Non-competitive anti-oestrogenic activity of progesterone antagonists in primate models

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We have summarized some of the studies containing basic biological data suggesting potential therapeutic utility of the anti-proliferative activity of antiprogestins on uterine tissues. The non-competitive anti-oestrogenic effects of RU486 were examined using oestradiol-treated ovariectomized monkeys given RU486, progesterone or both. The oestradiol-induced luteinizing hormone surge of control animals was abrogated by progesterone and/or RU486. Secretory transformation by progesterone was inhibited by RU486 co-administration. RU486 alone (1 mg/kg) induced endometrial secretory transformation, but higher doses (5 mg/kg) induced inhibited proliferation and secretory activity. Thus, in the presence of progesterone, RU486 is antagonistic but, in its absence, RU486 exhibits endometrial progestational effects at low doses and an anti-proliferative (anti-oestrogenic) effect at higher doses. These data encourage continued evaluation of RU486 as a potential contraceptive agent acting at the pituitary and/or endometrial level. Our study also demonstrates that after physiological oestradiol replacement therapy, oestradiol receptor concentrations rise dramatically following antiprogestin treatment; this effect was dose-dependent.

Key words: antagonist/anti-oestrogen/antiprogestin/endometrium/proliferation

Introduction

That some progesterone antagonists express other biological activities, besides being anti-progestin, was revealed by the antiglucocorticoid functions of RU486 (mifepristone; Healy *et al.*, 1983, 1985). More recently, we reported that RU486 has a non-competitive anti-oestrogenic activity that blocks oestrogen-induced endometrial proliferation in primates (van Uem *et al.*, 1989). This action of the antiprogestin was later found to be dose-dependent in the presence of physiological oestradiol (Wolf *et al.*, 1989). Paradoxically, we found that RU486 elevates the concentration of oestrogen receptors in monkey endometrium, yet the mitogenic (proliferative) impact of oestrogen on endometrial growth was negated (Neulen *et al.*, 1990).

These observations are consistent with other monkey and human data that may substantiate this anti-proliferative activity of RU486 on primate endometrium (Haluska *et al.*, 1990; Kettel *et al.*, 1991; Murphy *et al.*, 1991; Batista *et al.*, 1992). Apparently, other progesterone antagonists may also possess this property. Based on the report that ZK 299 (onapristone) also curtails endometrial growth (Chwalisz *et al.*, 1991), we wonder how general this activity may be among a wider spectrum of anti-progestin compounds.

Here, we have summarized some of the studies containing basic biological data suggesting potential therapeutic utility of the anti-proliferative activity of antiprogestins on uterine tissues.

Initial evidence of non-competitive anti-oestrogenic activity of RU486

In previous studies, RU486 administration arrested spontaneous folliculogenesis. To investigate the central versus peripheral effects of RU486 on the ovarian/menstrual cycle, including endometrial proliferation, RU486 was administered daily (10

mg/kg/day, i.m.) from menstrual cycle day 3 or 7 to day 25 in normal adult cynomolgus monkeys receiving human menopausal gonadotrophin (HMG) treatment (37.5 IU/day) from days 3–8 (n = 6) (Figure 1).

RU486 administration with HMG/human chorionic gonadotrophin (HCG) therapy did not inhibit ovarian response, as shown by the occurrence of steroidogenesis and ovulation. Nine of 23 oocytes retrieved by lavage or follicular aspiration at laparotomy after ovulation induction were morphologically classified as mature pre-ovulatory status. Whereas endometrial biopsy performed on cycle day 25 in control monkeys revealed an in-phase mature secretory endometrium, histological sections from RU486 plus HMG/HCG-treated females uniformly demonstrated atrophic to weakly proliferative endometrium on cycle day 25, despite serum oestradiol concentrations >300 pg/ml (Figure 1). Three months after the initial 25-day study, endometrial biopsies revealed persistent atrophic endometrium, even though repeated ovulation induction with HMG/HCG therapy elevated serum oestrogen concentrations. The findings prevailed whether RU486 treatment began on cycle day 3 or 7. The inter-menstrual interval was significantly (P < 0.01) lengthened by RU486 treatments (28.5 + 2.0 for control versus 131.3 + 11.5 days for RU486). In summary, RU486 consistently blocked ovulation unless HCG was provided, and it elicited a persistent retardation of early proliferative endometrium when administered daily beginning in early or mid-follicular phase.

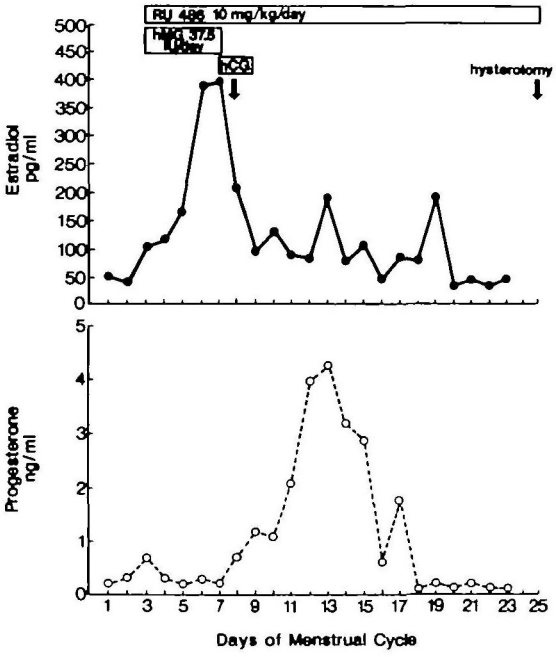

Fig. 1. Protocols for assessing the non-competitive anti-oestrogenic activity of RU486 HMG = human menopausal gonadotrophin, HCG = human chorionic gonadotrophin

The normal mitogenic effects of elevated ovarian oestrogen secretion on endometrial tissue were quelled, uniformly resulting in amenorrhoea (Figure 2). The long-lasting action of RU486, causing ovulation inhibition and atrophic endometrium, may be due to the depot effect of i.m injection In addition, RU486 did not prevent ovarian steroidogenesis, ovulation or oocyte maturation when an ovulation induction regimen of HMG/HCG was given. These findings show that RU486 prevented ovulation by diminishing pituitary gonadotrophin secretion, rather than by direct effects on ovarian folliculogenesis, and induced amenorrhoea by inhibiting oestrogen-induced endometrial proliferation.

Dose-dependent blockade of the proliferative action of oestradiol on endometrium by RU486

The non-competitive anti-oestrogenic effects of RU486 were examined using oestradiol-treated ovariectomized monkeys given RU486, progesterone, or

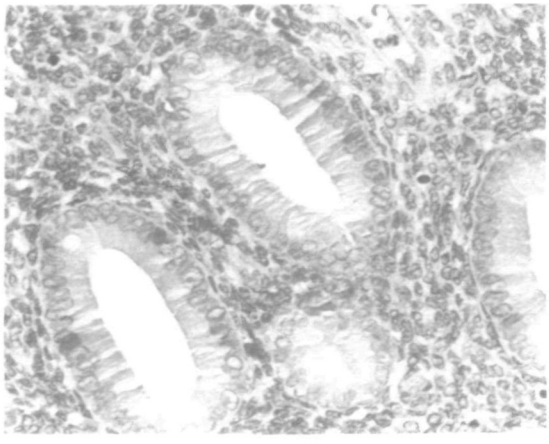

Fig. 2. Endometrial glands in cross-section from a monkey receiving RU486 from cycle day 7, the stroma is dense, the glands are lined by low cuboidal cells with oval nuclei and showing moderate amounts of eosinophilic cytoplasm Again, non-proliferative status is maintained in the face of oestradiol elevations (>300 pg/ml) in serum (×400)

Table I. Histological classification of the endometrium developed under the different hormonal treatments, using standard morphological criteria

Treatment	Thickness (mm)	Gland morphology	Epithelial stratification	Mitotic activity	Secretion	Stroma	Histological phase
Control	1 5 ± 0 5	Tubular	Pseudo	+++	0	Compact	Late proliferative
Progesterone (1 mg/kg/day)	1 8 ± 0 1	Tortuous	Mono	+	++	Oedema	Early secretory
RU486 (5 mg/kg/day)	0 8 ± 0 1	Tubular	Pseudo	0	0	Compact	Early proliferative
RU486-P_4 (5 mg/kg/day)	1 0 ± 0 1	Tubular	Pseudo	+	0	Dense	Mid proliferative
RU486 (1 mg/kg/day)	1 4 ± 0 1	Tubular	Mono	+	+	Dense	Interval endometrium

P_4 = progesterone

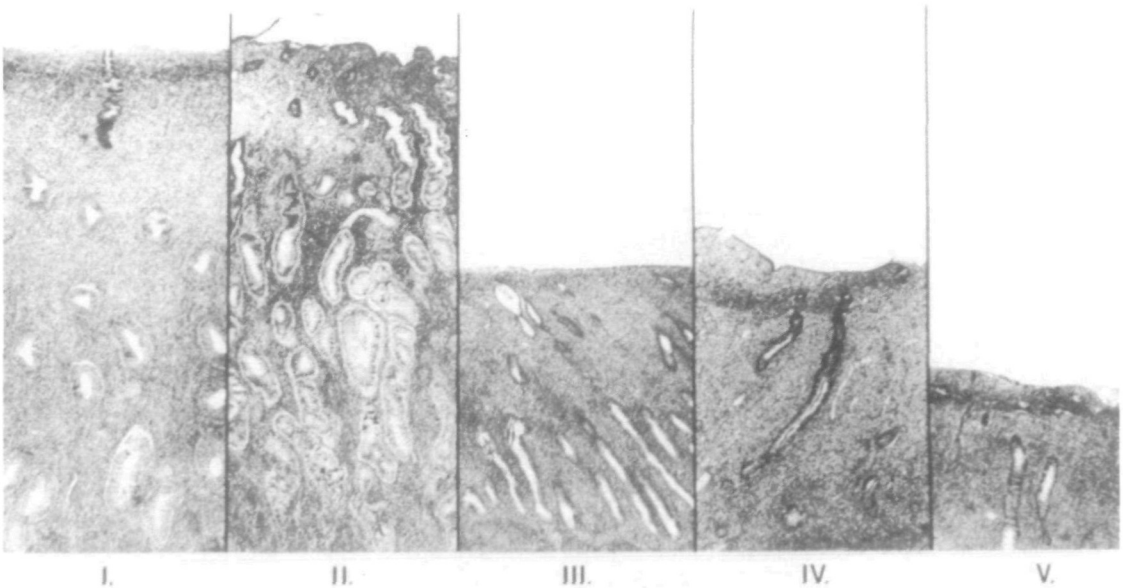

Fig. 3. Sections of the endometrium resting on its myometrium from the five groups After oestradiol treatment, the endometrium is thick, the stroma dense and the glands tubular (panel I). With progesterone stimulation, glands became tortuous and the stroma oedematous (panel II) Association of progesterone with RU486 resulted in a mid-proliferative endometrium (panel III), whereas RU486 induced a dose-dependent inhibition of glandular development and endometrium growth (panels IV and V) (×40)

both. The oestradiol-induced luteinizing hormone (LH) surge of control animals was abrogated by progesterone and/or RU486. Secretory transformation by progesterone was inhibited by RU486 co-administration. RU486 alone (1 mg/kg) induced endometrial secretory transformation, but higher doses (5 mg/kg) inhibited proliferation and secretory activity (Table I).

Thus, in the presence of progesterone, RU486 is antagonistic but, in its absence, RU486 exhibits endometrial progestational effects at low doses and an anti-proliferative (anti-oestrogenic) effect at higher

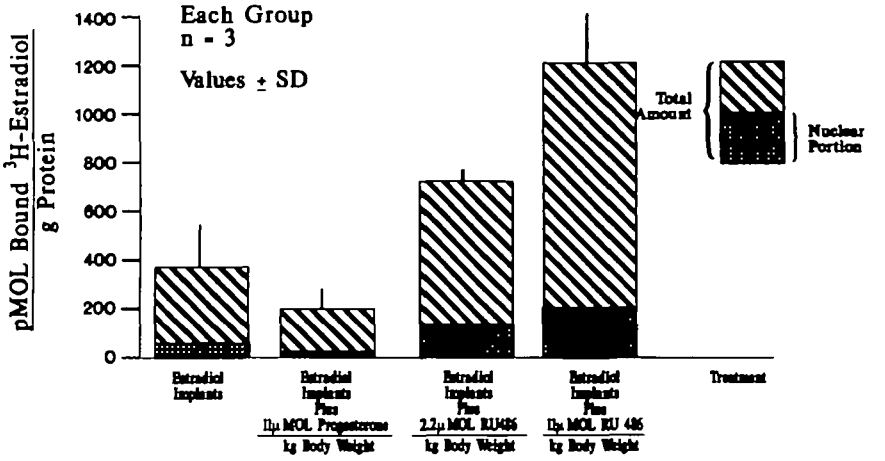

Fig. 4. Endometrial oestradiol receptor concentrations measured after different treatment regimens (oestradiol implants versus oestradiol implants plus 11 μM progesterone, or oestradiol implants plus 2 2 μM or 11 μM RU486)

doses (Figure 3). These data encourage continued evaluation of RU486 as a potential contraceptive agent acting at the pituitary and/or endometrial level.

RU486 induces elevations of oestrogen receptor in primate endometrium

This study was designed to investigate the effect of the antiprogestin RU486 on oestradiol receptor concentrations in the endometrium of monkeys given physiological oestrogen replacement therapy. Oestradiol-17β silastic implants were inserted interscapularly into 12 long-term ovariectomized cynomolgus monkeys (*Macaca fascicularis*), resulting in an average peripheral serum concentration of ~100 pg/ml oestradiol. On day 6 of oestradiol treatment, four treatment groups were initiated: group I, oestradiol implants only; group II, oestradiol implants plus 11 μmol progesterone/kg body weight in sesame oil via i.m. injections on days 6–8; group III, oestradiol implants plus 2.2 μmol RU486/kg in sesame oil via i.m. injections on days 6–8; group IV, oestradiol implants plus 11 μmol RU486/kg via i.m injections on days 6–8. On treatment day 9, endometrial biopsies were removed by hysterotomies Cytosolic and nuclear oestradiol receptor contents of tissues were estimated by the charcoal method (Figure 4).

In group I, the tissue contained 376 + 123 pmol bound [^{3}H]oestradiol/g protein; the nuclear portion was ~16%. In group II, the tissue contained 216 + 64 pmol bound [^{3}H]oestradiol/g protein; the nuclear portion was only 8%. In group III, tissue contained 654 + 47 pmol bound [^{3}H]oestradiol protein; the nuclear portion was ≈22%. In group IV, the tissue contained 1198 + 172 pmol bound [^{3}H]oestradiol/g protein; the nuclear portion was ~17%. Scatchard plot analysis indicated a $K_{d\ app}$ of 1.04×10^{-9} M. This study demonstrates that after physiological oestradiol replacement therapy, oestradiol receptor concentrations rise dramatically following antiprogestin treatment; this effect was dose-dependent.

Whether this non-competitive anti-oestrogenic (anti-proliferative) property of certain antiprogestins extends to breast cancers that are oestrogen or oestrogen–progestin-dependent is not known. Also, the effects of mifepristone, onapristone and other progesterone antagonists on oestrogen-dependent physiological functions, such as bone density conservation and lipid-related cardiovascular health, remain to be evaluated, especially in the context of long-term regimens.

'It is hoped that the political interference that has delayed antiprogestin research and development will not continue' (Hodgen, 1991).

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