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ANTITUMOR ACTIVITY AND MECHANISM OF ACTION OF DIFFERENT ANTIPROGESTINS IN EXPERIMENTAL BREAST CANCER MODELS

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Summary—Onapristone and other antiprogestins proved to possess a potent antitumor activity in several hormone-dependent experimental breast cancer models. This activity is as strong or even better than that of tamoxifen or ovariectomy in the MXT-mammary tumor of the mouse and the DMBA-and MNU-induced mammary tumor of the rat. The antitumor activity is evident in these models in spite of elevated serum levels of ovarian and pituitary hormones. The detailed analysis of all our data including the morphological (ultrastructure) studies of the mammary tumors of treated animals and the effects on growth and cell cycle kinetics using DNA flow cytometry indicates that the antitumor action of antiprogestins is mediated via the progesterone receptor and related to the induction of terminal cell differentiation leading to increased cell death. The strong antitumor activity of antiprogestins in our experimental breast cancer models does not primarily depend on a classical anti-hormonal mechanism. The antiprogestin-related reduction of the number of mammary tumor cells in the S-phase in our experimental tumor models (G_0G_1 arrest) emphasizes the unique innovative mechanism of action of these new agents in the treatment of human breast cancer.

INTRODUCTION

First choice for endocrine therapy of advanced postmenopausal breast cancer is the antiestrogen tamoxifen, whilst the second choice falls on the use of high-dose progestins and aminoglutethimide. A new approach for treatment of breast cancer could be the use of antiprogestins, compounds which were developed originally for the inhibition of progesterone-dependent processes as, for example, for interruption of pregnancy. First studies in progesterone receptor positive mammary carcinoma cell lines showed, however, that the antiprogestin, Mifepristone (RU 486, Fig. 1), had an inhibitory effect on cell growth [1] and it also proved to have a growth-inhibiting effect on the DMBA-induced mammary carcinoma of the rat [2]. In a preliminary clinical trial with heavily-pretreated patients some did respond to treatment with RU 486 [3].

Whilst the natural configuration of the steroid skeleton is maintained in Mifepristone (Roussell Uclaf), a further group of antiprogestins has been developed in which the link between the C and D rings is *cis* rather than *trans*. Furthest developed of the compounds in this group is Onapristone (ZK 98.299, Schering AG), which has a somewhat stronger antiprogestagenic effect but reduced antiglucocorticoid activity when compared to Mifepristone [4].

In order to obtain an insight into the tumor-inhibiting potential of this new class of compounds the effect of this antiprogestin was compared with that of Mifepristone in relevant experimental mammary carcinomas. The intention was also to discover the mechanism of action underlying the tumor-inhibiting effect of antiprogestins, particularly because estrogens, but to a lesser degree, progestins are known to be responsible for the growth of hormone-dependent mammary carcinomas. Therefore, Onapristone (ZK 98.299) and Mifepristone (RU 486) were compared with standard mammary carcinomas therapies in a number of different experimental arrangements with the hormone-dependent transplantable MXT(+)-mammary tumor of the mouse as well as with DMBA- and NMU-induced mammary carcinomas of the rat. To clarify the mechanism of action of antiprogestins their effect on endocrine-dependent organs and their histology as well as on the morphologic reaction pattern of these tumors was investigated by means of light and electron microscopy. Moreover, a study on cell cycle kinetics of mammary tumors treated by various

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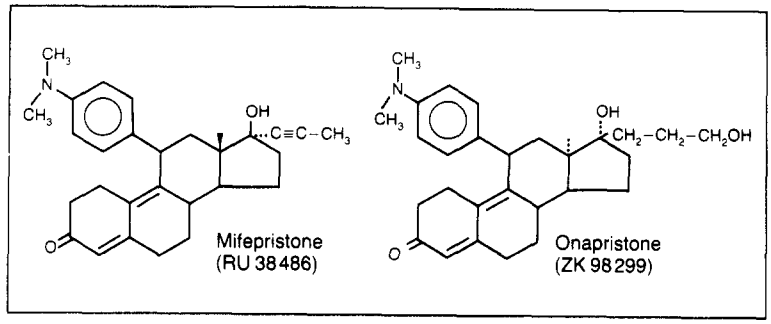

Fig. 1. Chemical structure of Onapristone (Schering AG) and Mifepristone (Roussel-Uclaf).

endocrine therapies including antiprogestins was performed. The importance of hormone deprivation and the importance of a mechanism of action unknown up to now for induction of the antiprogestin tumor-inhibiting potency was investigated in detailed studies with MXT(+)-mammary tumors in ovariectomized, hormone-substituted mice.

INHIBITORY EFFECT ON EXPERIMENTAL MAMMARY TUMORS

Hormone-dependent MXT(+)-mammary tumor of the mouse

The antiprogestin Onapristone (5 mg/kg) was first compared with tamoxifen (5 mg/kg), and diethylstilbestrol (2.5 mg/kg) as a high-dose estrogen therapy and ovariectomy for its tumor-inhibiting effect on the hormone-dependent, estrogen and progesterone receptor positive MXT(+)-mammary tumor in the mouse. In the experimental system employed (prophylaxis model) treatment started immediately after implantation of 2 tumors per mouse and was carried out over a period of 6 weeks. Treatment with tamoxifen and DES resulted only in a retardation of tumor growth as compared with the intact control. Onapristone had a pronounced antitumor effect and caused a strong inhibition of tumor growth comparable to that after ovariectomy (Fig. 2).

Because of this considerable effect an experimental arrangement was chosen in which treatment was started 3 weeks after implantation of the tumors (therapy of established tumors). In this experiment treatment with tamoxifen (4 mg/kg) for 3 weeks again led to a retardation of tumor growth, high-dose progestins (medroxyprogesterone acetate, 100 mg/kg, megestrol acetate, 25 and 50 mg/kg) had no significant effect, whereas ovariectomy resulted in tumor inhibition of 70%. Onapristone and Mifepristone were tested in doses of 1 and 10 mg/kg. Onapristone and Mifepristone had a strong antitumor effect comparable to that of ovariectomy and significantly better than that of tamoxifen [5]. In a further study on established MXT(+)-tumors over a period of only 2 weeks the two antiprogestins were tested against tamoxifen (10 mg/kg) in doses of 50 mg/kg (Fig. 3). The antiestrogen again had only a weak inhibitory effect; but the two antiprogestins achieved an inhibitory effect even superior to that of ovariectomy [5]. This result is all the more remarkable because endogenous estrogens are generally considered responsible for the growth of hormone-dependent mammary carcinomas, progestins only to a secondary extent.

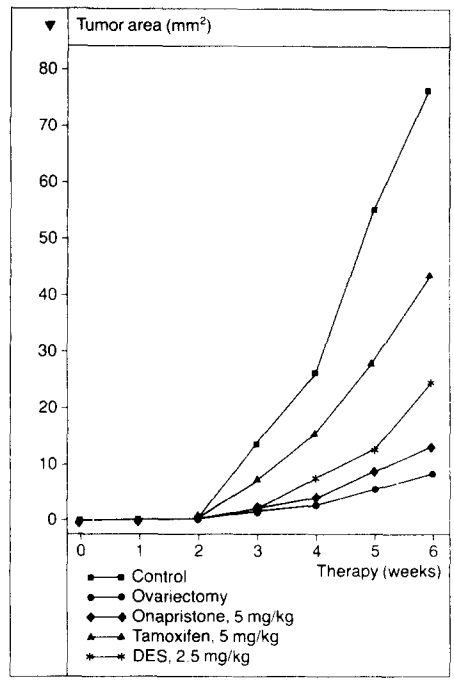

Fig. 2. Effect of Onapristone, tamoxifen, diethylstilbestrol (DES), and of ovariectomy on growth of the hormone dependent MXT(+)-mammary tumor of the mouse (prophylaxis model). Compounds were administered six times weekly s.c. for 6 weeks.

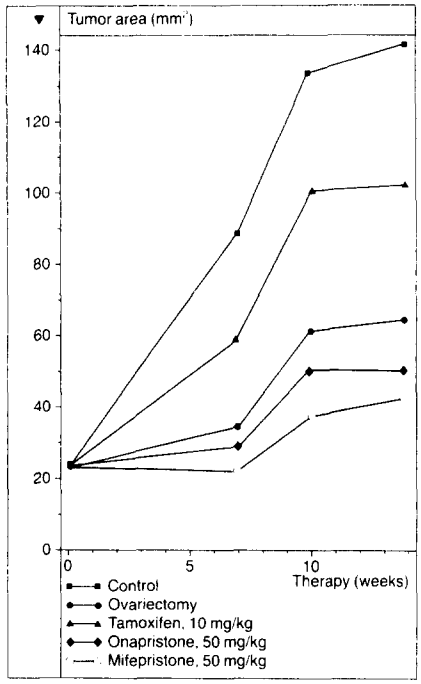

Fig. 3. Effect of Onapristone, Mifepristone and of ovariectomy on growth of established MXT(+)-mammary tumors of the mouse. Compounds were administered six times weekly s.c. for 2 weeks.

Hormone-dependent DMBA- and NMU-induced mammary carcinoma in the rat

The dimethylbenzanthracene (DMBA-) induced mammary carcinoma of the rat was used to compare the two antiprogestins in doses of 10 mg/kg daily s.c. with ovariectomy [5]. Four to 8 weeks following induction with 10 mg DMBA, the animals were allocated to individual test groups if their largest tumor has a surface area of at least 150 mm^2. Treatment of established tumors was performed for 4 weeks. Whilst tumor growth was progressive in the intact controls ovariectomy resulted in almost complete regression of the tumors. Treatment with Onapristone caused strong and very uniform inhibition of tumor growth almost comparable to that after ovariectomy, whereas therapy with Mifepristone resulted in an inhomogeneous tumor inhibition [5].

Both antiprogestins were further compared with ovariectomy using the nitrosomethyl urea (NMU-) induced mammary carcinoma [5]. This tumor differs from the DMBA-mammary carcinoma in its lesser degree of prolactin-dependence and its more aggressive growth. Seven to 14 weeks after induction by a single i.v. injection of 50 mg/kg of NMU the animals were allocated to groups as in the DMBA model. Treatment of established tumors is carried out over a period of 3 or 6 weeks. In contrast to the progressive growth in the intact controls, ovariectomy led to complete inhibition of the tumor. Therapy with 10 mg/kg Mifepristone led only to a non-significant retardation of tumor growth so that the animals had to be removed from the trial after 3 weeks (Fig. 4). Onapristone (10 mg/kg), however, caused a marked and highly significant tumor inhibition which enabled treatment to proceed for 6 weeks as it did with the ovariectomized animals [5].

To summarize, these antiprogestins have pronounced tumor-inhibiting effects in a number of different hormone-dependent mammary carcinoma models. These effects are superior to those of tamoxifen and high-dose progestins and almost equal to the effect of ovariectomy.

MECHANISM OF TUMOR INHIBITION

According to theoretical considerations the mechanism of the tumor inhibiting potency of antiprogestins can depend on the following possibilities:

Antagonism of the effect of progesterone: classical antihormonal (="antiprogestagenic") action;
Blocking of pituitary and ovary function (antigonadotrophic activity);

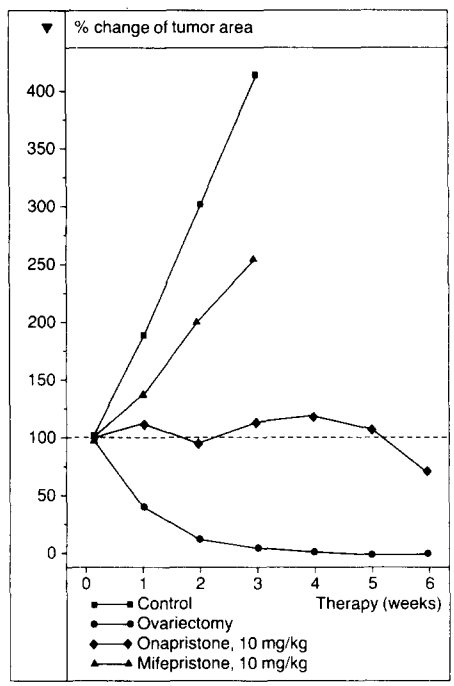

Fig. 4. Effect of Onapristone, Mifepristone and of ovariectomy on growth of hormone-dependent, MNU-induced mammary tumors of the SD-rat. Compounds were administered six times weekly s.c.

Non-receptor-mediated cytotoxic effects; and
Progesterone receptor-mediated blockade of tumor cell growth.

In the following studies these possibilities were systematically examined.

After treatment of the mammary carcinoma-bearing animals with Onapristone and Mifepristone an exact analysis of their effect on endocrine parameters was carried out [5, 6]. The studies revealed a stimulation of the pituitary and ovary function recorded in the activation of morphologic parameters (weight, number and histology of the corpora lutea) and increased levels of pituitary (prolactin, LH) and ovarian (estradiol, progesterone) hormones. Therefore, the tumor-inhibiting effect of the antiprogestins is not based on the blocking of pituitary and ovary function [6]. As a reaction to the activation of the ovary functions the target organs, uterus, vagina and mamma, exhibited characteristic estrogen-dependent features. In the light of these estrogenic reactions the simultaneous inhibition of hormone-dependent mammary carcinomas after administration of antiprogestins was even more surprising.

This prompted enquiry into whether the action of antiprogestins is based on non-receptor-mediated cytotoxic effects. Tests in the hormone-independent, receptor negative MXT-OVEX mammary carcinoma of the mouse revealed no indication of tumor inhibition by non-receptor-mediated cytotoxicity [6].

Important clues to the mechanism of action arose from studies with ovariectomized animals following hormonal substitution with an estrogen and/or progestin and simultaneous administration of the antiprogestin Onapristone [7]. The hormone-dependent MXT(+)-tumor model of the mouse was chosen for these investigations. Hormone substitution was carried out on the day following tumor implantation. On substitution with a progestin (medroxyprogesterone acetate) in ovariectomized animals tumor growth was only marginally stimulated above the low level found with ovariectomy alone. This provides a first indication that hormone deprivation hardly contributes to any great extent to the tumor inhibiting capacity of antiprogestins. In fact the slight stimulation by the progestin can only be influenced minimally by simultaneous administration of the antiprogestin Onapristone [7]. In substitution experiments with estrogen (estradiol benzoate)—in contrast to substitution with progestins—tumor growth in ovariectomized animals was stimulated up to the level of intact controls [7] (Fig. 5). Surprisingly, this effect was completely antagonized by antiprogestins, even though no progestins were present in this experimental arrangement. This result shows that the tumor inhibiting mechanism of the antiprogestins cannot primarily depend on a classical antihormonal (=antiprogestagenic) effect or to progesterone deprivation. As the progesterone receptor content in mammary carcinomas is known to be strongly induced by administration of estrogens, the tumor inhibiting mechanism of antiprogestins depends on a progesterone receptor-mediated, as yet unknown effect. Further experiments with substitution of an estrogen and a progestagen in ovariectomized mice with MXT(+)-mammary carcinomas underline the importance of the progesterone receptor for the induction of tumor inhibition by antiprogestins [7]. These experiments with different concentrations of a progestin clearly reveal that a tumor inhibiting activity of antiprogestins is only given if a sufficient concentration of available progesterone receptors is present. This was also demonstrable using the human postmenopausal T61 mammary tumor implanted in nude mice. Only after stimulation of the parently low progesterone receptor content by a

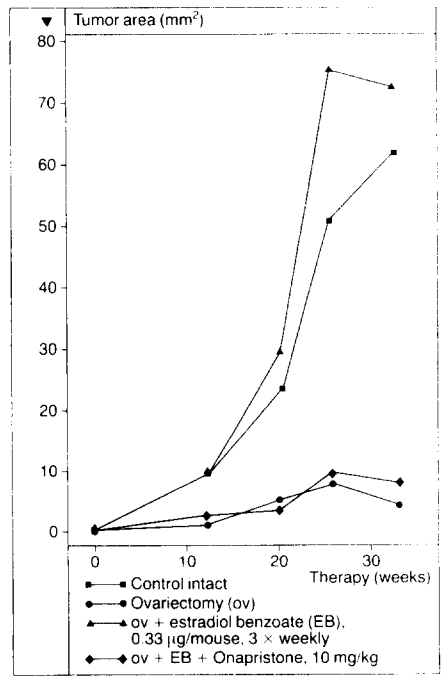

Fig. 5. Effect of Onapristone (six times weekly s.c.) on growth of MXT(+)-mammary tumors in ovariectomized, estradiol benzoate-substituted mice.

low dose of estrogen, a tumor-inhibiting effect of antiprogestins was given [8].

In these estrogen (and progestin) substitution experiments as well as in all experiments in intact animals (MXT-, DMBA-, NMU-tumors) detailed light and electron microscopic studies of the tumors revealed that after administration of antiprogestins the morphology was clearly distinguishable from that after ovariectomy [5–7]. Whilst necrobiotic degenerations are the characteristic features of mammary carcinomas following ovariectomy, those mammary carcinomas whose growth has been inhibited by antiprogestins display clear signs of differentiation. The outstanding feature of their morphology is the massive presence of acinar nodules which regularly are secretory activated. As regards the morphology of the mammary carcinomas in intact and ovariectomized animals, all the light and electron microscopy findings in these tumor experiments support a concept of the induction of terminal differentiation in progesterone receptor positive mammary carcinomas after administration of antiprogestins. These findings show that a differentiation of polygonal, actively dividing individual cells to highly secretory, mitotically inactive dysplastic acini and glandular ducts takes place. It is well-known that in a large number of tissues the relationship between proliferation and differentiation—here: the performance of specific cell functions—is one of mutual exclusion. After loss of cell contact the epithelial cells of these acini regularly undergo cell death. The appearance of apoptoses may also be considered as a typical feature of programmed cell death. Thus, these tumor cells go through the complete physiological differentiation program after proliferation commences.

The treatment of experimental hormone dependent mammary tumors with antiprogestins induced an accumulation of cells in G_0 G_1 phase together with a significant and biologically relevant reduction in the number of cells in the S and G_2 M phase [9]. Interestingly, there are observations in some stem cell types that hormonal control of G_1 and cell differentiation are somehow linked and a differentiation specific arrest was already proposed. Keeping this in mind, the accumulation of the tumor cells in G_0 G_1 may display differentiation and this correlates with all our quantitative light- and electron-microscopical data indicating that the antitumor action of antiprogestin is mainly related to the induction of terminal differentiation leading to cell death.

We conclude that the tumor inhibiting capacity of antiprogestins is based on their ability to trigger the mechanism of terminal differentiation probably by removing the intrinsic block at the genomic site. In light of the tumor experiments conducted, this action of the antiprogestins depends on the presence of a sufficient number of available progesterone receptors.

In consequence, in the clinical situation only progesterone receptor positive breast cancer patients should be selected for therapy with antiprogestins. According to the data obtained for the tumor inhibiting potency and mechanism of action of antiprogestins, these compounds are expected to provide an innovative and effective therapy for hormone dependent breast cancer.

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