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SHORT COMMUNICATION

EFFECT OF EARLY LUTEAL PHASE ADMINISTRATION OF A SINGLE DOSE MIFEPRISTONE ON IMMUNOHISTOCHEMICAL DISTRIBUTION OF INTERLEUKIN 1 ALPHA (IL-1α) AND TRANSFORMING GROWTH FACTOR BETA 1 (TGF-β1) IN MID-LUTEAL PHASE OVARY OF THE RHESUS MONKEY[1]

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Abstract : A single low dose administration of a high affinity anti-progestin agent like mifepristone during the early luteal phase inhibits blastocyst implantation in human and non-human primates. Though it has been observed that luteal phase serum concentrations of estradiol and progesterone were not affected by the application of anti-nidatory dose of early luteal phase mifepristone suggesting that ovarian steroidogenic function is not compromised, it is nevertheless possible that ovarian physiology at the local tissue level is affected in this treatment schedule. In the present study, healthy, mature, proven fertile female rhesus monkeys were divided into two groups. Group 2 animals were treated with a single dose of mifepristone (2 mg/kg body weight), while group 1 animals were injected with vehicle (1:4 benzoy1 benzoate: olive oil, v/v, s.c.) on day 2 post-ovulation. The morphological examination including that of vascularity, as well as, histometric determination of profiles of immunopositivity for IL-1α and TGF-β1 in stromal, follicular and luteal compartments of mid-luteal phase ovaries from animals with or without a single, anti-nidatory dose of mifepristone applied on day 2 after ovulation failed to reveal any significant change between the two groups. Thus, it appears that early luteal phase administration of a single antinidatory dose of mifepristone does not affect the ovarian physiology in the treatment cycle.

Key words : IL-1α luteinization mifepristone ovary progesterone rhesus monkey TGF-β

INTRODUCTION

It has been demonstrated earlier by several groups that a single low dose administration of a high affinity antiprogesterone agent like mifepristone

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[1]A part of this work was presented in APPICON 2001 where it was adjudged the best paper presented by an undergraduatestudent for the Harish Gupta Prize.

during early luteal phase inhibits blastocyst implantation in the human and non-human primates (1–4). The anti-implantation effect of early luteal phase mifepristone appears to be mediated by its antiprogestin action on implantation stage endometrium under progesterone dominance rendering it hostile (5, 6), as well as, indirectly affecting the growth and viability of pre-implantation stage embryos (7, 8). Furthermore, it was observed that the luteal phase serum concentrations of estradiol and progesterone were not affected by the application of anti-nidatory dose of early luteal phase mifepristone suggesting that ovarian steroidogenic function was not affected in this treatment schedule (1–4). However, it appears possible that ovarian physiology may be affected at subclinical level during luteal phase after early luteal phase treatment with mifepristone, especially in view of the fact that progesterone plays a critical role in the process of luteinization (9,10). While several cytokines are known to regulate the process of luteinization (11, 12) by their actions on matrix metalloproteineases and their inhibitors (13, 14), progesterone has been shown to regulate the expression of these proteases and inhibitors towards tissue remodelling during luteinization (15). Several studies indicate that interleukin 1 alpha (IL-1α) and transforming growth factor beta (TGF-β) are important candidates in this regard (13, 16). In the present study, we examined the pattern of distribution of immunopositive IL-1α and TGF-β in mid-luteal phase ovary of the rhesus monkey with and without a single dose administration of mifepristone on day 2 after ovulation.

METHODS

Paraffin embedded ovarian samples were obtained from the tissue archives of the laboratory. The experimental details have been described earlier (5, 6, 8). Briefly, female rhesus monkeys showing at least two consecutive ovulatory menstrual cycle of normal length were allocated in two groups. Animals in group 1 (n = 4) were treated with vehicle (1:4, benzoyl benzoate:olive oil, v/v, s.c.) on day 2 after ovulation. Animals in group 2 (n=6), on the other hand, were treated with a single dose of mifepristone (2 mg/kg body weight) on day 2 after ovulation. The day of ovulation was designated from serum profiles of estradiol and progesterone as described earlier (1, 2, 4). The ovulated ovaries were collected by laparotomy under ketamine (12 mg/kg body weight) anaesthesia on day 6 after ovulation from both groups of animals as described elsewhere (5, 6, 8). The tissue samples were fixed and paraffin embedded according to the method described previously (5, 6).

5 μm paraffin sections were collected on poly-l-lysine coated glass slides and employed for immunohistochemistry for IL-1α and TGF-β1 using polyclonal primary antibodies raised in goat and mouse respectively against recombinant human antigens (R & D Systems, Inc. Minneapolis, MN, USA) followed by visualization using Vectastain ABC Peroxidase kit from Vector Laboratories (Burlingame, CA, USA) according to the method described previously (5, 17). The areas of immunoprecipitation in follicular, stromal and luteal compartments were determined using a pre-calibrated computer

assisted video image analysis system attached to a Leica microscope (6). Parallel sections were stained with haematoxylin for morphological examination. Statistical analyses of morphometric data were performed using a modified *t*-test (18) and the results are expressed as means ± SD.

RESULTS AND DISCUSSION

There was no marked histological difference in stromal, follicular, luteal and vascular compartments between the two groups of tissue samples. As shown in Table I, the areas of precipitation for IL-1α and TGF-β1 in stromal, follicular and luteal compartments in ovaries from both the groups of animals did not reveal any statistically significant change. Despite occasional venular dilatation in group 2 ovarian samples, there was no detectable change in the level of immunopositivity for IL-1α and TGF-β1 in the vascular compartment of corpus luteum in tissue samples from both the groups. Thus, in the present study, we could not detect any marked change both in morphology and in the distribution pattern of IL-1α and TGF-β1 immunopositive stromal, follicular and luteal cells in monkey ovaries with or without early luteal phase mifepristone administration. This observation is congruent with the earlier reports that a single, low dose mifepristone administration during early luteal phase inhibits blastocyst implantation but it does not affect the serum concentrations of estradiol and progesterone during the luteal phase of the treatment cycle (1–4). Thus, we conclude that administration of mifepristone (2 mg/kg body weight) on day 2 after ovulation has no effect on ovarian morphology and in the expression of IL-1α and TGF-β1 in ovarian cells during the mid-luteal period of the treatment cycle in the rhesus monkey.

TABLE I : Morphometric analysis of immunohistochemical staining in follicular, stromal and luteal compartments.

Cytokine	*Follicular compartment*			*Stromal compartment*			*Luteal compartment*		
	Group (Mean ± S.D.)		*P value*	*Group (Mean ± S.D.)*		*P value*	*Group (Mean ± S.D.)*		*P value*
	1 (n = 4)	*2 (n = 6)*		*1 (n = 4)*	*2 (n = 6)*		*1 (n = 4)*	*2 (n = 6)*	
IL-1a	17.6±1.3	17.1±6.2	NS	19.4±2.7	24.3±6.6	NS	22.3±3.7	25.3±4.1	NS
TGF-b1	13.3±3.3	10.9±1.5	NS	15.4±4.9	13.0±2.2	NS	12.0±2.5	12.5±2.0	NS

NS, Not Significant

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