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Review Article

Progesterone receptor antagonists (antiprogestins)

Holger Hess-Stumpp*, Jens Hoffmann and Ulrike Fuhrmann

*Research Laboratories of Schering AG, 13342 Berlin, Germany. *Correspondence*

CONTENTS

Abstract

Progesterone is an essential regulator of female reproductive functions mediated by the progesterone receptor. Therefore, its blockade by using an antagonist (antiprogestin) allows modulation of various reproductive processes. Since the first description of RU -486 numerous antiprogestins were synthesized with varying pharmacological characteristics. In this review the current knowledge is summarized on the basic *in vitro* and *in vivo* profile of antiprogestins with a special focus on a recently identified antiprogestin, ZK 230211. In addition, the use of antiprogestins in a new concept of fertility control in combination with an iNOS inhibitor is discussed. Since antiprogestins have a profound antiproliferative effect on primate endometrium the target for the antiprogestagenic action in the primate endometrium remains to be elucidated. However, the results obtained so far indicate that the endometrial vasculature may be the target for antiprogestins. The implications of these findings for endometrial disorders such as endometriosis are discussed. Numerous studies in animal models and results from first clinical trials demonstrated the efficacy of antiprogestins in treatment of breast cancer. The basic biological principles of the use of antiprogestins in oncological therapy are summarized and recent developments of the use of this important class of anti-hormones in cancer therapy are discussed.

Introduction

The steroid hormone progesterone is a major regulator of reproductive processes in females. It is involved in the control of ovulation (1), regulation of the function of the corpus luteum (2), initiation of decidualization (3), maintenance of uterine quiescence during pregnancy (4), ripening of the cervix before delivery of the fetus (5), *etc.* In addition, progesterone is strongly involved in proliferation and differentiation of the mammary gland (6).

The biological activity of progesterone is mediated by the progesterone receptor (PR), a member of the large gene family of nuclear receptors. After binding of the natural ligand progesterone to the PR, the transcriptional machinery is formed and subsequently the expression of various genes is induced (7-9).

The blockade of PR function might allow the modulation of various reproductive processes. On this basis, PR antagonists (PRAs) or antiprogestins were developed to disrupt the normal transcriptional processes which are initiated after progesterone binding to the PR. Due to the ability of antiprogestins to block normal progesterone, these compounds have considerable potential as therapeutic drugs in gynecological, obstetrical and oncological indications. It has been shown that antiprogestins are able to block ovulation (10-15), prevent luteolysis (16), induce cervical ripening (17) and inhibit endometrial and mammary gland proliferation and differentiation (18-25).

The first antiprogestin, RU-38486 (better known as RU-486 or mifepristone), was reported in 1981 (26-28). Since then, several 100 related analogues have been synthesized showing all degrees of antiprogestagenic activity. The pharmacological profile of antiprogestins ranges from pure antagonists to mixed antagonist/agonist activities. RU-486 is an antiprogestin with only marginal agonist activity, whereas the so-called mesoprogestins show agonistic activity *in vivo* (16). Onapristone (ZK-98299) is an example of a pure antagonist (29). Just recently, the structure of a novel highly potent pure antiprogestin, ZK-230211, was published (25). This compound is practically devoid of endocrine-related side effects *in vivo*.

This review discusses the biological activities of antiprogestins with regard to new approaches for fertility control, hormone replacement therapy and treatment of

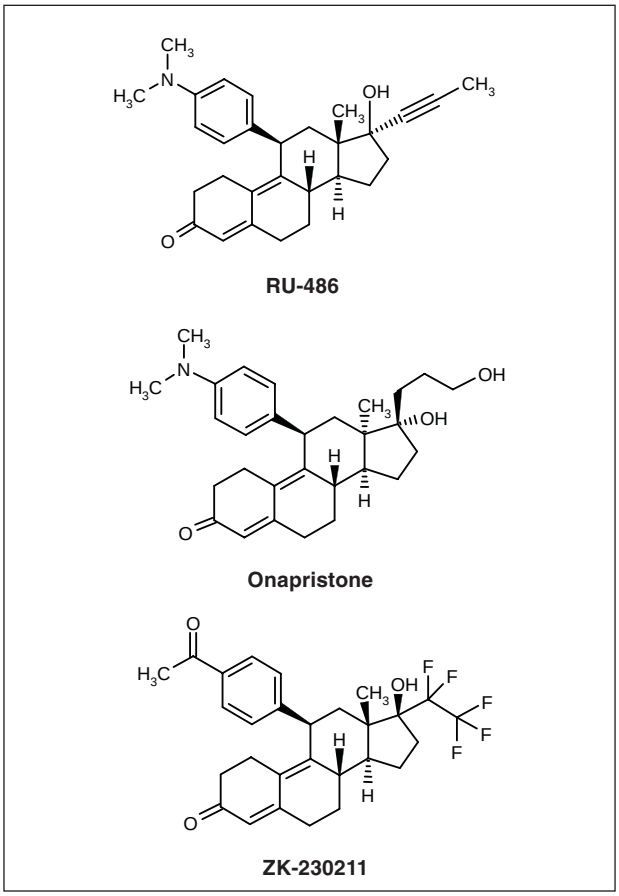

Fig. 1. Chemical structures of RU-486 (mifepristone), onapristone (ZK-98299) and ZK-230211.

breast cancer. Special emphasis is placed on the characteristics of the novel antagonist ZK-230211.

Chemical modifications

RU-486 is a derivative of 19-nortestosterone and has an additional 4-(dimethylamino)-phenyl group at the 11β position, a Δ^9 double bond and a 1-propynyl chain at the 17α position (Fig. 1). Since its first description, much effort has been devoted to optimize antiprogestational structures with regard to steroid receptor selectivity. Various modifications of the steroid nucleus were studied, *e.g.*, 13α- (30), 14β (31), 9,10-dihydro-, 10,11-bridged- (32) and 10β-methyl-antiprogestins (33) have been synthesized. Regarding the side chains, the replacement of the dimethylamino function in the 11β substituent of RU-486 by acetyl (34), cyanophenyl or heteroaryl substituents led to potent antiprogestins. In addition to position 11, the D-ring of the steroid was also a common site for chemical modifications of antiprogestins (27, 28, 30, 31, 35). It allows various modifications without a loss of activity. Exchange of the propynyl 17-side chain into hydroxypropenyl or spiroether (35) moieties in some cases enhanced antiprogestagenic activity. In addition, unwanted hormonal partial activities, *e.g.*, antiglucocorticoid activities, were reduced by these structural elements at C-17. Stimulated by these findings, position 17 was chosen for a detailed analysis of structure-activity relationships in order to find highly potent antiprogestins with considerably reduced endocrine side effects.

Modification of the 17α-pentafluoroethyl side chain (Fig. 1) led to a new antiprogestin (ZK-230211) which revealed a high antiprogestagenic activity and low or no other hormonal effects (25).

Molecular mechanism of action

Progesterone receptor isoforms

All of the currently available antiprogestins interact directly with the ligand binding domain of the PR, thereby competitively inhibiting progesterone binding. When discussing the molecular mechanism of action of antiprogestins and their potential clinical use, it should be considered that the human PR (hPR) is expressed in 2 forms: a B-form (hPR-B) which is 933 amino acids in length, and an A-form (hPR-A) which lacks the first 164 amino acids at the N-terminal end of the B receptor. These 2 isoforms are generated either by transcription directed from different promoters (36) or as a result of alternative initiation of transcription from the same mRNA (37). The manner in which the functional differences between these proteins are likely to impact on the overall physiologic role of the receptor in reproductive systems have been discussed in detail by Conneely *et al.* (6).

Ligand-dependent structural alterations of the progesterone receptor

Why the complex of PR and antiprogestin is functionally inactive is not clear. In comparison with an agonist, the antagonist induces a different conformation of the PR. The conformation adopted by the receptor following interaction with an agonist or antagonist was demonstrated by limited proteolytic digestion and monoclonal antibody-epitope mapping (38-40). Agonist binding led to a compact structure rendering the ligand binding domain resistant to protease digestion and antibody recognition. The conformation induced by antagonists is rather loose and is characterized by a 3 kD region within the ligand binding domain that is accessible to protease digestion and antibody recognition (38, 39, 41). These changes may be due to the ability of the agonists and antagonists to bind to different regions in the ligand binding domain of the hPR as shown by various mutational analyses (39, 42). Various ligands can be grouped into agonists or antagonist on the basis of the conformation they impose on the PR. Recently, Wagner *et al.* (43) analyzed a new class of antiprogestins, 16-substituted analogues of RU-486 that induced a conformation different from that achieved by

RU-486. This conformation resulted in mixed agonist/antagonist activity in cell culture since the compounds did not only slightly stimulate but also antagonized a PR regulated gene.

Although there are differences between agonist- and antagonist-induced PR structures, both types of ligands permit the first 2 steps of receptor activation, *i.e.*, dissociation of heat shock proteins and receptor dimerization (44, 45). However, it is still a matter of debate as to whether antiprogestins block PR-mediated transcriptional activation by inhibiting the next step of receptor activation, the association of the receptor with DNA or at some step downstream of DNA binding.

Different types of antagonists

Prevalent models assume the existence of 2 types of antiprogestins. Type I antiprogestins (13α-methyl substituted 19-nor-steroids) represented by onapristone (46), have been shown to prevent the association of the hPR to DNA. Type II antagonists (13β-methyl 19-nor-steroids) represented by RU-486 or ZK-98734 (lilopristone), appeared to promote stable binding of the receptor to DNA (29, 47, 48) in *in vitro* binding studies. Based on these findings, it was proposed that the type I compounds may hamper hPR-mediated transactivation by preventing binding of hPR to progesterone response elements (PRE), whereas the type II compounds may act at steps downstream of DNA binding by preventing interaction with coactivators and/or enhancing interaction with corepressors (29, 44, 49).

Additional support of this *in vitro* classification was the observation that type II antiprogestins such as RU-486 can function as PR agonists in the presence of activators of the protein kinase A signaling pathway like cAMP (50-52). This observation might explain the agonist-like proliferative effects reported for RU-486 in the endometrium of postmenopausal women. It is noteworthy that cAMP failed to change the antagonistic activity of the type I antiprogestin onapristone (51). Therefore, type I antagonists have been classified as pure antagonists in contrast to type II antagonists.

ZK-230211 behaved differently compared to the 2 types of antiprogestins described thus far and therefore belongs to a novel class of antiprogestins. By using gel shift analysis we showed that the receptor bound to ZK-230211 can bind to its responsive elements with a considerably higher affinity in comparison to agonist-, RU-486- or ZK-98299-bound receptor. Interestingly, in contrast to RU-486, ZK-230211 did not show any agonist activity after treating the cells with 8-Br-cAMP. In addition, the ZK-230211-liganded PR can bind to the corepressor NCoR with a higher affinity than RU-486- or onapristone-bound receptor in a mammalian 2-hybrid assay. Furthermore, GST pulldown assays showed a strong interaction between ZK-230211-liganded PR and NCoR in contrast to the weak interaction of NCoR with unliganded, agonist- or RU-486-liganded PR. We therefore hypothesize that the interaction of ZK-230211-bound receptor with NCoR is due to a distinct conformation of the PR that enables the receptor to actively recruit NCoR.

It will be interesting to determine whether the observed mechanistic differences between the 3 types of antiprogestins *in vitro* are reflected by distinct biological activities *in vivo*. Such different biological activities *in vivo* were observed for a fourth class of antiprogestins, the so-called mesoprogestins. These compounds showed partial agonist effects *in vivo* (see below) but were pure antagonists in transactivation assays using the MMTV promotor. The molecular mechanism of this phenomenon is still unknown.

In vivo profile

A classical model to quantify the antiprogestagenic activity of antiprogestins is a modified assay in juvenile rabbits according to McPhail (53). In this model, ZK-230211 displayed an almost complete antagonism of the progesterone-induced differentiation of the endometrial glands already at a low dose (0.3 mg/kg). Experiments conducted in our laboratories showed a ten times lesser potency for RU-486 (25). Partial PR agonists such as mesoprogestins were not able to neutralize the progesterone-induced differentiation of the endometrium and showed a medium grade of differentiation (16). This model also allows the analysis of the progestagenic activity of an antiprogestin. ZK-230211 displayed no intrinsic progestagenic activity supporting the notion that this antiprogestin is a pure antagonist. A second model which allows discrimination between pure antagonists and partial agonists *in vivo* is the luteolysis inhibition assay in cycling guinea pigs (16). Pure PR antagonists abolish luteolysis of the corpus luteum, PR agonists are less active or inactive in this assay. The secretion of $PGF_{2\alpha}$ which induces corpus luteum regression is under control of progesterone (for more details see 16). A complete cessation of progesterone secretion via antiprogestins terminates secretion of $PGF_{2\alpha}$ thus preventing luteolysis whereas exogenous progestins increase and prolong prostaglandin secretion thereby inducing luteolysis. Accordingly, mesoprogestins as partial agonists did not prevent luteolysis (16) whereas the pure antiprogestin ZK-230211 acted as an antiluteolytic agent.

An important issue in the pharmacology of antiprogestins is their ability to inhibit ovulation. Studies conducted with nonhuman primates using Onapristone and ZK-137316 indicated that it is possible to achieve an amenorrhea as a consequence of endometrial atrophy without disturbing the ovarian cycle (15, 19, 54-56). However, the degree of antiovulatory activity is dose- and species-dependent. In this regard the ability of ZK-230211 was analyzed to inhibit ovulation in rats and monkeys. After application of ZK-230211 to natural cycling rats, a full inhibition of ovulation was achieved only with high doses (15 mg/kg). However, the situation in

primates was different. To study the antiovulatory activity of ZK-230211, experiments were conducted with cynomolgus monkeys (*Macaca fascicularis*). The ovarian cycle was monitored by determination of the serum concentrations of estradiol and progesterone. An inhibition of ovulation was indicated by a suppression of the progesterone peak which should arise from a secreting corpus luteum after a successful ovulation. It was found that a low dose of 0.03 mg/kg was already able to inhibit ovulation (57). This is a factor 500 lower than the dose needed to inhibit ovulation in rats.

Preliminary experiments indicated that mesoprogestins did not inhibit ovulation consistently. In a first experiment where the mesoprogestin J-1042 (16) was applied to normal cycling cynomolgus monkeys (3 mg/kg) ovulation was inhibited in only half of the animals whereas ZK-137316 and ZK-230211 inhibited ovulation completely (20).

Additional pharmacological characteristics of ZK-230211 were explored in various other models. ZK-230211 showed neither androgenic nor estrogenic activity and only weak antiglucocorticoid (weaker than RU-486) and antiandrogenic activity (for details see 25).

Antiprogestins for fertility control

Shortly after the description of RU-486 in 1981, the first clinical trials were reported (58). One result of these studies was that RU-486 taken at a dose of 200 mg/kg for 4 days in early pregnancy resulted in complete abortion in 8 of 11 women. Although these studies showed that antiprogestins have a therapeutic potential, the controversies surrounding their use as abortifacients did not support a fast development of these compounds (59). Efforts to develop RU-486 as an abortifacient are still ongoing (60, 61), but so far, RU-486 is only available in a few countries for this indication. However, the research efforts of the last 2 decades revealed other potential uses of antiprogestins for fertility control which are not related to abortion (62, 63). A recent review discussed a progestin-only contraception with a coadministered antiprogestin (64). Because a progestin only contraception leads to irregular bleeding in some women the coadministration of an antiprogestin at regular intervals may cause menses induction and leads to a regular cycle. Studies performed in cynomolgus monkeys where the antiprogestin Org-31710 was combined with the progestin desogestrel revealed a reproducible induction of menses. However, nonconfirmed signs of ovulation were observed also with high doses of the antiprogestin. Thus, it remains to be elucidated whether this approach may be an alternative to established contraceptive methods. In this review, we concentrate on an approach which is able to inhibit decidualization as presented below. The decidua is a highly specialized and well-vascularized tissue surrounding the developing embryo and is formed in early pregnancy by a massive differentiation of the endometrial stroma. In later stages of pregnancy, the decidua forms the maternal part of the placenta. It is believed that decidual cells provide the young embryo with nutrients and control trophoblast invasion (65, 66). In rodents, the decidual reaction can occur in response to the blastocyst or artificial stimuli. Many factors were found in the last decade which may control or are involved in implantation (67-71). However, in which manner these factors do interact to control differentiation from stromal cells to decidual cells is still not clear. The complex process of decidualization with the invasion of the cytotrophoblast, the infiltration of the decidua with natural killer cells in primates and the dynamic changes in cell-cell and cell-matrix interactions can be viewed as an inflammatory reaction.

Prostaglandins and the cyclooxygenase (COX) system as major mediators of inflammatory processes and their role in implantation are described elsewhere (72-76). Another important factor in inflammation is nitric oxide (NO). NO is released by the activity of 3 different enzymes known as NO synthases (NOS) which are endothelial NOS (eNOS), cytokine-inducible NOS (iNOS) and neural or brain NOS (nNOS or bNOS) (77). The extensive research regarding the role of NO as an important second messenger molecule revealed also a role in various stages of gestation (78). During pregnancy, NO is important for controlling uterine contractility, cervical ripening and utero-placental blood flow (79, 80). Among the different forms of NOS it has been shown that iNOS is the major NO synthase in the pregnant uterus, cervix and placenta (81-85). It was also shown that the expression of iNOS during rat gestation is under control of progesterone (78). Taken together, decidualization as an inflammatory process, the subsequent involvement of NO in this process, the control of NO synthesis by the regulation of iNOS expression through progesterone and the well established important function of progesterone led to the hypothesis that a combination of these 2 principles may provide a promising target for female contraception. Hence, experiments were carried out to evaluate the contraceptive potential of blocking iNOS and PR activity by using a combination of an iNOS inhibitor and an antiprogestin. Chwalisz *et al.* (86) analyzed in various rat models of early gestation a combination of onapristone and either a nonspecific NOS inhibitor (L-NAME [N^G-nitro-L-arginine methyl ester]) or a specific iNOS inhibitor (aminoguanidine). When the compounds were applied alone during the periimplantation period, neither the antiprogestin nor the NOS inhibitors prevented a pregnancy. However, when the compounds were applied together either in the periimplantation or the preimplantation period, the combination completely prevented a pregnancy in the case of aminoguanidine or reduced the pregnancy rate to approximately 50% in the case of L-NAME. These experiments showed that NO does not only play a major role in later stages but also in early stages of pregnancy. Obviously, this pathway has to be taken into consideration regarding the current concept of implantation in rodents in which molecules such as LIF, HB-EGF, COX and others play a major role (71). Additionally, these results indicate a dramatic synergy

between iNOS inhibition and progesterone withdrawal. It remains to be elucidated by further research how this concept may be valid for contraception in primates. If this is the case, it may also be taken into consideration that an increased synthesis of NO in the uterus either alone or in combination with progestins is an opportunity for treatment of early pregnancy disorders such as recurrent abortions.

Antiprogestins in hormone therapy

The rationale for the use of antiprogestins in indications such as endometriosis, dysfunctional bleeding, hormone replacement and cancer treatment is their antiproliferative action. Estradiol acts on the endometrium as an inducer of proliferation. This activity of estradiol is competed by progesterone which is the basis for the use of progestins in hormone replacement therapy (87). An antiprogestin which counteracts the effects of progesterone should act like an estrogen. Indeed, this has been demonstrated in some rodent models (88). However, antiprogestins do not have any intrinsic estrogenic activity (88-90) and the situation in primate endometrium is apparently different. Antiprogestins administered chronically at low doses inhibited the mitotic activity of the endometrial epithelium and induced a stromal compaction. These effects were dose-dependent and were found in both spayed as well as intact normal cycling monkeys (91-94). Paradoxically, long-term treatment with progestins and antiprogestins led to an endometrial atrophy. It has to be mentioned that the antiproliferative effect of antiprogestins is tissue-specific. The studies conducted with RU-486 (94), ZK-137316 (92) and ZK-230211 (20) showed that the estrogenic stimulation of the oviductal and vaginal growth or differentiation was not inhibited by antiprogestins. Thus, as reviewed by Chwalisz *et al.* the term “endometrial antiproliferative effect” was proposed for the action of antiprogestins on the primate female reproductive tract, since the effect appears to be restricted to the primate endometrium (20).

The target for the antiprogestagenic action in the primate endometrium remains to be elucidated. However, the results obtained with ZK-137316 by Slayden *et al.* (92) indicated that the endometrial vasculature may be the target for the antiprogestins. The spiral arteries of the endometrium were found to be degenerated upon long-term treatment with antiprogestins. Own experiments conducted with other antiprogestins such as ZK-230211 (20) confirmed these observations and argue for a general antiprogestagenic effect on endometrial vasculature. Other factors which may contribute to antiproliferative effects of antiprogestins on the primate endometrium such as downregulation of NOS, inhibition of VEGF synthesis or block of the cell cycle were reviewed elsewhere (20).

As mentioned above, one potential indication for antiprogestins is the treatment of endometriosis, an important gynecological indication which affects about 5-10% of women in the reproductive age (95, 96). Among a variety of symptoms women mainly suffer from pain and infertility (97, 98). Besides surgical intervention medical treatments are available in form of Danazol, progestins and GnRH agonists (99). These compounds are effective in treatment of endometriosis, however, they induce more or less severe side effects, such as androgenic side effects (hirsutism, *etc.*) in the case of danazol and climacteric symptoms and osteopenia in the case of GnRH agonists. First clinical trials conducted with RU-486 revealed pain relief in treated patients (100-102). These studies provide antiprogestins as a possible alternative for the treatment of endometriosis.

Antiprogestins in oncology

Endocrine therapy of breast cancer has been established for decades. However it is still not yet fully recognized that, in addition to estradiol, progesterone in physiological concentrations participates in the proliferation of mammary carcinomas. Therefore, it is expected that antiprogestins can block the growth of breast tumors and might be promising new tools for breast cancer therapy. These compounds clearly need a functionally expressed PR to block tumor growth, but there is strong experimental evidence that their tumor inhibition is based on more than just progesterone antagonism.

Basic biological principles for use in oncology

To clearly define those physiological events that are specifically attributable to progesterone *in vivo*, a mouse model carrying a null mutation of the PR gene was generated using embryonic stem cell/gene targeting techniques (103, 104). The PRKO model was used to define the controversial role of progesterone-initiated intracellular signaling in mammary gland tumorigenesis (105). In combination studies with tissue transplantation and an established carcinogen-induced (7,12-dimethylbenz(a)-anthracene, DMBA) mammary tumorigenesis system, it was shown, that there was a marked reduction in mammary tumor incidence in PRKO mice as compared with isogenic wild types.

This observation demonstrated that in the absence of the PR function, prolactin alone is not sufficient to induce the neoplastic transformation and that progesterone may activate mitogenic mediators of the prolactin pathway. Under these conditions the epithelial cells might exhibit a low proliferative index and, at the time of carcinogen administration, be a poor candidate for malignant transformation.

The luminal epithelial compartment has been considered not only to be primarily responsive to the progesterone induced proliferative signals and to be the primary site for the initial carcinogenic insult, but additionally PR expression has been localized predominantly to these cells. One interpretation for the reduction of mammary tumorigenesis could be that the progenitor cells for

alveologenesis, the PR-expressing epithelial cells are absent in the PRKO mice. Because the majority of mammary tumors are of alveolar origin the absence of these progenitor cells might reduce the number of target cells for neoplastic transformation.

These results give strong support for the use of antiprogestins in breast cancer since they might inhibit the prolactin mitogenic action on the luminal epithelium.

There is considerable evidence linking the EGF and progesterone signaling pathways in breast cancer. This includes attenuation of progestin responsiveness and decreases in PR levels in cells treated with EGF (106), and progestin-specific regulation of EGF and EGFR levels (106, 107).

Depending on the tissue, progesterone is classified as a proliferative or a differentiative hormone. Studies in cultured human T47D breast cancer cells focusing on the initial growth stimulatory components by progestins show that growth stimulation is restricted to one cycle, however, and is followed by growth arrest at the G_1/S boundary of the second cycle, resistant to growth regulatory effects of additional progesterone (108, 109). During the progesterone-arrested state, cells upregulate EGFR 3- to 5-fold and acquire a progestin potentiated sensitivity to the proliferative effects of EGF (108, 110, 111). This led to the model put forward by Horwitz and coworkers, that progesterone is a competence factor that switches breast cancer growth from steroid hormone to growth factor dependence.

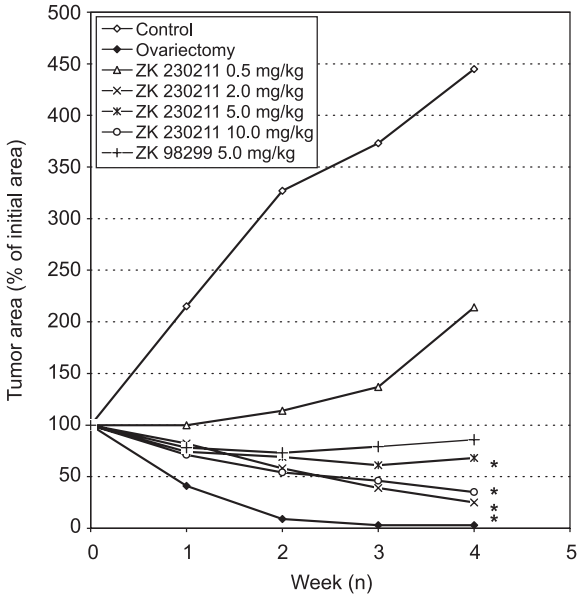

Fig. 2. Antitumor effect of ZK-230211 and onapristone (ZK-98299) in the DMBA-induced mammary tumor model in rats. Mammary tumors were induced by a single oral administration of 10 mg DMBA. Rats with at least one established tumor were treated for 4 weeks and the tumor growth was measured. (Reprinted with permission from J Med Chem 2000, 43: 5010-16. Copyright 2000 American Chemical Society.)

Therapeutic efficacy in breast cancer models

ZK-230211 demonstrated inhibition of E_2-stimulated growth in human T47D breast cancer cells and was able to inhibit dose-dependently E_2-stimulated cell proliferation even superior in comparison to 4-OH-tamoxifen.

Experimental mammary tumors induced by chemical carcinogens like DMBA in female rats have a similarity to breast cancers in women. These tumors are estrogen and progesterone receptor-positive and their growth is hormone-responsive (*i.e.*, estrogen can stimulate, whereas ovariectomy suppresses tumor growth). Antiestrogens and aromatase inhibitors are effective in this tumor model.

In intact control animals, progressive tumor growth was observed, whereas ovariectomy caused a complete tumor regression in 90% of the animals. Treatment with ZK-230211 at doses of 1.0, 5.0 and 10.0 mg/kg resulted in a significant inhibition of tumor growth compared with the control (Fig. 2). Treatment with 0.2 mg/kg resulted in a growth inhibition which was improved with 1.0 mg/kg. At this dose maximal growth inhibition was observed so that a further dose increase did not lead to better tumor growth control. In these groups a complete tumor regression was seen in 30-45% of the rats.

In earlier studies using onapristone, it was established by morphometric procedures that treatment with progesterone antagonists can trigger differentiation of the mitotically active polygonal tumor epithelial cell towards secretory active glandular structures and acini. All quantitative light and electron microscopic data indicated that the antitumor action of antiprogestins is accompanied by the initiation of terminal differentiation leading to apoptotic cell death (112, 113). Surprisingly, the antitumor activity of antiprogestins is evident in spite of elevated serum levels of ovarian and pituitary hormones.

In addition growth inhibition of several other breast cancer models was observed, *i.e.*, the aggressively growing methylnitrosourea (NMU)-induced mammary carcinoma of the rat, the mouse MXT mammary tumor and the human postmenopausal, estrogen and progesterone receptor positive T 47 and MCF-7 mammary carcinomas.

Comparison with standard hormone therapy

Observations from preclinical experiments with onapristone in different model systems led to the conclusion that the strong antitumor activity of these “pure” antiprogestins in breast cancer might not only depend on a primarily classical antihormonal mechanism but rather differs totally from that after treatment with tamoxifen, high dose estrogen, or ovariectomy. Michna *et al.* (112, 113) reported that the antitumor action of antiprogestins is accompanied by the initiation of an accumulation of the tumor cells in the G_0-G_1 phase of the cell cycle, terminal

differentiation and with the appearance of apoptotic cell death.

The ability of progesterone antagonists to reduce the number of cells in S phase may offer a significant clinical advantage, since it is established that the S-phase fraction is a highly significant predictor of disease-free survival among axillary node-negative patients with diploid mammary tumors (114).

Clinical studies were initiated to investigate onapristone as first- and second-line endocrine therapy in patients with breast cancer. In an explorative phase II clinical trial (115), 19 patients with either locally advanced breast cancer (n=12) or who were elderly, unfit patients with primary breast cancer (n=7) received 100 mg onapristone per day. Seventeen of the 19 tumors expressed estrogen receptors (ER) while 12 of the 18 tumors tested expressed progesterone receptors (PgR). Whereas one patient was withdrawn from the study, 10 patients (56%) showed a partial response and 2 (11%) durable static disease (≥6 months), giving an overall tumor remission rate of 67% confirming that progesterone antagonists can induce tumor responses in human breast cancer. The median duration of remission was 70 weeks. These clinical results suggest a potential benefit of adding the antiprogestins to the panel of endocrine breast cancer therapeutics especially to extend the therapeutic options in *i.e.*, antiestrogen refractory diseases.

The effects of antiprogestins were investigated in other tumor types, both classical endocrine sensitive tumors (*i.e.*, prostate cancer) and nonclassically endocrine sensitive (*i.e.*, gastrointestinal tumors). In prostate tumors that have relapsed from androgen ablation therapies, the androgen receptor (AR) is still expressed and, compared to the primary tumors, its level is often even enhanced (116). Mutated AR that can be activated by other compounds such as adrenal steroids, estrogens, progestins and even antiandrogens. Thus, relapse of tumors under the selective pressure of common androgen ablation therapies can be caused by acquired androgen hypersensitivity and AR activation by ligands other than (dihydro)testosterone. There is a clinical need for future compounds that are effective inducers of apoptosis in recurrent tumors. Published data indicated that RU-486 could inhibit prostate cancer cell growth *in vitro* and *in vivo* (117, 118). As concluded from these results, it appears that RU-486 may be an effective inducer of apoptosis and may represent a novel therapeutic approach, not directed towards the androgen receptor, and suitable to overcome a potential intrinsic apoptosis resistance of androgen-independent prostate cancer cells.

Conclusions

Antiprogestins are an important class of antihormones. The recent findings of new and highly potent antiprogestins may stimulate further research and exploration of new therapeutic concepts in gynecology and oncology. The combination of an antiprogestin with an inhibitor of iNOS revealed a new approach for fertility control and may enhance further work on the cross-talk of signaling pathways and other processes in very early pregnancy. The antiproliferative effects of antiprogestins provide a solid rationale for the treatment of endometriosis and others major indications in gynecology and oncology. The efficacy in the treatment of breast cancer might be improved by the use of antiprogestins and should also induce much less side effects for patients than treatment with cytostatic agents.

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