicin was used in combination with 2-MeO-E2, the antagonistic effect of 2-MeO-E2 was somewhat antagonized by doxorubicin when it was present at high concentrations. Our results showed that 2-MeO-E2 at nontoxic or subtoxic doses selectively enhanced the effects of certain microtubule-disrupting agents (such as paclitaxel and vinorelbine) against the growth of the estrogen receptor-negative human breast cancer cell lines in culture and also in athymic nude mice. (Cancer Res 2005; 65(2): 387-93)

Introduction
2-Methoxyestradiol (2-MeO-E2), a well-known nonpolar metabolite of 17β-estradiol, is formed by the catechol-O-methyltransferase–mediated O-methylation of 2-hydroxyestradiol in humans (reviewed in refs. 1, 2). Many studies have shown that 2-MeO-E2 at pharmacologic concentrations has strong antiproliferative and apoptotic actions in a variety of human cancer cell lines in vitro (3–11). Among the cancer cell lines tested, several human breast cancer cell lines seemed to be highly sensitive to the in vitro growth-inhibitory actions of 2-MeO-E2 (4, 5, 7–9). Moreover, 2-MeO-E2 has strong antiangiogenic effects both in vitro and in vivo at pharmacologic doses (3, 6). Because of these unique biological properties of 2-MeO-E2, a great deal of research efforts have been initiated in the past several years to explore the usefulness of 2-MeO-E2 as an effective, low-toxicity chemotherapeutic agent for human breast cancer as well as for other types of human cancers (8). Another potential application of 2-MeO-E2 is its use as an adjunct to enhance the anticancer actions of other commonly used chemotherapeutic agents when they are given in certain combinations. This possibility is explored in the present study in several human breast cancer cell lines, including the estrogen receptor (ER)–negative MDA-MB-435s and MDA-MB-231 cells and the ER-positive MCF-7 and T-47D cells. The rationale for using these human breast cancer cell lines in our present study was primarily based on our recent data showing that these cell lines were all sensitive to the growth-inhibitory actions of 2-MeO-E2 (7). The anticancer agents tested in this study included paclitaxel, vinorelbine, 5-fluorouracil (FU), methotrexate, doxorubicin, and mitomycin C. These anticancer agents are widely used in the treatment of human breast cancers and are selected from three representative classes of anticancer agents with different mechanisms of anticancer actions. Their inclusion would be advantageous for us to determine if 2-MeO-E2 has the potential to be used as an adjunct to beneficially enhance the anticancer actions of certain clinically useful chemotherapeutic agents in human breast cancer. Our results showed that 2-MeO-E2, when used in combination with paclitaxel or vinorelbine, exhibited a marked synergistic effect against the growth of human breast cancer cells in culture or after transplantation into athymic nude mice.

Materials and Methods
Chemicals. Paclitaxel, FU, methotrexate, doxorubicin, mitomycin C, 17β-estradiol, crystal violet, 50% glutaraldehyde, Triton X-100, dextran-coated charcoal, and fetal bovine serum were all obtained from the Sigma Chemical Co. (St. Louis, MO). Vinorelbine tartrate (vinorelbine) was provided by GlaxoSmithKline (Research Triangle Park, NC). 2-MeO-E2 was obtained from Steraloids (Newport, RI). Our high-performance liquid chromatography (HPLC) analysis showed that the large batch of 2-MeO-E2 provided by GlaxoSmithKline (Research Triangle Park, NC) was all sensitive to the growth-inhibitory actions of 2-MeO-E2 in human breast cancer cell lines (3, 8). We obtained the reference and standard 2-MeO-E2 from Steraloids (Newport, RI) and found that these two batches were identical. Our HPLC results showed that the large batch of 2-MeO-E2 obtained from Steraloids (Newport, RI) were all sensitive to the growth-inhibitory actions of 2-MeO-E2 without any impurities.

Culture of Human Breast Cancer Cell Lines. The ER-positive MCF-7 and T-47D and the ER-negative MDA-MB-231 and MDA-MB-435s human breast cancer cell lines were all obtained from the American Type Culture Collection (Manassas, VA). The methods for the in vitro culture of these cell lines were described in our recent study (7). The human breast cancer cells were first propagated in 75-cm² flasks under 37°C air with 5% CO2 and 95% humidity to 80% confluence. They were then detached from the flask by
treatment with 3 mL of Trypsin-Versene mixture (containing 0.25% trypsin and 0.02% EDTA) for ~5 minutes. Cell suspensions were centrifuged and the cell sediments were resuspended in the culture medium at the desired 10^5 cells/mL density. A 0.1-mL aliquot of the cell suspension was then added to each well of the 96-well microplate at a final density of 10^3 cells per well. After the cells were allowed to attach and grow for 48 hours, the cell culture medium was changed and different treatments were given at that time. In most experiments, the drug treatment lasted for 4 days with one medium change on the third day following the initial drug treatment.

**Preparation of the Anticancer Drug Solutions.** Due to their high lipophilicity, the stock solutions of 2-MeO-E2 (10 mmol/L) and paclitaxel (0.2 mmol/L) were prepared in pure ethanol (200 proof). The stock solutions of doxorubicin (5 mmol/L), mitomycin C (0.1 mg/mL), and vinorelbine (1 mmol/L) were prepared in phosphate buffer (pH 7.4). The stock solutions containing 10 mmol/L of FU or methotrexate were prepared by first making a 50-mmol/L drug concentration in 1.0 N potassium hydroxide and followed by dilution with phosphate buffer (pH 7.4) to a 10-mmol/L drug concentration. All these stock solutions were filtered with a Millex syringe filter (0.22-μm acetate cellulose membrane), and the filtrates were stored at ~20°C in tightly sealed sterile tubes. Shortly before introducing the anticancer agents to the cultured cancer cells, each chemical was freshly diluted with a buffer to the desired concentrations and an aliquot (usually 10 μL) of the drug-containing solution was added to each well. Usually <0.1% of the original solvent of the stock solution was present in the final cell culture medium.

**Measurement of Cell Growth In vitro.** The cell density in the 96-well microplates was determined by using the crystal violet staining method (7). Briefly, the culture medium in the microplates was first removed by aspiration and then the cells in each well were fixed with 1% glutaraldehyde for 15 minutes. After removing the fixation solution, each well was rinsed with PBS buffer and allowed to dry at room temperature. The cells in each well were then stained with 50 μL of 5% crystal violet (dissolved in 20% methanol and 80% deionized water) for 15 minutes at room temperature, and the plates were rinsed carefully with tap water to remove residual crystal violet. The stained dye was then dissolved in 100 μL of 0.5% Triton X-100 for overnight. After addition of 50 μL of 200-proof ethanol, the absorbance values of each well were measured at 560 and 405 nm with a UVmax microplate reader (Molecular Devices, Palo Alto, CA), and the difference in the absorbance values at these two wavelengths were used to represent the cell density. As described in our recent study (7), we have compared the crystal violet staining method with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (12) for quantification of the cell density in 97-well culture plates, and the data obtained from both methods matched closely. Moreover, we noted that the crystal violet staining method was not only convenient and cost far less, but it also yielded more reproducible data than did the MTT assay.

**Inhibition of the Growth of Human Breast Cancer Xenografts in Athymic Mice.** The original breeders of the female athymic BALB/c nu/nu mice were obtained from the National Cancer Institute (Fredrick, MD). Six-week-old female athymic mice were used in the present study, and they were caged in sterilized cages with sterilized bedding and filtered air, with free access to specially treated water and animal feed. The ER-negative MDA-MB-231 cells (at 5×10^6 cells in 100 μL) were s.c. injected into the mammary fat pad of the female athymic mice. Tumors were allowed to grow to a size larger than 1.2 cm in diameter. At the end of the *in vivo* tumor growth study, macroscopic pathologic examination of the animals was done, and microscopic examination was also done for some of the animals. The procedures for animal care and use were in accordance with institutional and NIH guidelines.

**Statistical Analysis.** In the cell growth inhibition experiments, the growth rate of drug-treated cells was expressed as mean ± SE of the values obtained from six to eight replicate wells. The IC50 values were calculated according to the equation for sigmoidal dose-response curves (with variable slopes) using the nonlinear regression curve-fitting model of the Prism software. Unless otherwise indicated, one-way ANOVA was used for multiple comparisons and the Tukey's test was used for the pairwise comparisons. A P value < 0.05 was considered to be statistically significant, and a P value < 0.01 was considered statistically very significant.

**Results**

**Antiproliferative Actions of 2-MeO-E2 in Human Breast Cancer Cells.** Four human breast cancer cell lines were used in this study, which included two ER-negative lines (MDA-MB-231 and MDA-MB-435s) and two ER-positive lines (MCF-7 and T-47D). 2-MeO-E2 inhibited the growth of the ER-negative MDA-MB-435s cells in a concentration-dependent manner, with an average IC50 value of 0.8 μmol/L. A near-complete growth inhibition of MDA-MB-435s cells was obtained when 2 μmol/L 2-MeO-E2 was present. In comparison, the MDA-MB-231 cells were somewhat less sensitive to the antiproliferative actions of 2-MeO-E2. The average IC50 value of 2-MeO-E2 in MDA-MB-231 cells was 1.4 μmol/L, and only ~70% of growth inhibition of MDA-MB-435s cells was obtained when 2 μmol/L 2-MeO-E2 was present. Notably, the presence or absence of exogenous E2 (at a 10 nmol/L concentration) in the cell culture medium did not affect the potency and efficacy of the antiproliferative action of 2-MeO-E2 in these two ER-negative cell lines.

2-MeO-E2 also inhibited the growth of the ER-positive MCF-7 and T-47D cells in a concentration-dependent manner when 10 nmol/L of exogenous E2 was present in the culture medium. The average IC50 values were 0.8 and 1.2 μmol/L, respectively. A near-complete growth inhibition of these two cell lines was obtained when 2 μmol/L 2-MeO-E2 was present.

**Growth-Inhibitory Effect of Paclitaxel Alone or in Combination with 2-MeO-E2.** When each of the four human breast cancer cell lines was treated with increasing concentrations (0.01-100 nmol/L) of paclitaxel, similarly precipitous dose-response curves were observed. The estimated average IC50 values for paclitaxel were 2.7, 4.2, 2.2, and 8.2 nmol/L in MDA-MB-231, MDA-MB-435s, MCF-7, and T-47D cells, respectively. A representative dose-response curve in MDA-MB-231 cells is shown in Fig. 1A.

In the ER-negative MDA-MB-231 cells, combination of 2-MeO-E2 with paclitaxel (at 1, 2, or 4 nmol/L) exhibited a synergetic antiproliferative effect compared with each drug used alone. Notably, this synergy in growth inhibition was more pronounced when each of the two drugs was used at relatively low concentrations that would only produce a weak effect individually. For instance, 1 nmol/L paclitaxel or 0.5 μmol/L 2-MeO-E2 produced no appreciable growth-inhibitory effect when present alone, but the combination of both drugs at these ineffective concentrations produced a ~50% growth inhibition (Fig. 1B). Similarly, paclitaxel

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3 The term synergy (or synergism) used in this article only refers to our gross observation that treatment with two drug combinations exerted a growth-inhibitory effect that was greater than what was expected from the sum of effects of the individual compounds used, but an isobologram analysis for synergy as described in Pharmacol Rev 1989 41:93 and Biometrics 1994 50:834 was not done.
alone at 2 nmol/L only produced a rather weak growth inhibition (~20%), but the co-presence of 0.5 μmol/L 2-MeO-E2 resulted in ~80% growth inhibition (Fig. 1B). In additional experiments, we repeated the experiments by selectively testing their synergistic effect when two very low concentrations of paclitaxel (0.5 and 1 nmol/L) were present (data summarized in Fig. 1C). Paclitaxel at these two low concentrations or 2-MeO-E2 at 0.5 or 0.75 μmol/L did not have appreciable growth-inhibitory effect when present alone, but the combination of both agents at these ineffective concentrations produced strong growth inhibition (Fig. 1C).

We also determined the effects of paclitaxel in combination with 2-MeO-E2 in the ER-negative MDA-MB-435s breast cancer cells (representative data are shown in Fig. 1D). Similar patterns of enhancement were observed in this cell line, but the synergy between paclitaxel and 2-MeO-E2 seemed to be less pronounced than that in the MDA-MB-231 cells. In addition, similar patterns of a synergistic antiproliferative action between paclitaxel and 2-MeO-E2 were observed in the ER-positive MCF-7 and T-47D cells (some representative data are shown in Fig. 1E and F).

Growth-Inhibitory Effect of Vinorelbine Alone or in Combination with 2-MeO-E2. When each of the human breast cancer cell lines was treated with increasing concentrations (0.01-100 nmol/L) of vinorelbine alone, highly precipitous dose-response curves were also observed, which were similar to the curve patterns observed with paclitaxel. The average IC50 values for vinorelbine were 5.1, 4.4, 4.5, and 6.5 nmol/L in MDA-MB-231, MDA-MB-435s, MCF-7 cells, and T-47D cells, respectively. A representative dose-response curve in MDA-MB-231 cells is shown in Fig. 2A.

In the ER-negative MDA-MB-231 cells, combination of 2-MeO-E2 with vinorelbine at 1 or 2 nmol/L had a synergistic

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**Figure 1.** Enhancement by 2-MeO-E2 of the antiproliferative effect of paclitaxel in the ER-negative MDA-MB-231 and MDA-MB-435s cells and in the ER-positive MCF-7 and T-47D cells. The ER-negative MDA-MB-231 and MDA-MB-435s cells were cultured in the absence of exogenous E2, and the ER-positive MCF-7 and T-47D cells were cultured in the presence of 10 nmol/L E2. The rate of cell growth in the absence of 2-MeO-E2 and paclitaxel was assigned to be 100%, and the rate of cell growth in the presence of 2-MeO-E2 or paclitaxel or a combination of both agents was expressed as percent of the control. Columns, mean of six to seven replicate determinations; bars, SE. *, or **, marked enhancement of the growth inhibition when a given concentration of 2-MeO-E2 was co-present with paclitaxel.
antiproliferative effect compared with each drug present alone (data summarized in Fig. 2B). For instance, when 1 nmol/L vinorelbine was used alone, it showed no appreciable growth-inhibitory effect, but when it was used together with 0.5 μmol/L 2-MeO-E2, a ~40% growth inhibition was observed (Fig. 2B). Similarly, vinorelbine alone at 2 nmol/L only produced a rather weak growth inhibition (~20%), but co-presence of 0.5 μmol/L 2-MeO-E2 produced ~60% growth inhibition (Fig. 2B). We repeated similar experiments by testing the enhancing effect of 2-MeO-E2 when low concentrations of vinorelbine (at 0.5 and 1 nmol/L) were present (data summarized in Fig. 2C). It is apparent that combination of vinorelbine with 2-MeO-E2 at low concentrations produced higher synergistic effects (data summarized in Fig. 2C).

We also determined the effects of vinorelbine in combination with 2-MeO-E2 in the ER-negative MDA-MB-435s cells (data summarized in Fig. 2D), but the synergy between 2-MeO-E2 and vinorelbine in this cell line seemed to be less pronounced than that seen in MAD-MB-231 cells. In addition, similar patterns of a synergistic antiproliferative action between paclitaxel and 2-MeO-E2 were also observed in the ER-positive MCF-7 and T-47D cells (data summarized in Fig. 2E and F).

Growth-Inhibitory Effect of FU or Doxorubicin in Combination with 2-MeO-E2. For the purpose of comparison, we also determine the growth-inhibitory effects of 2-MeO-E2 in combination with several commonly used anticancer agents (including FU, methotrexate, doxorubicin, and mitomycin C) under the same experimental conditions. No appreciable synergistic actions were observed between 2-MeO-E2 and any of these anticancer agents. Some of the data for FU and doxorubicin are briefly described below.

When FU at different concentrations were used in combination with 2-MeO-E2, only weak additive effect was observed when relatively low concentrations of each drug were combined. No synergistic actions were observed in any of the four cell lines tested. The representative data obtained for the two ER-negative cell lines are summarized in Fig. 3 (top). Notably, a similar additive effect was
observed when 2-MeO-E2 was used in combination with methotrexate, another commonly used antimetabolism anticancer agent (data not shown).

Interestingly, when doxorubicin was used in combination with 2-MeO-E2, we observed, for the first time, that this drug at higher concentrations seemed to antagonize the actions of 2-MeO-E2. Representative data for this unique phenomenon in the two ER-negative cell lines are shown in Fig. 3 (bottom). The same experiments were repeated multiple times, and highly consistent patterns were observed.

Inhibition of the Growth of Human Breast Cancer Xenografts in Athymic Mice by Paclitaxel or Vinorelbine in Combination with 2-MeO-E2. To evaluate the in vivo synergistic anticancer effects of 2-MeO-E2 in combination with paclitaxel or vinorelbine, we used the growth of the ER-negative MDA-MB-231 human breast cancer cell xenografts in female athymic BALB/c nu/nu mice as an in vivo model. In a series of preliminary experiments using five to six animals per group, we first estimated the dose-response curves in this in vivo model for the anticancer effect of 2-MeO-E2, paclitaxel, or vinorelbine when given individually. P.o. administration of 2-MeO-E2 alone at 30 mg/kg body weight once weekly for 6 weeks produced no appreciable anticancer activity, whereas the average body weight of the control animals (without tumor xenografts) was only slightly decreased (6.7%). When the animals were given 75 or 150 mg/kg body weight of 2-MeO-E2 once weekly for 6 weeks, it produced a 32% and 53% reduction of the overall tumor volume, but the control animals (without tumor) lost 15% to 25% of body weight. When paclitaxel and vinorelbine were tested in this animal model, we found that i.p. injection of 5 mg/kg body weight of paclitaxel or 3 mg/kg body weight of vinorelbine once weekly for 6 weeks produced a weak but detectable growth inhibition of the human cancer xenografts.

Following these initial experiments, we devised our experiments by using 30 animals per treatment group and selected the doses of 2-MeO-E2 at 30 mg/kg body weight, paclitaxel at 5 mg/kg body weight, and vinorelbine at 3 mg/kg body weight for testing. P.o. administration of 2-MeO-E2 once weekly for 6 weeks had only weak anticancer activity in these animals (Fig. 4). Treatment of the animals with 5 mg/kg body weight of paclitaxel or 3 mg/kg body weight of vinorelbine once weekly for 6 weeks produced 33% ($P < 0.05$) and 22% ($P < 0.05$) inhibition of the tumor volume, respectively (Fig. 4). Combination of paclitaxel or vinorelbine with 2-MeO-E2 (30 mg/kg body weight) significantly enhanced the anticancer activity in the animal model. Notably, during the first 2 weeks following the two-drug combination therapy, the tumor growth was almost completely inhibited, and after that, the tumor started to grow at a relatively slow rate. At the end of the 6 weeks of chemotherapy, the tumor volumes were significantly smaller with the two-drug combination therapy than what was expected according to the anticancer effects of the individual drugs. Notably, the average body weights of the animals treated with paclitaxel + 2-MeO-E2 or vinorelbine + 2-MeO-E2 for 6 weeks were slightly lower (6.4 ± 4.1% or 5.2 ± 4.2%, respectively) than the body weights of animals treated with paclitaxel or vinorelbine alone. From a comparison of the tumor size changes with the body weight changes in various treatment groups, it was evident that the decrease in tumor size in the combination drug treatment groups was far greater than the decrease of body weight in these groups. These observations suggested that the observed stronger anticancer

![Figure 3. Antiproliferative actions of 2-MeO-E2 in combination with FU or doxorubicin in the ER-negative MDA-MB-231 and MDA-BM-435 cells. The rate of cell growth in the absence of 2-MeO-E2 and the anticancer agent was assigned to be 100%, and the rate of cell growth in the presence of 2-MeO-E2 or an anticancer agent or a combination of both was expressed as percent of the control. Columns, mean of six to seven replicate determinations; bars, SE.](image-url)
actions of 2-MeO-E2 (which has low toxicity) and paclitaxel or vinorelbine, intolerance to their toxicity has often been the main cause for early discontinuation of the chemotherapy and, thus, the observed synergism may be attributable to the similar mechanisms of action shared by these anticancer agents, namely, through the disruption of the normal microtubule functions during mitosis. It is highly possible that 2-MeO-E2 may have a different target site on the microtubule for exerting its anticancer action than the target sites of action for paclitaxel and vinorelbine, thereby causing a synergistic enhancement of the disruption of the mitotic microtubule functions. This explanation seems to be in line with our observation that the synergistic effect between 2-MeO-E2 and paclitaxel or vinorelbine was more pronounced when each of the two drugs was present at relatively lower concentrations, which would only produce a very weak growth inhibition individually. Moreover, this explanation also agrees with our observation that the maximum anticancer efficacy of the two-drug combinations was not markedly different from one drug alone when present at higher concentrations.

It should be noted that we have also compared in the present study the effects of combining 2-MeO-E2 with several other anticancer agents, including FU, methotrexate, doxorubicin, and mitomycin C. These commonly used anticancer agents have very different mechanisms of anticancer actions, and their inclusion may assist in determining whether 2-MeO-E2 can broadly or selectively enhance the anticancer actions of chemotherapeutic agents in human breast cancer. Besides a weak additive effect between 2-MeO-E2 and FU, no synergistic effect was observed in any of the four cell lines tested. Interestingly, when doxorubicin was used in combination with 2-MeO-E2, this drug at higher concentrations was found to antagonize the growth-inhibitory effect of 2-MeO-E2. This observation is rather intriguing, and more studies are needed to understand the mechanism of such an antagonistic interaction.

In summary, 2-MeO-E2 in combination with paclitaxel or vinorelbine exhibited a synergistic anticancer effect in several human breast cancer cell lines in vitro, and this synergistic effect was more pronounced when each of the drugs was present at relatively low concentrations. Oral administration of 2-MeO-E2 at 30 mg/kg body weight, once weekly for 6 weeks, also markedly enhanced the anticancer activity of paclitaxel or vinorelbine against the ER-negative MDA-MB-231 human breast cancer xenografts grown in athymic female BALB/c nu/nu mice. By contrast, combination of 2-MeO-E2 with FU or doxorubicin had no synergistic effects. The results of our present study raised the possibility that 2-MeO-E2, at nontoxic or subtoxic doses, may be used as an effective adjunct to enhance the chemotherapeutic effects of certain microtubule-disrupting agents such as paclitaxel and vinorelbine in the treatment of ER-negative, late-stage human breast cancer. This novel and highly practical idea warrants further clinical testing in the receptor-negative breast cancer patients.

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Figure 4. Enhancement by 2-MeO-E2 of the anticancer effects of paclitaxel or vinorelbine in the ER-negative MDA-MB-231 breast cancer cell xenografts grown in female athymic BALB/c nu/nu mice. The ER-negative MDA-MB-231 cells (5 × 10⁶ cells in 100 μL) were subcutaneously injected into the mammary fat pad of the animals. Tumors were allowed to develop for 20 days and the mice were then randomly grouped, and the animals received one of the following treatments: paclitaxel (i.p., at 5 mg/kg body weight per injection, once a week for 6 weeks), vinorelbine (i.p., at 3 mg/kg body weight per injection, once a week for 6 weeks), 2-MeO-E2 (p.o. gavage, at 30 mg/kg body weight, once a week for 6 weeks), paclitaxel in combination with 2-MeO-E2 at the same doses, or vinorelbine in combination with 2-MeO-E2 at the same doses. Thirty animals per group were used in the experiment. Both the maximum and minimum diameters of the resulting tumors were measured once a week using a slide caliper. Tumor volumes were calculated by assuming a spherical shape and using the formula: volume = 4/3πr³/3, where r = 1/2 of the mean tumor diameter measured in two dimensions. Points, mean; bars, SD. Right, the line for 2-MeO-E2 alone was not shown (which is the same as the line in the left).

Discussion

Paclitaxel and vinorelbine are among the commonly used microtubule-disrupting agents for the treatment of late-stage human breast cancer (13–18). Mechanistically, they exert their anticancer actions primarily through disturbing the disassembly of microtubules (for paclitaxel) or the polymerization of tubulins (for vinorelbine), consequently resulting in mitotic arrest and cell death. The results of our present study showed that 2-MeO-E2, a well-known nonpolar endogenous metabolite of 17β-estradiol with strong antiproliferative and apoptotic actions in cancer cells, enhanced the growth-inhibitory effect of paclitaxel and vinorelbine in vitro and in vivo in a synergistic manner. Notably, our results are in line with an earlier study that reported a certain degree of synergy between the antiangiogenic actions of paclitaxel and 2-MeO-E2 when tested in vitro (19).

Despite the strong chemotherapeutic efficacy of paclitaxel and vinorelbine, intolerance to their toxicity has often been the main cause for early discontinuation of the chemotherapy and, thus, clinical failure. Our finding of a synergy between the anticancer actions of 2-MeO-E2 (which has low toxicity) and paclitaxel or vinorelbine in human breast cancer cells suggests the possibility that a combined use of 2-MeO-E2 with paclitaxel or vinorelbine may significantly enhance the anticancer activity or maintain the effective anticancer activity of paclitaxel or vinorelbine, yet without increasing the drug dose and their overall toxicity.

Although it has been suggested that the mechanism of the antiproliferative actions of 2-MeO-E2 at pharmacologic concentrations results from its antitubulin activity (4, 5), the mechanism underlying the synergic actions between 2-MeO-E2 and paclitaxel or vinorelbine is not understood at present. However, it is reasonable to suggest that the observed synergism may be attributable to the similar mechanisms of action shared by these anticancer agents, namely, through the disruption of the normal microtubule functions during mitosis. It is highly possible that 2-MeO-E2 may have a different target site on the microtubule for exerting its anticancer action than the target sites of action for paclitaxel and vinorelbine, thereby causing a synergistic enhancement of the disruption of the mitotic microtubule functions. This explanation seems to be in line with our observation that the synergistic effect between 2-MeO-E2 and paclitaxel or vinorelbine was more pronounced when each of the two drugs was present at relatively lower concentrations, which would only produce a very weak growth inhibition individually. Moreover, this explanation also agrees with our observation that the maximum anticancer efficacy of the two-drug combinations was not markedly different from one drug alone when present at higher concentrations.

In summary, 2-MeO-E2 in combination with paclitaxel or vinorelbine exhibited a synergistic anticancer effect in several human breast cancer cell lines in vitro, and this synergistic effect was more pronounced when each of the drugs was present at relatively low concentrations. Oral administration of 2-MeO-E2 at 30 mg/kg body weight, once weekly for 6 weeks, also markedly enhanced the anticancer activity of paclitaxel or vinorelbine against the ER-negative MDA-MB-231 human breast cancer xenografts grown in athymic female BALB/c nu/nu mice. By contrast, combination of 2-MeO-E2 with FU or doxorubicin had no synergistic effects. The results of our present study raised the possibility that 2-MeO-E2, at nontoxic or subtoxic doses, may be used as an effective adjunct to enhance the chemotherapeutic effects of certain microtubule-disrupting agents such as paclitaxel and vinorelbine in the treatment of ER-negative, late-stage human breast cancer. This novel and highly practical idea warrants further clinical testing in the receptor-negative breast cancer patients.
References

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