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Potentiation of the antitumor effect of tamoxifen by combination with the antiprogestin onapristone

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ABSTRACT

The present study was undertaken to investigate the antitumor effect of a combination of the antiestrogen tamoxifen (TAM) and either the antiprogestin onapristone (ON) or the progestogen megestrol acetate (MEG) in experimental mammary tumor models. Rats bearing DMBA- or NMU-induced mammary tumors were treated with ON or MEG either alone or in combination with TAM for four weeks. In the DMBA-tumor model, treatment with ON or TAM alone caused tumor remissions, whereas the combination of ON and TAM was almost as effective as ovariectomy (100% remission) and led to a remission of 86–100%. The combination of TAM and ON was distinctly more effective than that of TAM and MEG. A similar potentiation of the antitumor effect of TAM and ON was observed in the NMU-tumor model. In DMBA-tumors, the concentration of progesterone receptors was found to increase after treatment for three days with TAM and ON. In hosts bearing DMBA-tumors, treatment with the combination of TAM and ON caused a reduction in ovarian and uterine weights. In the same animals, the basal level of progesterone was decreased in spite of a slight increase in the LH level. These findings suggest that the high antitumor effect of the combination of TAM and ON compared to the corresponding monotherapies can be related not only to the interaction of antihormones and receptors, but also to the up-regulation of PR and to a decrease in progesterone production. These data clearly suggest the sense of a combination of TAM with an antiprogestin in breast cancer treatment.

1. Introduction

In cancer therapy combinations of cytotoxic drugs are generally used. So far however no combination of endocrine therapeutics has been routinely used in the treatment of hormone-sensitive breast cancer. It is reasonable to assume that an appropriate combination of hormones or antihormones with different modes of action might be more effective than the respective monotherapies. In publications, we have reported on the enhancement of the antitumor effect of the progesterone antagonist onapristone by combination with pure antiestrogens [1,2] or aromatase inhibitor, atamestane [3,4]. To investigate the clinical effect of a combined hormone therapy, tamoxifen is the first choice for use, since this antiestrogen is the standard first line therapy of estrogen-receptor-positive mammary carcinoma. We have therefore conducted studies in experimental mammary tumors to investigate the effect of a combination of tamoxifen and onapristone.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats (Tierzucht Schönwalde GmbH/Schönwalde, Germany) were used throughout the experiment. Mammary tumors were induced by treatment of animals with DMBA or NMU as described previously [5]. Tumor size was measured once weekly with calipers. When the largest tumor in each animal had reached 1.5 cm in diameter, treatment by subcutaneous injections with ON, MEG or TAM was initiated, or ovariectomy was performed. Treatment was continued for four weeks. Ovariectomized and control animals were treated with vehicle. One day after the last treatment the animals were sacrificed by decapitation and blood was collected. The organ weights of uterus, vagina and adrenal glands were determined.

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Table 1
Remission rates of DMBA-induced mammary tumors in rats treated with onapristone, tamoxifen or a combination of both antihormones.

	Complete remission (%)	Partial remission (%)	Progression remission (%)
Control	0	0	0
Ovariectomy	100	0	0
Onapristone (3 mg/kg[a])	10	30	60
Tamoxifen (5 mg/kg[a])	20	10	70
Onapristone + tamoxifen[a]	73	27	0

[a] Daily s.c.-treatment over four weeks.

2.2. Compounds and formulation

Onapristone (ON) and megesterol acetate (MEG) were synthesized in the laboratory of Schering AG. Tamoxifen (TAM) was purchased from Sigma Chemicals Co. (St. Louis, USA). 7,12-Dimethylbenz(α)anthracene (DMBA) and methylnitrosourea (NMU) were purchased from ICN Biochemicals (OH, USA) and Sigma Chemicals Co. (St. Louis, USA), respectively. ON, MEG and TAM were dissolved in castor oil containing 20% benzyl benzoate DMBA or NMU were dissolved in peanut oil and saline, respectively.

2.3. Radioimmunoassay (RIA)

Serum levels of estradiol and progesterone were determined by Kits from H. Biermann GmbH (Bad Nauheim, Germany). Serum LH and prolactin were determined using RIA reagents, kindly provided by Dr. A. F. Parlow (NIAMDD).

2.4. Determination of progesterone receptors (PR) in DMBA-tumor tissues

To determine the PR content by ligand binding assay (LBA), frozen tumor tissues were pulverized and homogenized in "high-salt" buffer containing 20 mM Tris, 10 mM Na_2MoO_4, 10% glycerol, 1.5 mM EDTA, 400 mM KCl and a protease cocktail (pH 7.5). The homogenate was centrifuged at 100.0 g (1 h, 4 °C). After protein determination, the supernatant was diluted to a final salt concentration of 50 mM KCl for LBA. Thereafter, a single dose saturation assay was performed using 10 nM [^{3}H]-ORG 2058 with/without a 200-fold excess of unlabelled ORG 2058. The reaction mixtures were then incubated at 4 °C for 16 h. After separation of unbound steroid by the usual dextran coated charcoal method the specific binding and the PR content were calculated.

2.5. Statistical analyses

The Dunnett-test was used for multiple comparison among groups (10 animals per group). The statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Effect on the growth of experimental mammary tumors in rats

3.1.1. NMU- and DMBA-induced mammary tumor (Tab.1 DMBA und Fig. 1 NMU)

To find a suitable dose of TAM for combination with ON, doses of TAM were investigated (Fig. 1). TAM-dose 6 mg/kg showed the highest antitumor activity, but was not able to cause an ovariectomy-like effect. Higher as well as a lower doses of TAM (1, 3 and 10 mg/kg) were less effective. As can be seen in Table 1, treatment with ON or TAM alone caused complete remissions of 10% and 20%, respectively. Compared to the monotherapies, the combination of ON and TAM was effective in inhibiting tumor growth and led to a complete remission of 73% while ovariectomy resulted in 100% complete remission. A combination of TAM and MEG had no effect on tumor growth, while the combination of TAM and ON was again highly effective (Fig. 2).

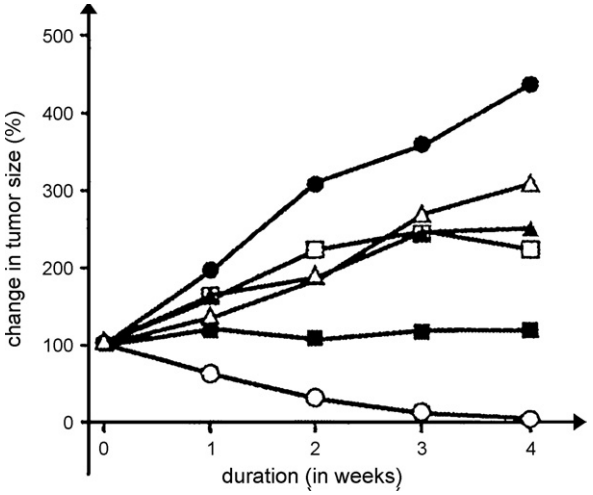

Fig. 1. Effect of tamoxifen on the growth of NMU-induced mammary tumors in rats. Animals were treated s.c. once daily with varying doses of tamoxifen (TAM) for four weeks: (□) 1 mg/kg, (△) 3 mg/kg, (■) 6 mg/kg, (▲) 10 mg/kg. Control animals (●) and ovariectomized animals (○) received only vehicle.

3.1.2. NMU-induced mammary tumor

Monotherapy with either ON or TAM caused partial remission of tumors in only 30% of animals. When both antihormones were administered concomitantly, an 80% partial and 20% complete remission was achieved while ovariectomy induced 22% partial and 67% complete remission (Table 2). With respect to tumor growth the combination was as effective as ovariectomy (Fig. 3).

3.1.3. Influence on the progesterone receptor (PR) status in DMBA-tumor tissues after treatment for three days

After treatment with the combination of ON and TAM for three days the PR content in tumor tissues was slightly higher than the

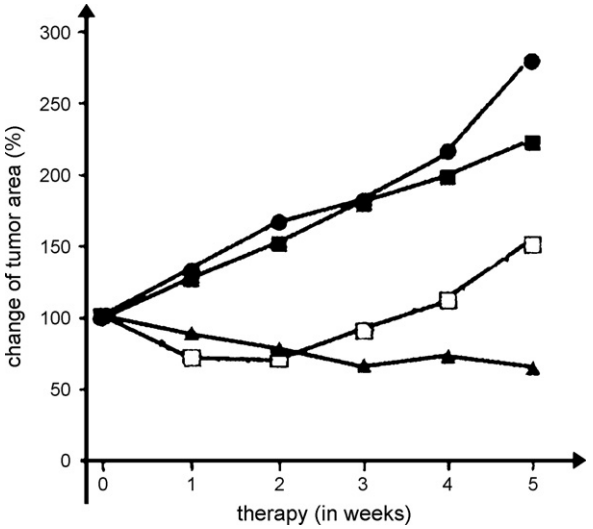

Fig. 2. Effect of onapristone and megestrol acetate in combination with tamoxifen on the growth of DMBA-induced mammary tumors in rats. Animals were treated s.c. with onapristone (5 mg/kg) + tamoxifen (5 mg/kg) (▲) or megestrol acetate (50 mg/kg) + tamoxifen (5 mg/kg) (●). Control animals (■) and ovariectomized animals (□) received only vehicle.

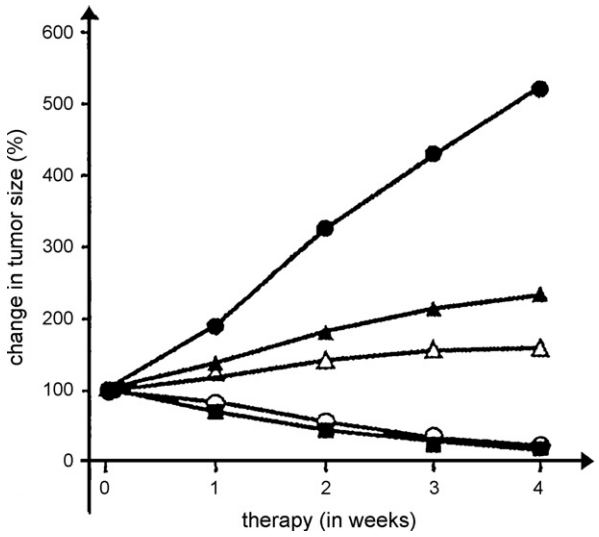

Fig. 3. Effect of onapristone and tamoxifen on the growth of NMU-induced mammary tumors in rats. Animals were treated s.c. once daily with onapristone (4 mg/kg) (△), tamoxifen (4 mg/kg) (▲) or a combination of both antihormones (■). Control animals (●) and ovariectomized animals (○) received only vehicle.

Table 2
Remission rates of NMU-induced mammary tumors in rats treated with onapristone, tamoxifen or a combination of both antihormones.

	Complete remission (%)	Partial remission (%)	Progression remission (%)
Control	0	0	0
Ovariectomy	67	22	11
Onapristone (4 mg/kg[a])	0	30	70
Tamoxifen (4 mg/kg[a])	0	30	70
Onapristone + tamoxifen[a]	20	80	0

[a] Daily s.c.-treatment over four weeks.

control value (Table 3). No difference in the tumor PR contents was found between groups treated with TAM and with vehicle (control).

3.1.4. Influence on organ weights and peripheral hormone levels at treatment in rats bearing DMBA-tumors

3.1.4.1. Organ weights. In animals bearing DMBA-tumors ovariectomy and treatment with TAM caused a significant decrease in the uterine weight (Table 4). The effect of TAM was not altered by concomitant administration of ON. ON alone had no effect on uterine weights. Ovarian weights were decreased by treatment with TAM.

3.1.4.2. Peripheral hormone levels. ON, administered alone, caused a slight increase in progesterone and LH levels (Table 4). The effect of ON on the LH level was not altered by TAM. TAM and TAM/ON-combination significantly lowered the progesterone level.

Table 3
Progesterone receptor concentration in DMBA-induced mammary tumors in rats after treatment with tamoxifen or a combination of both antihormones onapristone and tamoxifen for three days[*].

	Progesterone receptor in fmol/mg protein
Control	334 ± 44
Tamoxifen	334 ± 60
Onapristone + tamoxifen	429 ± 95

The PR-values were determined four days after the beginning of the treatments.
[*] Significant ($p < 0.05$).

Table 4
Organ weights and peripheral hormone levels in DMBA-tumor bearing rats after treatment with onapristone, tamoxifen or a combination of both antihormones for four weeks.

	Organ weight in mg		
	Ovary	Uterus	Adrenal
Control	124 ± 12	373 ± 96	71 ± 9
Ovariectomy	–	122 ± 12	80 ± 9
Onapristone (3 mg/kg)	149 ± 45	358 ± 118	74 ± 16
Tamoxifen (5 mg/kg)	81 ± 17	216 ± 32	62 ± 14
Onapristone + tamoxifen	89 ± 11	210 ± 14	67 ± 16
	Serum level		
	LH (μl/l)	Estradiol (nmol/l)	Progesterone (nmol/l)
Control	25 ± 8	82 ± 57	33 ± 14
Ovariectomy	674 ± 83	16 ± 7	6 ± 3
Onapristone (3 mg/kg)	54 ± 21	73 ± 33	59 ± 52
Tamoxifen (5 mg/kg)	26 ± 9	48 ± 22	8 ± 5
Onapristone + tamoxifen	47 ± 20	82 ± 34	5 ± 4

4. Discussion

A synergistic effect of the antiestrogen TAM and the antiprogestin mifepristone in inhibiting the *in vitro* proliferation of MCF-7 cells has been found by Thomas and Monet [6]. *In vivo* data by Bakker et al. [7] also indicate an additive antitumor effect of TAM and mifepristone on DMBA-induced mammary tumors in rats. The mechanism(s) for this combination effect is not clear. The present results also support these reported effects. TAM is known as an antiestrogen with partial estrogenic activity. Because of its inherent estrogenicity the antitumor effect of TAM is limited. The present results clearly demonstrate that 10 mg/kg of TAM are less effective than a lower dose of 6 mg/kg suggesting a predominating estrogenic effect at the higher dose. Because of its estrogenicity, TAM is, moreover, able to activate certain estrogen-regulated genes such as the PR gene [8] and to induce the PR in breast tumors [9–12]. According to Robustelli della cuna et al. [13] and Pouillart et al. [14], the increase in PR number produced by TAM could prime endogenous progestins and result in a tumor stimulation. This might be a reason why TAM cannot exert a complete inhibition of tumor growth and why the combination of TAM and MEG is ineffective in the present study.

The growth of hormone-dependent mammary tumors is stimulated not only by estrogens but also by progesterone [15–17]. It is, therefore, reasonable to expect that the antitumor effect of TAM may be enhanced – via an increase in PR – better by an appropriate combination with antiprogestins than with progestins. In our recent studies, we have shown that antiprogestins inhibit the growth of experimental mammary tumors and induce cell differentiation leading to terminal cell death (apoptosis), as the result of interaction with PR [18,19]. To ascertain the possibility of up-regulation of PR by TAM, we determined the PR concentration in tumor tissues. Although the PR level in DMBA-tumors form TAM-treated animals was not higher than the control value, a significantly higher level of PR was recognized in animals treated with the combination of TAM and ON which is distinctly more effective in inhibiting tumor growth than are the monotherapies. In a previous study, Madjno et al. [20] have found an up-regulation of PR in NMU-induced mammary tumors of rats treated with TAM for three days. In the present study using NMU-induced mammary tumor models, treatment with the combination of TAM and ON was also much more effective than the corresponding monotherapies and was almost as effective as ovariectomy. Thus, it is conceivable, that the enhancement of the tumor-inhibiting effect of TAM by the combination with ON might be due not only to the interactions of estrogen receptor and TAM but

also partly to the increase in PR for the binding with ON. In the hosts bearing DMBA-tumors, ON alone had no effect on the organ weights of ovaries, uteri and adrenal glands. The slight increase in basal LH and progesterone levels was observed in ON-treated animals, probably due to a counter-regulation of the negative feedback effect of progesterone by ON [21–23]. By contrast, TAM and the combination of TAM and ON caused a significant decrease in the peripheral level of progesterone in animals bearing DMBA-tumors. This decrease in the progesterone level may also be one part of the mechanism of the potentiation of the antitumor effect by the TAM/ON-combination.

In view of the present results, the combination of TAM and a progesterone antagonist may be worthy of consideration for the management of breast cancer.

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