Effect of mifepristone on steroid receptor expression and biotransformation of oestrogen and progesterone in rat uterus and deciduoma

URMILA VIJ, ANAND KUMAR, KANHAIYA SHARMA, MISHI KAUSHAL, RAJ MEHRA

ABSTRACT

Background. Mifepristone is a synthetic antiprogestin which terminates early pregnancy. Since it interferes with the progesterone maintained decidua, we compared the effect of mifepristone on oestrogen and progesterone receptors, and on the biotransformation of these hormones in normal and deciduous uterus.

Methods. Ovariectomized rats were treated with an oestrogen–progesterone hormone regimen and deciduoma was induced by trauma in one horn of the rat uterus while the other served as a control under an identical hormonal milieu. Hormone receptor and biotransformation studies were done using radiolabelled oestradiol and progesterone with high specific activity.

Results. The artificially formed decidual tissue was comparable with that of early pregnancy. Mifepristone replenished oestrogen and progesterone receptors which were suppressed by progesterone in both the normal and decidualized uterine horns. Inhibition of oestrogen receptors by progesterone correlated with decreased oestradiol levels at the site of action. Metabolism of progesterone to less potent compounds was promoted by mifepristone. The enzymatic activities of 17β-hydroxysteroid dehydrogenase (which metabolizes oestradiol), and 20α-hydroxysteroid dehydrogenase and 5α-reductase (which metabolize progesterone) were altered by mifepristone.

Conclusion. The effect of mifepristone in varying the hormone receptor population and the availability of different levels of active metabolites of ovarian hormones have an important role in the antiprogestin action of mifepristone.

Natl Med J India 2006;19:64–9

INTRODUCTION

Progesterone (P) is crucial for the maintenance of pregnancy. An effective antiprogestin would act as a specific abortifacient by interacting with the decidual tissue of early pregnancy. Mifepristone (M, RU 486), well known for its use as an abortifacient pill, is a derivative of norethindrone with an α side chain and an 11β ring substitution. It possesses a high binding capacity and progesterone antagonist activity.[1] The abortifacient action of M is a result of its interaction with uterine hormone receptors.[2–6] Since M is an early abortifacient, its action, specifically on the decidual tissue, is of importance.

We developed a deciduoma in ovariectomized rats by traumatizing one uterine horn. Decidualization or decidual cell reaction (DCR) is the proliferation of peri- and subluminal cells following a traumatic stimulus, either by the blastocyst or artificially by mechanical or chemical means; hormonal priming is a prerequisite for decidualization. Stromal cells derived from unprimed uteri fail to differentiate to decidual ones *in vivo*.[7] It has been shown that, in ovariectomized rats, the hormonal requirements for normal pregnancy and decidualization induced by an artificial stimulus are identical.[8] Using such decidual tissue, we studied the interaction of M with ovarian hormone receptors and the metabolic biotransformation of oestradiol (E2) and P, and compared these with normal uterine tissue.

METHODS

Induction of deciduoma and hormone treatment

The preparation of animals and induction of deciduoma was done as described earlier with some modifications.[9] Briefly, rats were ovariectomized and 2 weeks later were treated with hormones. The rats were primed with E2 (2 µg/50 µl olive oil/rat, subcutaneously) from day 1 and P (2 mg/50 µl olive oil/rat) was started from day 6. On day 10, the rats underwent a laparotomy and one uterine horn was traumatized for decidualization while the other horn was left untouched to serve as a control.

In a pilot experiment, rats were sacrificed each day for 11 days post-traumatization (as described above) to estimate the change in weight of the decidualized horn and to confirm the validity of this animal model. Also, to standardize the dose, 0.25, 0.5, 1, 2 and 4 mg of P was injected in separate sets of animals.

After standardization, the protocol followed was the same as described above till day 10, when one horn was traumatized. P was continued till day 14. At this point the rats were divided into 3 groups of 6 rats each and treated as follows:

1. E2 treated: Given E2 2 µg/50 µl/rat for 3 days
2. P treated: Given E2 2 µg/50 µl/rat with 4 mg P/rat for 3 days
3. M treated: Given E2 2 µg/50 µl/rat, 4 mg P/rat and 4 mg M/rat for 3 days

Twenty-fours hours after the last injection the animals were sacrificed, the traumatized and control uterine horns were removed and processed. The control and decidual tissues for each experiment were pooled from 5–6 rats and the results of each experiment were calculated from 3–4 observations. DCR in the traumatized horn was calculated from the per cent increase in decidual weight over the control uterine horn as follows:

All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India
URMILA VIJ, ANAND KUMAR Department of Reproductive Biology
KANHAIYA SHARMA, MISHI KAUSHAL, RAJ MEHRA Department of Anatomy
Correspondence to URMILA VIJ

$$\% \text{ increase} = \frac{\text{weight of (traumatized horn–control horn)} \times 100}{\text{weight of traumatized horn}}$$

Receptor assay

For accurate quantitative analysis, radioreceptor assay using high specific activity radioligands were preferred to immunohistochemical assay. Cell fractionation and oestrogen (ER) and progesterone receptor (PR) assays in nuclear and cytosol fractions were done as described earlier.[10,11] Briefly, 100 µl cytosol or 100 µl nuclear suspension was incubated with 200 nM 3H P for PR assay and 20 nM 3H E2 for ER assay with or without 2 µM non-radioactive P and E2, respectively, at 0 °C for 12–16 hours. ER assay tubes were further incubated at 30 °C for 30 minutes for complete exchange. To eliminate cortisol-binding globulin (CBG) from interfering with PR, 1 µM of cortisol was added. At the end of incubation, free and bound steroids in the cytosol assay tubes were separated by the dextran charcoal method.[12]

For nuclear assay, at the end of incubation the nuclei were washed with buffer to remove unbound steroid. The steroid was extracted with ethanol and radioactivity counted in a liquid scintillation counter. For the cross-incubation study, different volumes (50 µl and 100 µl) of uterine cytosol of E2 treated rats was incubated with uterine nuclei of P treated rats to quantitate the functional or translocatable receptors in the nuclei. DNA and protein estimation were done as described earlier.[13,14] The results were expressed as total receptors (sum of nuclear and cytosol).

Biotransformation studies

Control and traumatized uterine horns were removed and collected on ice as soon as the rats were sacrificed. The tissues were freed of adhering fat, weighed, minced and incubated for 60 minutes with 20.9 pmol of 3H P or 25 pmol of 3H E2 in 5 ml of RPMI 1640 medium containing 33 µmol of glucose, 11 µmol glucose-6-phosphate, 2.3 µmol of ATP and 1.3 µmol of NADPH for P, and 10 µM each of NAD, ATP and NADPH for E biotransformation studies.[15] At the end of incubation the steroids were extracted from the tissue and analysed using thin layer chromatography. The chromatograms were developed in a hexane:ethyl acetate (5:2 v/v) and chloroform:acetone (9:1 v/v) solvent system for P and its metabolites. The steroids were located by UV and by exposure to iodine vapours. For E and its metabolites, the chromatograms were developed in a benzene:methanol (9:1) solvent system and steroids were located by iodine vapours. Confirmation of metabolites was carried out by acetylation, saponification of acetylated compounds, oxidation and recrystallization to constant specific activity as described earlier.[15]

Source of materials

1,2,6,7-3H P (86 Ci/mmol) and 2,4,6,7-3H E2 (99 Ci/mmol) were obtained from Amersham Pharmacia Biotech UK Ltd, England. Radio-inert E2 and oestrone were obtained from Sigma Chemical Co., St Louis, MO. Norgestrel was obtained from Wyeth Pharma, Munster, Germany; norethindrone and norethindrone acetate from Schering AG, Berlin, Germany. M was obtained from Roussel UCLAF, France. Other chemicals were purchased from commercial sources. Adult female Wistar rats bred in the animal house of our institution were used for the study. Ethical clearance was obtained from the Animal Ethics Committee.

RESULTS

Increase in uterine weight

In the pilot study, it was observed that following traumatization the weight of the decidualized horn increased till day 5 and then declined gradually by day 11 (Fig. 1). The weight gain of the deciduoma was dose dependent and with 2 and 4 mg doses of P there was >400% increase in the weight of the decidualized horn compared with the control horn (Fig. 2). Hence, in the main experiment, the deciduoma was maintained intially with 2 mg and later 4 mg of P. In the M treated rats, the deciduoma maintenance effect of P was antagonized although there was a mild uterotrophic effect in terms of increase in weight of both the uterine horns (Fig. 3). However, the uterotrophic effect was transient and showed a decrease at 48 hours (Fig. 3). Uninterrupted, continuous (15 days) treatment of intact rats with M led the normal adult rats to a state

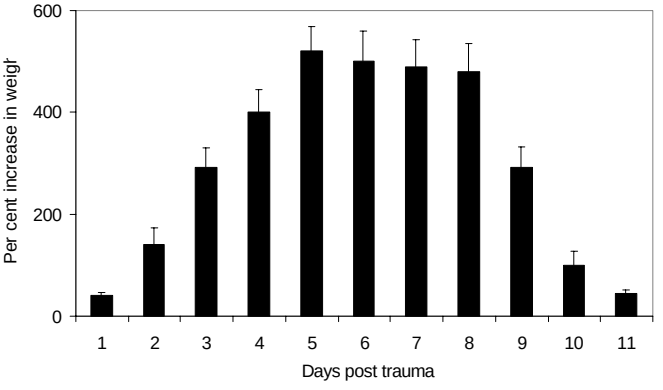

FIG 1. Per cent increase in weight of the decidualized uterine horn. The mean weight of the control horn was 25 mg. Results are mean (SD) of 3 observations on 5–6 rats on each day.

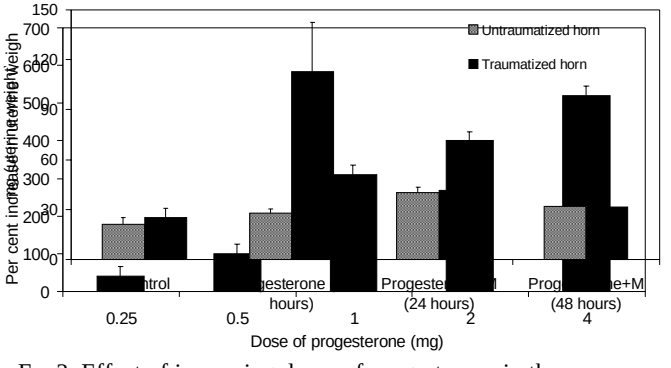

FIG 2. Effect of increasing doses of progesterone in the decidualized horn on day 5 post-trauma. Results are mean (SD) of 3 observations on 5–6 rats with each dose.

FIG 3. Effect of mifepristone (M) treatment on decidualization. Results are mean (SD) of 3 observations on 5–6 rats in each group.

of constant oestrus suggesting an E and P antagonistic action (results not shown).

Effect of P and antiprogestin on PR

Binding of P and antiprogestin to PR in control and decidual tissue revealed that M had significantly higher ($p<0.01$) relative binding affinity (RBA) than P (Table I). It did not elicit progestational activity as it did not maintain the decidua. Other progestins such as norgestrel, medroxyprogesterone acetate, norethindrone, etc. with known progestational activity, also had high RBA for P binding sites. However, E2 and cortisol did not show any binding to PR (Table I). Further antagonistic action of M on the total PR population was evaluated in the control and decidualized horns in all 3 groups (Fig. 4). P significantly suppressed total PR in the decidualized ($p<0.01$) and control horns ($p<0.001$) compared with E2 treated rats. Interestingly, M along with P (M treated group) antagonized the suppressive effect of P on PR in both the control and decidualized horns leading to a significant increase in the PR population in the decidualized horn ($p<0.01$) The direct proteolytic effect on receptor level was demonstrated by cross-incubation studies in which different volumes of treated and untreated cytosols were used for translocation of receptors to the nucleus (Table II). The functional or translocatable receptor levels in P treated nuclei incubated with 50 µl and 100 µl E2 treated cytosol was comparable with E2 treated nuclei incubated with E2 treated cytosol. However, if E2 treated nuclei were incubated with P treated cytosol the functional receptor population decreased in relation to the volume of cytosol. Also, the functional receptor level significantly decreased ($p<0.001$) when 100 µl of P treated cytosol was used instead of 50 µl (Table II). This proteolytic-like effect of P in cytosol was antagonized by M treatment (Table II) leading to receptor replenishment when E2 treated nuclei were incubated with M treated cytosol.

Effect of P and M on ER

The ER population in the decidualized uterine horn was significantly lower ($p<0.001$) than in the control horn (Fig. 5). P significantly decreased the ER population in the decidualized ($p<0.001$) and control ($p<0.001$) horns, and M antagonized this suppressive effect of P on ER. Thus, in the M treated group the P associated reduction was inhibited by M (Fig. 5). Moreover, this replenishment effect on ER by M, though significant, was much higher in the control ($p<0.001$) than the decidualized horns ($p<0.01$). Removal of the suppressive effect of P on PR and rescue of the same by M was confirmed by another experiment (Table III). At 24 hours of E2 treatment, the ER was about 10 times higher than that at 0 time. However, when at 24 hours of E2 treatment the rats were treated with P (4 mg), the ER decreased significantly ($p<0.001$) at 48 hours. In contrast, if M was injected along with P, at 24 hours it antagonized the inhibitory effect of P and the receptor

TABLE I. Mean (SD) relative binding affinity of progestins and antiprogestins for progesterone receptors in the control and decidualized uterine horns

Compound	Relative binding affinity	
	Control	Deciduoma
Progesterone	100	100
Norgestrel	196 (13.5)	155 (26)
Mifepristone	166 (25)	190 (20)
Medroxyprogesterone acetate	164 (15.5)	145 (15.1)
Norethindrone	98.6 (14.4)	85.3 (9.3)
Norethindrone acetate	8 (2)	5.7 (2.5)
Oestradiol	<0.1	<0.1
Cortisol	<0.1	<0.1

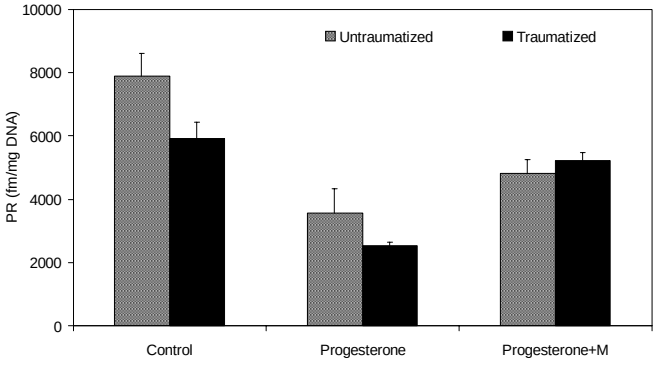

FIG 4. Effect of mifepristone (M) on progesterone receptors (PR) in rat uterine horns. Uterine tissue from 5–6 animals in each group was pooled for each observation. Results are mean (SD) of 4 observations.

TABLE II. Effect of progesterone (P) and mifepristone (M) on functional (translocatable) progesterone receptors in the uterus

Treatment	Receptor molecules	
	Used	Translocated
E treated nuclei+		
E treated 50 µl cytosol	4025 (500)	2500 (274)
E treated 100 µl cytosol	8800 (997)	3378 (296)
P treated nuclei+		
E treated 50 µl cytosol	4025 (500)	2480 (198)
E treated 100 µl cytosol	8800 (997)	3428 (325)*
E treated nuclei+		
P treated 50 µl cytosol	2150 (238)	1500 (116)
P treated 100 µl cytosol	3585 (362)	800 (99)†
E treated nuclei+		
M and P treated 50 µl cytosol	2895 (335)	2105 (194)
M and P treated 100 µl cytosol	4795 (349)	3159 (227)

* $p<0.001$ † $p<0.001$ Values are mean (SD)

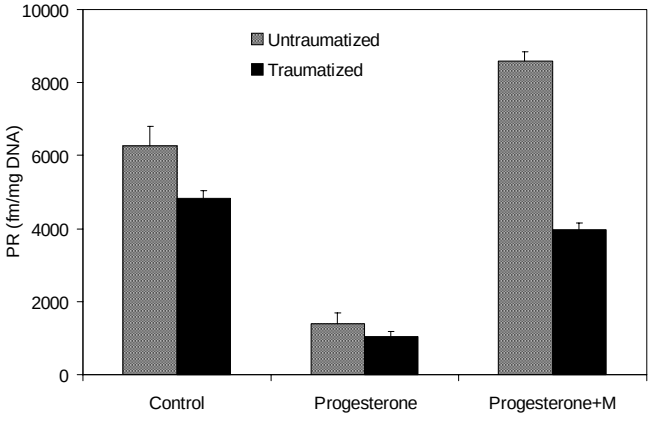

FIG 5. Effect of mifepristone on oestrogen receptors (ER) in rat uterine horns. Uterine tissue from 5–6 animals in each group was pooled for each observation. Results are mean (SD) of 4 observations.

TABLE III. Removal of suppressive effect of progesterone (P) on oestrogen receptors (ER) by mifepristone (M)

Treatment	Mean (SD) ER (fmol/mg DNA)		
	0 hours	24 hours	48 hours
E2 5 µg at 0 hours	347 (78)	3347 (478)	5084 (925)
E2 5 µg at 0 hours+P 4 mg at 24 hours	–	–	1283 (214)*
E2 5 µg at 0 hours+P 4mg + M 4 mg at 24 hours	–	–	5157 (315)†

E2 oestradiol * $p<0.001$ † $p<0.001$

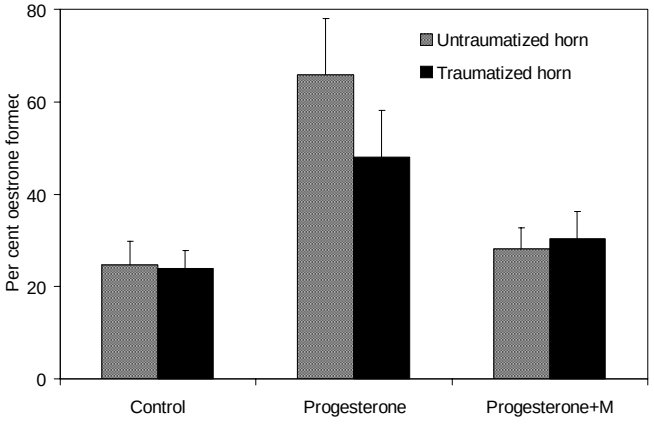

FIG 6. Effect of mifepristone on biotransformation of oestradiol in rat uterine horns. Uterine tissue from 5–6 animals in each group were pooled for each observation. Results are mean (SD) of 4 observations.

level increased significantly ($p<0.001$); the difference in ER level between the E2 treated and M treated uterine horns became insignificant ($p>0.05$).

Biotransformation of E2

The metabolic biotransformation of 3H E2 to oestrone (E1) was not different ($p>0.05$) in the control and traumatized horns of E2 treated rats, being 24.6% and 24%, respectively. In the P treated group the metabolism of E2 to E1 increased significantly in the control (65.8%, $p<0.001$) and decidualized (48%, $p<0.01$) uterine horns, respectively (Fig. 6). This showed that 17β-hydroxysteroid dehydrogenase (HSD) is a progestin dependent enzyme. This effect of P on increase in metabolic biotransformation of E2 was antagonized by antiprogestin and the formation of E1 decreased significantly to 28% in the control and 30% in the decidualized tissue ($p<0.001$). Thus, antiprogestin antagonized the action of P and more E2 was made available by decreasing the metabolism.

Biotransformation of P

The effect of progestin and antiprogestin on 20α-HSD and 5α-reductase was investigated in the control and decidualized uterine horns (Table IV). In E2 treated rats, more P was metabolized and only 56% and 59% remained unconverted in the control and decidualized uterine horns, respectively. Of the major metabolites identified, 20α-OH-P was less than 5α-pregnan compounds in both control and decidualized uterine horns. Under the influence of P, a significantly larger amount of P (81%) remained unconverted in the decidualized uterine horn ($p<0.01$) so that more P was available for biological action. P also influenced the pattern of metabolites since more 20α-OH-P was formed while less 5α-pregnan compounds were formed as compared with E2 treated rats. These results suggest that P increased the 20α-HSD activity and decreased the 5α-reductase activity. These effects of P on its biotransformation were antagonized by M.

DISCUSSION

This study was designed to establish a rat model in which one uterine horn was decidualized while the other horn was used as a control under identical hormonal manipulation. The DCR measured in terms of increase in uterine weight started on day 1 after the stimulus as the rats were maintained under proper hormonal conditions. This kept increasing till day 5 post-traumatization and declined after about day 8. Although the progression of deciduoma was hormone dependent, the decidual cells possessed a limited life span.[7] Hyperplasia was the characteristic feature of the DCR, leading to enormous growth of decidual tissue maintained under the effect of P, while M antagonized this action and inhibited maintenance of the deciduoma. However, there was some trophic effect of M *per se* since there was a slight increase in uterine weight in both the decidualized and control uterine horns. The uterotrophic effect was transient and showed a reversal with metabolic clearance of the drug. This effect has been reported in patients with Cushingoid features treated with long term M.[16] M has also been shown to lead to regression of leiomyoma, with some of the patients developing drug-associated simple endometrial hyperplasia.[17] Unlike onapristone, a paradoxical agonist effect of M on the post-menopausal endometrium has been reported, as well as an antiproliferative effect.[5,18] The uterotrophic effect of M in terms of a slight increase in uterine weight might be mediated by a transient interaction between M and the uterine ER. Ballooning and increase in weight of uteri has been explained by the 'unopposed' E effect after the action of P is blocked by progesterone antagonists.[19] The constant oestrus state observed in intact rats on continuous M reflects its oestrogenic action on epithelium that is not opposed by P. E upregulated the ER and PR in the uterus and decidua while P downregulated them.

Treatment with M upregulated the hormone receptors that had been suppressed by P in both control and decidualized uterine

TABLE IV. Effect of mifepristone (M) on biotransformation of progesterone (P) in control (C) and decidualized (D) uterine horns

Group	Progesterone		20α-OHP		5α-pregnan-3-20-dione		5α-pregnan 3β-ol-20-one	
	C	D	C	D	C	D	C	D
E treated	56 (5)	59 (4)	4.8 (1)	5.2 (0.8)	10.2 (1.8)	11.1 (1.2)	12.1 (1.7)	12.5 (2.1)
P treated	71 (6)	81 (6)	9.8 (2)	10.1 (1.2)	6.7 (0.7)	2.1 (0.3)	8.5 (1)	4.2 (0.7)
M treated	65 (2)	69 (5)	7.2 (2)	7.5 (0.6)	8.3 (1.1)	8.8 (1.3)	9.1 (1)	8.9 (0.9)

Results are expressed as mean (SD) from 4 observations and as per cent metabolites formed and per cent unmetabolized progesterone
Progesterone significantly decreased progesterone metabolism ($p<0.01$) E oestradiol

horns; an effect reported earlier.[3,6,20,21] In monkey uterus, M has antiproliferative, apoptotic and stromal compaction effects that decrease the ability of E2 to maintain the mass of endometrium.[20] The major effect of M was to block P-induced suppression of ER allowing E2 to exert its effects on cell differentiation.[20] The higher levels of PR in E2-maintained endometrium may allow M to act more rapidly than in the regenerating endometrium, which initally has lower PR levels. In the presence or absence of P, apoptotic cell death in the glandular epithelium increases, while the action of E2 results in a balance between the rates of cell division and death in the regenerating endometrial glands, similar to what is observed in primates. If M is present during endometrial regeneration, apoptosis increases but there is no inhibition of epithelial proliferation. Thus, M disturbs the baseline E2-regulated balance between cell division and death in favour of cell death.[22] We found a direct proteolytic effect of P which was inhibited by M. In the cross-incubation studies P treated uterine cytosol decreased the translocation of receptors to nuclei while M antagonized the effect of P by protecting the receptor protein. A similar increase in ER and PR protein expression due to M has been reported in hormonally replaced castrated monkeys, possibly by inhibiting degradation of mature protein.[23]

M has a half-life of about 20 hours. The liver metabolizes M by demethylation and hydroxylation. The enterohepatic circulation may account for the long half-life of the drug.[24] We found that M influenced the metabolic biotransformation of ovarian hormones in the deciduoma and endometrium. The maintenance of gestation and pseudopregnancy is dependent on the availability of P. If the metabolic biotransformation of P and E2 in the target tissues is altered, the optimal hormonal milieu required for maintenance of decidual tissue will be affected, adding to the abortifacient property of M. Under the influence of P, a high concentration of E2 was metabolized to E1; in decidual tissue E1 formation was even higher. This suggests that 17β-HSD, responsible for the conversion of E2 to E1, is a progestin dependent enzyme. The activity of 17β-HSD in the human endometrium has been shown to change during the menstrual cycle. It increases in the luteal phase due to progesterone secretion.[25–28] Progestins such as norgestrel and provera increase the activity of 17β-HSD while E and oestrogenic compounds decrease it.[29,30] The anti-oestrogenic effect of P in increasing the activity of 17β-HSD accelerated the conversion of E2 to E1, which is a biologically less active hormone. We have shown that the activity of 17β-HSD, which is P dependent in the rat uterus, was decreased due to P antagonism of M.

Thus, one of the ways by which an antiprogestin takes part in the interplay of the effects of E and P in the normal uterus and a deciduoma is by inducing 17β-HSD and influencing the metabolic biotransformation of E2 to E1. Moreover, M influenced the metabolism of P by increasing the activity of enzymes that metabolize P. Unmetabolized P was much higher in P treated control as well as decidual tissue. This may be one of the mechanisms by which more P was made available at the site of action. In contrast, M antagonized this action of P and metabolic biotransformation was increased both in control and decidual tissue. 5α-pregnan-3,20-dione was formed in larger amounts in the M treated uterine tissues compared with P treated ones. This suggests that the enzyme 5α-reductase was activated due to antiprogestin. Saturation of the C-4, C-5 double bond of P and consequent formation of 5α-pregnan-3,20-dione has been shown to be an E dependent step.[15,31] Unlike 5α-reductase, 20α-HSD increases under the influence of P. This increase was antagonized by M. P dependence of 20α-HSD in secretory endometrium has been reported earlier.[32,33] Since the equilibrium of the reaction catalysed by 20α-HSD is in favour of reduction of P, 20α-HSD could function as a regulator of progesterone activity in the target decidual cell. Our study suggests that different concentrations of 5α-pregnan-3,20-dione formed under the influence of progestin and antiprogestin may have a role in manifesting the action of P. Higher concentrations of 20α-OHP formed by P dependent 20α-HSD may influence the action of P in decreasing PR. Thus, the downregulation or masking of PR observed with P may be attributed to a dual effect of increased concentration of 20α-HP and decreased rate of P metabolism. A similar decrease in the PR concentration along with increase in 20α-HSD from days 11 to 22 of pregnancy has been reported.[34]

Our study has shown that the action of M on decidual tissue results in its disruption. E and P act on their target organs by interacting with their receptors[35–37] while M intervenes by blocking PR and antagonizing the suppressive effect of P on PR and ER. Increase in the metabolism of P by antiprogestin may also be due to local regulation of molecular events at the target site to decrease the effect of P by converting it to a biologically less active compound. Thus, variations in specific receptor and enzyme levels in decidual tissue under the effect of progestin and antiprogestin appear to play a critical role in the action of M due to a direct effect on the concentration of P and E and their metabolites in deciduoma and the uterus.

ACKNOWLEDGEMENT

Financial assistance for this study was received from the Indian Council of Medical Research (Project no.I-316).

REFERENCES

1 Wolf JP, Hsiu JG, Anderson TL, Ulmann A, Baulieu EE, Hodgen GD. Noncompetitive antiestrogenic effect of RU 486 in blocking the estrogen-stimulated luteinizing hormone surge and the proliferative action of estradiol on endometrium in castrated monkeys. *Fertil Steril* 1989;**52:**1055–60.

2 Marions L, Cekan SZ, Bygdeman M, Gemzell-Danielsson K. Effect of emergency contraception with levonorgestrel or mifepristone on ovarian function. *Contraception* 2004;**69:**373–7.

3 Gemzell-Danielsson K, Marions L. Mechanisms of action of mifepristone and levonorgestrel when used for emergency contraception. *Hum Reprod Update* 2004;**10:**341–8.

4 Vij U, Murugesan K, Kalita JC, Farooq A. Interaction of antiprogestins with progesterone receptors in rat uterus. *J Steroid Biochem* 1989;**32:**279–82.

5 Mahajan MA, Samuels HH. Nuclear hormone receptor coregulator: Role in hormone action, metabolism, growth and development. *Endocr Rev* 2005;**26:**583–97.

6 Baird DT, Brown A, Critchley HOD, Williams AR, Lin S, Cheng L. Effect of long-term treatment with low-dose mifepristone on the endometrium. *Hum Reprod* 2003;**18:** 61–8.

7 Bell SC, Searle RF. Differentiation of decidual cells in mouse endometrial cell cultures. *J Reprod Fertil* 1981;**61:**425–33.

8 Finn CA, Martin L. Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol Reprod* 1972;**7:**82–6.

9 Zarrow MX, Yochin JM, McCanthy JL. The progestogens. In: Zarrow MX, Yochin JM, McCanthy JL (ed). *Experimental endocrinology. A source book of basic endocrinology*. New York:Academic Press; 1964:65–108.

10 Bayard F, Damilano S, Robel P, Baulieu EE. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. *J Clin Endocrinol Metab* 1978;**46:** 635–48.

11 Verma U, Laumas KR. *In vitro* binding of progesterone to receptors in human endometrium and the myometrium. *Biochim Biophys Acta* 1973;**317:**403–19.

12 Verma U, Laumas KR. Screening of antiprogestins using *in vitro* human uterine progesterone receptor assay system. *J Steroid Biochem* 1981;**14:**733–40.

13 Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J* 1956;**62:**315–23.

14 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;**193:**265–75.

15 Verma U, Laumas KR. Effect of oestradiol -17beta and progesterone on the metabolism of 1,2-3H progesterone by the rabbit endometrium and myometrium. *Biochem J* 1976; **156:**55–62.

16 Newfield RS, Spitz, IM, Isacson E, New MI. Long-term mifepristone (RU 486) therapy resulting in massive benign endometrial hyperplasia. *Clin Endocrinol* 2001; **54:**399–404.

17 Eisinger SH, Meldrum S, Fiscella K, le Roux HD, Guzick DS. Low-dose mifepristone for uterine leiomyomata. *Obstet Gynecol* 2003;**10:**243–50.
18 Brenner RB, Slayden OD, Critchley HO. Anti-proliferative effects of progesterone antagonists in the primate endometrium: A potential role for the androgen receptor. *Reproduction* 2002;**124:**167–72.
19 Michna H, Schneider MR, Nishino Y, el Eterby FE. Antitumor activity of the anti-progestins ZK 98.299 and RU 38.486 in hormone dependent rat and mouse mammary tumors: Mechanistic studies. *Breast Cancer Res Treat* 1989;**14:**275–88.
20 Slayden OD, Brenner RM. RU 486 action after estrogen priming in the endometrium and oviducts of rhesus monkeys (*Macaca mulata*). *J Clin Endocrinol Metab* 1994;**78:** 440–8.
21 Narvekar N, Cameron S, Critchley HOD, Lin S, Cheng L, Baird DT. Low-dose mifepristone inhibits endometrial proliferation and upregulates androgen receptor. *J Clin Endocrinol Metab* 2004;**89:**2491–7.
22 Haluska GJ, West NB, Novy MJ, Brenner RM. Uterine estrogen receptors are increased by RU 486 in late pregnant rhesus macacques but not after spontaneous labor. *J Clin Endocrinol Metab* 1990;**70:**181–6.
23 Heikinheimo O, Hsiu JG, Gordon K, Kim S, Williams RF, Gibbons WE, *et al*. Endometrial effects of RU 486 in primates—antiproliferative action despite signs of estrogen action and increased cyclin-B expression. *J Steroid Biochem Mol Biol* 1996;**59:**179–90.
24 Baulieu EE. Contragestion by the progesterone antagonist RU 486 : A novel approach to human fertility control. *Contraception* 1987;**36** (Suppl):1–5.
25 Tseng L, Gurpide E. Nuclear localization of estradiol in superfused slices of human endometrium. *Am J Obstet Gynecol* 1972;**114:**995–1001.
26 Tseng L, Gurpide E. Effects of progestins on estradiol receptor levels in human endometrium. *J Clin Endocrinol Metab* 1975;**41:**402–4.
27 Tseng L, Gurpide E. Changes in the *in vitro* metabolism of estradiol by human endometrium during the menstrual cycle. *Am J Obstet Gynecol* 1972;**114:**1002–8.
28 Tseng L, Gurpide E. Estradiol and 20 alpha-dihydroprogesterone dehydrogenase activity in human endometrium during the menstrual cycle. *Endocrinology* 1974;**94:** 419–23.
29 Tseng L, Gurpide E. Induction of human endometrial estradiol dehydrogenase by progestins. *Endocrinology* 1975;**97:**825–33.
30 Maentausta O, Svalander P, Gemzell-Danielsson K, Bygdeman M, Vihko R. The effects of an antiprogestin, mifepristone and an antiestrogen tamoxifen on endometrial 17beta-hydroxysteroid dehydrogenase and progestin and estrogen receptors during the luteal phase of the menstrual cycle: An immunohistochemical study. *J Clin Endocrinol Metab* 1993;**77:**913–18.
31 Verma U, Laumas KR. Cellular and subcellular metabolism of progesterone by the human proliferative and secretory phase endometrium and myometrium. *J Steroid Biochem* 1976;**7:**275–82.
32 Pollow K, Lubbert H, Boquoi E, Pollow B. Progesterone metabolism in normal human endometrium during the menstrual cycle and in endometrial carcinoma. *J Clin Endocrinol Metab* 1975;**41:**729–37.
33 Vij U, Murugesan K, Laumas KR, Farooq A. Progestin and antiprogestin interactions with progesterone receptors in human myomas. *Int J Gynecol Obstet* 1990;**31:** 347–53.
34 Clementi MA, Deis RP, Telleria M. Luteal 3 beta-hydroxysteroid dehydrogenease and 20 alpha hydroxysteroid dehydrogenase activities in the rat corpus luteum of pseudo-pregnancy: Effect of the deciduoma reaction.*Reprod Biol Endocrinol* 2004;**2:**22–30.
35 Varayoud J, Ramos JG, Monje L, Bosquiazzo V, Munoz-de-Toro M, Luque EH. The estrogen receptor alpha sigma 3 mRNA splicing variant is differentially regulated by estrogen and progesterone in the rat uterus. *J Endocrinol* 2005;**186:**51–60.
36 Wu RC, Smith CL, O'Malley BW. Transcriptional regulation by steroid receptor coactivator phosphorylation. *Endocr Rev* 2005;**26:**393–9.
37 Bryant W, Snowhite AE, Rice LW, Shupnik MA. The estrogen receptor (ER) alpha variant delta 5 exhibits dominant positive activity on ER-regulated promoters in endometrial carcinoma cells. *Endocrinology* 2005;**146:**751–9.