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Report

Effect of antiprogestins and tamoxifen on growh inhibition of MCF-7 human breast cancer cells in nude mice

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Key words: antiestrogens (tamoxifen), antiprogestins (mifepristone, onapristone), breast cancer, MCF-7 cells, nude mice

Summary

This is the first report demonstrating an *in vivo* antitumor activity of antiprogestins (mifepristone, onapristone) alone and in combination with tamoxifen in the MCF-7 human breast cancer model. The MCF-7 cells produced progressive growing tumors in female nude mice supplemented with 17β-estradiol. Tumor regression was observed following either estrogen ablation alone or estrogen ablation in combination with tamoxifen. Monotherapy with tamoxifen or antiprogestins caused a retardation of estrogen-induced tumor progression. Complete inhibition or prevention of tumor growth occurred as a result of simultaneous administration of mifepristone and tamoxifen. The addition of mifepristone in this combination treatment was also effective in delaying or preventing tumor escape (relapse) from the antiestrogenic (antitumor) effect of tamoxifen. These results suggest a potential clinical benefit of adding an antiprogestin to antiestrogen therapy of breast cancer patients.

Introduction

A new approach for treatment of hormone-dependent breast cancer could be the use of antiprogestins, a new class of compounds (progesterone antagonists) which were developed originally for inhibition of progesterone-dependent processes (e.g. termination of pregnancy) [1]. In preliminary clinical studies, some breast cancer patients responded to treatment with the antiprogestin mifepristone (RU 486, Roussel Uclaf/Population Council). The beneficial effects were observed mainly in patients with progesterone (PR) and estrogen (ER) receptor positive (+) tumors [2–4]. Robertson et al. [5] have conducted a clinical study using the antiprogestin onapristone (ZK 98.299, Schering AG-100 mg/day) as a first-line endocrine therapy in either patients with locally advanced breast cancer (n = 12) or elderly patients with primary metastatic disease (n = 7). Seventeen of the 19 tumors expressed estrogen receptors. 56% of treated patients showed partial remission and 11% showed a static disease, giving an overall tumor remission rate of 67% [5].

While the antitumor activity of antiprogestins in rodent mammary tumor models has been the subject of intense research [2, 6–14], their effect in *in vivo* human breast cancer models has received much less attention [9, 12]. The authors could not find any published data demonstrating that antiprogestins will inhibit the hormone-dependent ER (+) and PR (+) MCF-7 human breast cancer model

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in nude mice. This human breast cancer model system has been used frequently to test the antitumor activity and mechanism of action of estrogen ablation and antiestrogen treatment [15–20]. Therefore, our objective in this study was to compare the antitumor activity of antiprogestins (mifepristone, onapristone) with that of tamoxifen or the combination of both utilizing the MCF-7 human breast cancer cell line inoculated into female nude mice supplemented with 17β estradiol. The effect of estrogen withdrawal (ablation) alone and in combination with tamoxifen treatment was also determined to evaluate the contribution of the antihormone (antiestrogen/antiprogestin) effect versus estrogen ablation. In this paper, results are presented to demonstrate for the first time an antitumor activity of antiprogestins alone and in combination with tamoxifen in the MCF-7 human breast cancer model in nude mice.

Materials and methods

Cell line and tumor growth

The MCF-7 human breast cancer cells (passage number 149) were obtained from American Type Culture Collection (ATCC), Rockville, MD. The cells were cultured in Dulbecco's Modified Eagle medium (DMEM), low glucose, containing 1 mM sodium pyruvate, 10 μg/ml bovine insulin, and 10% fetal bovine serum. They were maintained at 37° C, 5% CO_2 incubator. The MCF-7 cells (5×10^6 cells) were inoculated subcutaneously (s.c.) into the right flank of 6- to 8-week old intact female nude mice (BALB/c, nu/nu: Harlan, Indianapolis, IN). One day before inoculation of MCF-7 cells, the animals were implanted with 17β-estradiol (E_2) pellets (Innovative Research of America, Sarasota, FL) into the left flank. Different E_2 pellets were used in the various experiments (0.72 or 1.7 mg E_2/pellet for 60 or 90 days release) to maintain a constant high serum E_2 level for the duration of the experiments (see Results). The nude mice were examined for tumors by palpation. Tumor size was measured in three dimensions in millimeters once or twice weekly and tumor volumes were calculated according to the known formula: Volume = $\pi/6 \times$ [Length × Width × Height] and expressed in cubic millimeters ($\pi = 3.1416$). In all experiments, caliper measurement of tumor volume was performed in a blinded fashion (without knowledge of the different experimental groups) by two independent investigators. The tumors were permitted to grow in the presence of the E_2 pellets until they reached a volume of about 160–350 mm^3. The initial tumor volume (at the start of treatment) was different in our various experiments. This variable, as well as other specific conditions (e.g. E_2 pellets, number of tumor-bearing animals, etc.) of each experiment, are given in the legends of the figures, in the tables, and/or in the results of the different nude mouse experiments.

Experimental design

The animals were randomly assigned to different experimental groups of 3–5 animals each. A positive control group retained the E_2 pellets to allow continued tumor growth. E_2 pellets were removed in some groups to inhibit tumor growth (estrogen ablation) by puncturing the skin and retrieving the pellet with forceps. The other groups retained the E_2 pellets and were treated with mifepristone, onapristone (both generously provided by Schering AG, Berlin, Germany), tamoxifen, or their combinations. The treatments were continued for either 17 or 35 days (5 weeks) in various experiments. The animals in the antiprogestin treatment groups received mifepristone (50 mg/kg/day s.c.) or onapristone (30 mg/kg/day s.c.) suspended in 0.1 ml vehicle (0.1 ml ethanol, 0.0625 ml Tween 80, and 0.8375 ml 0.9% NaCl). The animals in the tamoxifen treatment groups received a tamoxifen pellet (15 mg/pellet) for 60 days release of 10 mg/kg/day (Innovative Research of America, Sarasota, FL). Control animals and animals in the tamoxifen monotherapy groups were treated daily with 0.1 ml of the above-mentioned vehicle s.c. To test the estrogen dependency of our MCF-7 tumor model system, a few animals were implanted with the E_2 pellets to allow continuous tumor progression, then the E_2 pellets were removed. Tumor regression was followed by

caliper measurement of tumor volume once weekly as described above. After the tumors became unpalpable for different periods of time (up to several months), reimplantation of the E_2 pellets was performed on these same animals to study the effect of estrogen-resubstitution on tumor growth. These cycles of E_2 pellet supplementation and E_2 pellet removal were repeated several times. At the end of the experiments, all animals were sacrificed by spinal elongation and the tumors were harvested and wet weights determined. Percentage inhibition of tumor volume or tumor weight was calculated according to the formula: T-C/C × 100 (T = treatment, C = Control).

Statistical analysis

Differences among groups for the antitumor activity were tested using one-way analysis of variance (ANOVA) with repeated measures over time. The assumption of analysis of variance was examined and nonparametric tests based on ranks used if needed. Values for tumor volume and tumor weight were reported as means ± standard errors of the mean (SEM). When ANOVA indicated significant treatment effects (F-ratio, $P < 0.05$), the Student Newman-Keuls multirange test was employed to compare individual treatment means.

Results

Effect of estrogen supplementation, estrogen ablation, and/or tamoxifen treatment

In a first experiment to test the estrogen-dependency of our MCF-7 tumor model system, the MCF-7 tumors showed a slow growth pattern in the presence of E_2 pellets (0.72 mg E_2/pellet to maintain serum E_2 levels between 300–400 pg/ml for 60 days). These tumors were permitted to grow for almost 8 weeks after subcutaneous injection of the MCF-7 cells in nude mice until they reached an average tumor volume of about 160 mm^3. In the control group, E_2 supplementation induced a significant time-dependent tumor progression. The average tumor volume increased from about 160 mm^3 (at start of treatment) to about 800 mm^3 after 5 weeks of continuous estrogen stimulation. Tamoxifen (10 mg/kg/day) caused a retardation of the E_2-induced tumor progression. At the end of treatment, tamoxifen induced approximately 50% inhibition of tumor growth. However, this inhibition was not significantly different ($P > 0.05$) as compared with the E_2 control group (Figure 1 and Table 1). In the group of animals in which the E_2 pellets were removed, estrogen ablation (withdrawal) induced 86 or 88% inhibition of tumor growth as compared with the E_2 control group ($P < 0.05$). The combination of tamoxifen treatment and estrogen withdrawal resulted in slightly more tumor growth inhibition as compared with estrogen ablation alone. However, the extent of inhibition of tumor volume and tumor weight in this combination group was only significantly different ($P < 0.05$) as compared with the E_2 control group (see Table 1). In all treatment groups (except the E_2 control), a slight decrease in tumor volume (tumor regression) was observed for the first few days of treatment. This tumor regression continued in both groups treated by estrogen ablation alone or estrogen ablation in combination with tamoxifen. The average tumor volume in these two groups at the end of the 5 weeks of treatment was 113 or 78 mm^3, clearly below the initial tumor volume at the start of treatment (about 160 mm^3). On the contrary, in the group treated with tamoxifen alone, the transient tumor regression was followed with a progressive increase in tumor volume (relapse), indicating an escape from the (antiestrogen) antitumor effect of tamoxifen.

The estrogen-dependency of this model was confirmed also by implanting the E_2 pellets in a few animals to allow continued tumor growth for several weeks. When the E_2 pellets were removed tumor growth was inhibited significantly and after about 10 weeks the tumors became unpalpable. After different periods of time (up to several months), the reimplantation of the E_2 pellets caused these tumors to regrow again and become palpable (data not shown). We repeated these E_2 supplementation and E_2 withdrawal cycles several times and the results were reproducible. The tumors disappeared with estrogen withdrawal and growth resumed with

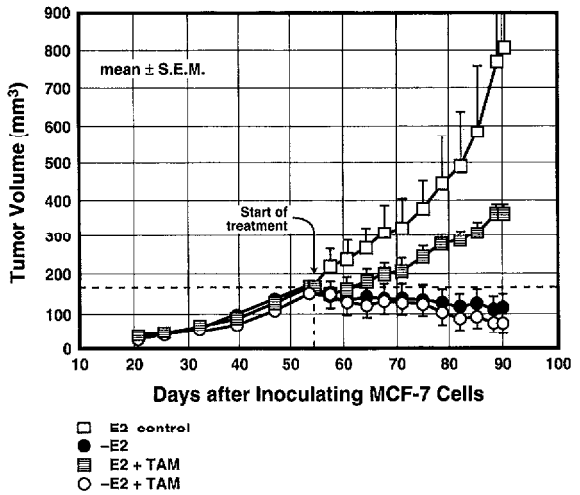

Figure 1. Effect of 35 days of estrogen ablation (– E_2) and/or tamoxifen (TAM) treatment on the growth of MCF-7 tumors in nude mice. This experiment consisted of the following four groups: (1) a positive control group retaining the E_2 pellets (E_2 control), (2) an estrogen ablation group in which the E_2 pellets were removed (– E_2), (3) a tamoxifen treatment group retaining the E_2 pellets and receiving a tamoxifen pellet (15 mg/pellet) for 60 days release of 10 mg/kg/day (E_2 + TAM), and (4) an E_2-ablation (E_2 pellets were removed) group which also received the same tamoxifen pellet as described above (– E_2 + TAM). Seventeen tumor-bearing mice were included in this experiment.

estrogen supplementation. These results confirm the estrogen dependency of our MCF-7 tumor model system and clearly indicate that tumor cells remained viable despite prolonged estrogen deprivation. Similar observations were made before [15].

Effect of mifepristone and/or tamoxifen treatment

In a second experiment, we compared the antitumor activity of mifepristone with that of tamoxifen and the combination of both. The MCF-7 tumors showed a rapid growth pattern in this experiment. These tumors were permitted to grow for only about 3 weeks after subcutaneous inoculation of tumor cells in nude mice (in the presence of the E_2 pellets: 1.7 mg E_2/pellet to maintain serum E_2 levels above 900 pg/ml for 60 days) until they reached an average tumor volume of about 200 mm^3. In the control group, E_2 supplementation induced a significant time-dependent rapid tumor progression. The average tumor volume increased from about 200 mm^3 (at start of treatment) up to about 750 mm^3 after 5 weeks of continuous estrogen stimulation. In all treatment groups (except the E_2 control), a slight decrease in initial tumor volume (tumor regression) was observed for the first few days of treatment. In the tamoxifen and mifepristone monotherapy groups, this initial tumor response was followed by a progressive increase in tumor volume (relapse) indicating an escape from the antitumor effect of tamoxifen or mifepristone. Nevertheless, the administration of tamoxifen (10 mg/kg/day) or mifepristone (50 mg/kg/day) as monotherapy, induced a significant retardation of tumor growth. At the end of treatment, monotherapy induced about 50% inhibition of tumor volume as compared with the E_2 control group ($P < 0.05$) (Figure 2 and Table 2). The inhibition of tumor volume

Table 1. Effect of estrogen supplementation (17β-estradiol = E_2 control), estrogen ablation (– E_2), and/or tamoxifen (TAM) treatment on growth of MCF-7 tumors in nude mice

Groups	Results after 5 weeks of treatment			
	Average tumor volume (mm^3)[a]	% Inhibition	Average tumor weight (mg)	% Inhibition
E_2 control	801 ± 311		914 ± 308	
E_2 + TAM	363 ± 33	55	451 ± 69	51
– E_2 (E_2 ablation)	113 ± 42[b]	86	113 ± 47[b]	88
– E_2 + TAM	78 ± 16[b]	90 (31/79)[c]	82 ± 24[b]	91 (27/82)[c]

For details of experimental design see Figure 1. Mean ± S.E.M.

[a] = Initial average tumor volume at the start of treatment was about 160 mm^3.

[b] = Significant difference ($P < 0.05$) as compared with E_2 control.

[c] – Numbers between brackets are % inhibition as compared with E_2 ablation or E_2 + TAM groups.

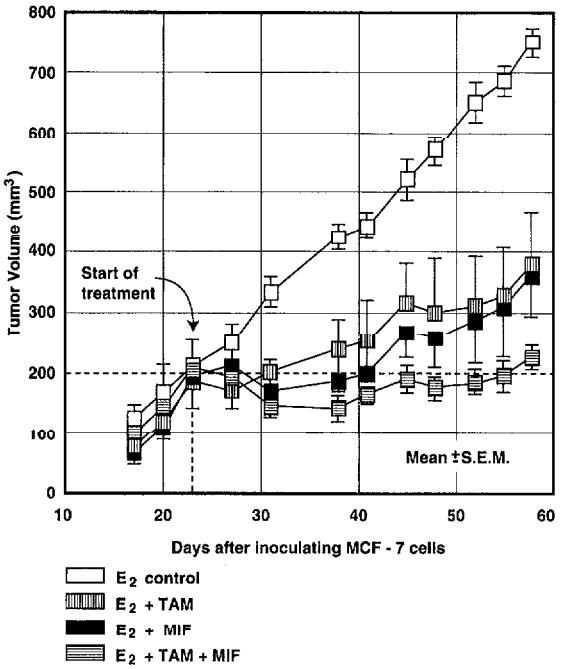

Figure 2. Effect of 35 days of treatment with tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen (17β-estradiol = E_2)-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the E_2 pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. (E_2 control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day + the vehicle s.c. (E_2 + TAM), (3) a mifepristone treated group receiving 50 mg/kg/day s.c. (E_2 + MIF), and (4) a combination group treated with the same tamoxifen pellet in combination with mifepristone in the same dose as in group 3 (E_2 + TAM + MIF). Twenty tumor bearing mice were included in this experiment.

and tumor weight was amplified to 70 or 74% by simultaneous administration of tamoxifen and mifepristone. The effect of the various drug treatments on the reduction of tumor volume started to become significantly different ($p < 0.05$) from the control group as early as 8 days after the start of treatment. The antitumor activity in the combination group resulted in complete growth inhibition delaying or preventing the escape phenomenon (relapse) for about 5 weeks. These results suggest that mifepristone (50 mg/kg/day) in combination with tamoxifen (10 mg/kg/day) was slightly more effective as compared with tamoxifen (10 mg/kg//day) or mifepristone (50 mg/kg/day) monotherapy in this particular experiment. However, the extent of inhibition in this combination group was only significantly different ($P < 0.05$) from the E_2 control group (see Table 2).

Effect of mifepristone, onapristone, and/or tamoxifen treatment

In a third experiment, the antitumor activity of antiprogestins (mifepristone and onapristone), tamoxifen and their combination was tested. The MCF-7 tumors showed a rapid growth pattern in this experiment in the presence of E_2 pellets (1.7 mg E_2/pellet to maintain serum E_2 levels at about 500–600 pg/ml for 90 days). These tumors were permitted to grow for about 8 weeks after subcutaneous

Table 2. Effect of tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen-(17β-estradiol – E_2) induced growth of MCF-7 tumors in nude mice

Groups	Results after 5 weeks of treatment			
	Average tumor volume (mm^3)[a]	% Inhibition	Average tumor weight (mg)	% Inhibition
E_2 control	752 ± 17		1013 ± 302	
E_2 + TAM	384 ± 115[b]	49	506 ± 216	51
E_2 + MIF	376 ± 188[b]	50	607 ± 34	40
E_2 + TAM + MIF	223 ± 12[b,c]	70 (42/41)[d]	266 ± 5[b,c]	74 (47/56)[d]

For details of experimental design see Figure 2. Mean ± S.E.M.

[a] = Initial average tumor volume at the start of treatment was about 200 mm^3.

[b] = Significant difference ($P < 0.05$) as compared with the control group.

[c] = Difference between combination and single drug treatments is not statistically significant.

[d] = Numbers between brackets are % inhibition as compared with tamoxifen or mifepristone monotherapy group.

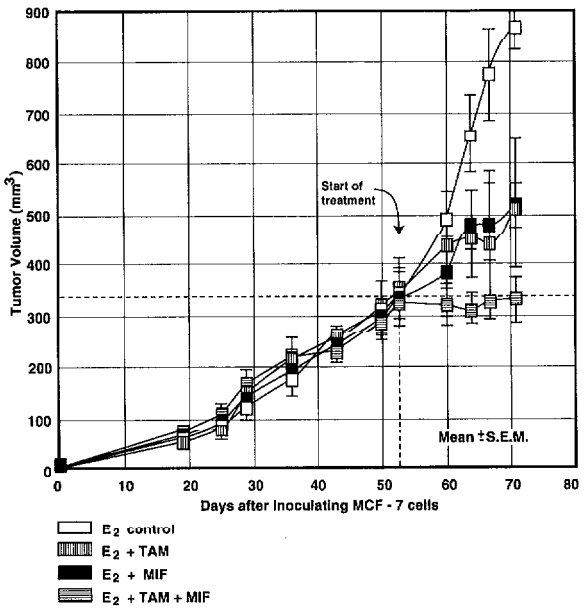

Figure 3. Effect of 17 days of treatment with tamoxifen (TAM), mifepristone (MIF), and their combination on estrogen (17β-estradiol = E_2)-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the E_2 pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. (E_2 control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day, + the vehicle s.c. (E_2 + TAM), (3) a mifepristone treated group receiving 50 mg/kg/day s.c. (E_2 + MIF), and (4) a combination group treated with the same tamoxifen pellet in combination with mifepristone in the same dose as in group 3 (E_2 + TAM + MIF). The rest of the results of this experiment are shown in Figure 4. Twenty-six tumor-bearing mice were included in this experiment.

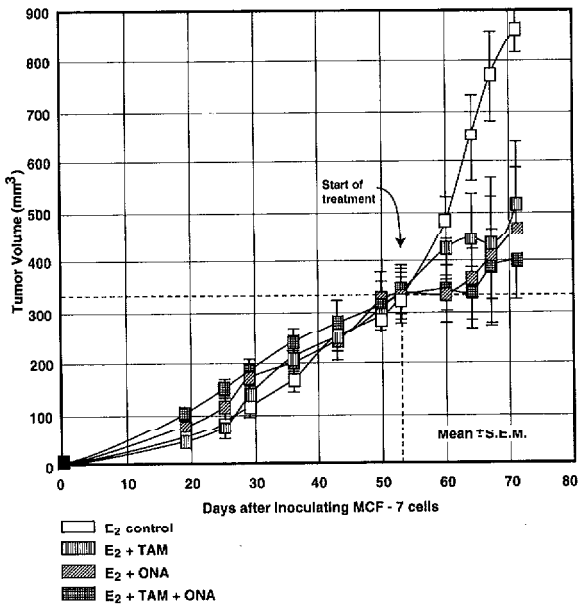

Figure 4. Effect of 17 days treatment with tamoxifen (TAM), onapristone (ONA), and their combination on estrogen (17β-estradiol = E_2)-induced tumor growth of MCF-7 tumors in nude mice. All animals retained the E_2 pellet and were randomly assigned to 4 groups: (1) a control group receiving the vehicle s.c. (E_2 control), (2) a tamoxifen treated group receiving 15 mg tamoxifen/pellet which is releasing 10 mg/kg/day + the vehicle s.c. (E_2 + TAM), (3) an onapristone treated group receiving 30 mg/kg/day s.c. (E_2 + ONA), and (4) a combination group treated with the same tamoxifen pellet in combination with onapristone in the same dose as in group 3 (E_2 + TAM + ONA). The rest of the results of this experiment are shown in Figure 3. Twenty-six tumor-bearing mice were included in this experiment.

injection of the tumor cells in nude mice until they reached an average tumor volume of about 350 mm^3. In the control group, E_2 supplementation induced a significant time-dependent rapid tumor progression. The average tumor volume increased from about 350 mm^3 (at start of treatment) to about 860 mm^3 after only 17 days of continuous estrogen stimulation. The administration of tamoxifen (10 mg/kg/day), mifepristone (50 mg/kg/day), or onapristone (30 mg/kg/day) for 17 days as a monotherapy induced approximately 30 or 40% inhibition of tumor growth ($P < 0.05$) as compared with the E_2 control group (Figures 3 and 4 and Table 3). This tumor inhibition was slightly amplified by the simultaneous administration of mifepristone in combination with tamoxifen. This combination treatment resulted in complete growth inhibition of these rapid growing MCF-7 tumors in nude mice (see Figure 3 and Table 3). In this experiment, both tumor volume and tumor weight at the end of the treatment was significantly different ($P < 0.05$) in all treated groups as compared with the control group (see Table 3). Most probably due to the large tumor volume at the start of treatment (300–400 mm^3) and due to their rapid growth under estrogen stimulation, no treatment-related transient tumor regression was observed. However, the first measurement of tumor volume in this experiment was one week after the start of treatment, so that we may have missed the transient tumor regression phase, which could have been observed within the first few days of treatment.

Discussion

This is the first report demonstrating an antitumor activity of antiprogestins (mifepristone/onapristone) alone and in combination with tamoxifen in the MCF-7 human breast cancer model in nude mice. Our results were consistent and reproducible in two different experiments, independent of the initial tumor load at the start of treatment and the rate of tumor growth and progression. The estrogen-dependency of our MCF-7 tumor model was clearly evident in all our experiments. In agreement with many previously published studies [15–20], progressively growing tumors were produced only when the animals were supplemented with exogenous E_2. Without estrogen supplementation, no tumor growth was observed in our intact female nude mice, most probably due to low estrogen levels, unable to support the growth of the MCF-7 tumors. Also, the extent of tumor growth in control animals was different in various experiments, depending on the type of the E_2 pellets used and the serum E_2 levels maintained (see Figures 1–4). Furthermore, in this and other investigations [16–18], tumor regression was observed as a result of estrogen ablation. In our study, the tumor regression as a result of the removal of the E_2 pellets could also be slightly amplified by the simultaneous administration of tamoxifen. On the contrary, in a previous study estrogen ablation and/or antiestrogen treatment resulted only in cessation of tumor growth, but not in tumor regression [15]. However, other groups reported, in agreement with our results, a rapid reduction in the MCF-7 tumor volume in nude mice treated by estrogen withdrawal and/or tamoxifen [19, 20].

Based on our own previously published studies and in agreement with data of other investigators, the addition of tamoxifen or a pure antiestrogen (ICI 164,384) to the antiprogestin (mifepristone, onapristone, etc.) treatment showed a striking additive antitumor effect in several rodent hormone-dependent breast cancer models [2, 10, 11, 13]. Using the MCF-7 human breast cancer model in nude mice, our data in the present investigations also shows that the combination of tamoxifen and mifepristone completely prevents or abolishes tumor growth, while monotherapy results only in inhibition of tumor growth as compared with the estrogen-treated controls. Moreover, there is a good evidence that the simultaneous administration of mifepristone in combination with tamoxifen is effective in delaying or preventing tumor escape (relapse) from the antiestrogenic (antitumor) effect of tamoxifen. These results mimic the clinical situation and suggest that: (a) adjuvant treatment by antiprogestins in combination with tamoxifen may prevent tumor escape, which usually develops after

Table 3. Effect of tamoxifen (TAM), mifepristone (MIF), onapristone (ONA), and their combinations on estrogen-(17β-estradiol = E_2) induced growth of MCF-7 tumors in nude mice

Groups	Results after 17 days of treatment			
	Average tumor volume (mm^3)[a]	% Inhibition	Average tumor weight (mg)	% Inhibition
E_2 Control	863 ± 41		1013 ± 137	
E_2 + TAM	515 ± 128[b]	40	605 ± 185[b]	40
E_2 + MIF	512 ± 32[b]	41	700 ± 20[b]	31
E_2 + ONA	464 ± 117[b]	46	644 ± 103[b]	36
E_2 + TAM + MIF	325 ± 21[b,c]	62 (37/37)[d]	465 ± 22[b,c]	53 (22/32)[d]
E_2 + TAM + ONA	403 ± 76[b,c]	53 (21/13)[d]	576 ± 34[b,c]	43 (5/11)[d]

For details of experimental design see Figures 3 and 4. Mean ± S.E.M.

[a] = Initial average tumor volume at the start of treatment was about 350 mm^3.

[b] = Significant difference ($P < 0.05$) as compared with the control group.

[c] = Difference between combination and single drug treatments is not statistically significant.

[d] = Numbers between brackets are % inhibition as compared with tamoxifen or the corresponding antiprogestin monotherapy groups.

a long-term treatment with tamoxifen, and (b) the antitumor effect of tamoxifen can be enhanced by simultaneous administration of antiprogestins. This may increase the rate and magnitude of the clinical response, reduce malignant progression and relapse, as well as increase the survival of breast cancer patients. These assumptions have been recently strengthened by results of *in vitro* studies from our laboratories. In these studies, MCF-7 cells growing in culture were used to explore the mechanism of the antiproliferative activity of mifepristone, as compared with 4-hydroxytamoxifen or the combination of both. These steroid antagonists induced a significant time- and dose-dependent cell growth inhibition (cytotoxicity). This inhibition of cell survival was associated with a significant increase in DNA fragmentation (apoptosis), downregulation of bcl_2, and induction of $TGF\beta_1$ protein [21]. Abrogation of the mifepristone- and/or 4-hydroxytamoxifen-induced cytotoxicity by $TGF\beta_1$ neutralizing antibody confirms the correlation between induction of active $TGF\beta_1$ and subsequent cell death [21]. The effect of a combination of mifepristone and 4-hydroxytamoxifen on cell growth inhibition, on the increase in DNA fragmentation, bcl_2 downregulation and induction of $TGF\beta_1$ protein was additive and significantly different ($P < 0.05$) from the effect of monotherapy [21]. A translocation of protein kinase C (PKC) activity from the soluble to the particulate and/or nuclear fraction appeared to be also additive in cells treated with a combination of both 4-hydroxytamoxifen and mifepristone [21]. These results suggest that the mechanism of the additive antiproliferative activity of mifepristone and tamoxifen could be explained at least in part by an additive induction of apoptosis in both ER and PR positive MCF-7 breast cancer cells. A bcl_2 downregulation, the PKC transduction pathway, and $TGF\beta_1$ expression seem to be involved in this additive mechanism of action [21]. However, all clinical trials of concurrent combination endocrine therapy in breast cancer have so far not shown a confirmed evidence of increase either in the mean duration of remission or survival. Therefore, sequential endocrine therapy has been preferred because it keeps some effective therapy in reserve and may also delay the onset of tumor autonomy [22, 23]. Furthermore, there are no clinical data supporting that combinations of antiestrogens and antiprogestins should be better than monotherapy. Therefore, a lot of caution is needed in extrapolations of data from our experimental models to the clinical situation. Also, the precise molecular biochemical mechanism of the *in vivo* growth inhibitory effect of tamoxifen versus mifepristone in the MCF-7 tumor model needs to be clarified in future studies. These studies are currently going on in our laboratories using the breast cancer tissue harvested from the different nude mouse experiments described in this publication.

Acknowledgements

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