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Short Communication

Antifertility effect of tamoxifen as tested in the female bonnet monkey (*Macaca radiata*)

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Abstract. The administration of a potent antiestrogen, tamoxifen at a dose of 3 mg/kg body weight/day orally post-coitally to cycling mated bonnet monkeys (*Macaca radiata*) from days 18–30 of cycle resulted in inhibition of establishment of pregnancy in 9 out of 10 monkeys. Tamoxifen effect was not due to interference with luteal function. The effect was specific to tamoxifen as exogenously administered progesterone could not reverse it. In addition to suggesting a role for estrogen in maintenance of early pregnancy in the primate the present study could be a prelude to the development of an effective post-ovulatory approach for regulation of fertility in the human female.

Keywords. Antiestrogen; implantation; pregnancy; primate; post-coital contraception.

Introduction

The need for estrogen for maintenance of early pregnancy in the primate is presently questioned. The idea that estrogen probably is not required for implantation and immediate postimplantation survival of the blastocyst in primates (possibly including the human) stems from the observation that monkeys ovariectomized within 4–5 days of mating or luteectomy on day 6 post-coitum will continue with pregnancy if supplemented with progesterone alone (Meyer *et al.,* 1969; Bosu and Johansson, 1975). A variety of antiestrogens which are effective in inhibiting implantation in the rat are ineffective when administered post-coitally in the monkey (Morris *et al.,* 1967). However, most of the compounds used thus far—clomiphene, MER-25, centchroman and others are known to have, in addition to antagonistic, pronounced estrogenic activity. Further, as the time of administration of these drugs does not overrule the effect they may have on tubal motility, blastocyst survival etc., the need of estrogen for implantation *per se* and postimplantation survival of the blastocyst remains to be convincingly demonstrated (Prasad, 1985). There is a suggestion that in the primate the preovulatory estrogen surge in itself may be adequate to sensitize the uterus for implantation to occur (Prasad, 1985). In the context of present world-wide interest in

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Abbreviations used: MPA, Medroxyprogesterone acetate; mCG, monkey chorionic gonadotropin.

the use of antiprogestins to terminate early pregnancy and the lack of clarity regarding the need for estrogen in maintaining early pregnancy in the primate, it was decided to study the effect of administration of a relatively pure antiestrogen such as tamoxifen in mated bonnet monkeys, starting from mid luteal phase, on the establishment of pregnancy. Tamoxifen [(Z)-2-{4-(l,2-diphenylbut-l-enyl) phenoxy}-N, N-dimethyl-ethanamine] (gift of Dr B. J. A. Furr, I.C.I., UK) is an antiestrogenic compound having apparently no estrogenic activity as tested in the pig tailed monkey and the human (Furr *et al.,* 1979). It is presently primarily used as a therapeutic agent in controlling estrogen dependent breast cancer (Furr *et al.,* 1979).

Methods

A group of 32 cycling bonnet monkeys with a proven history of normal cycles and pregnancies were mated between days 9–14 of cycle with proven fertile males. Information regarding the husbandry of bonnet monkeys and their gonadal hormone profiles during the cycle and early pregnancy have been reported earlier (Moudgal *et al.,* 1978). In particular, the ovulatory nature of cycles during the treatment phase was adjudged by monitoring serum estrogen levels between days 7–10 and progesterone levels between days 16–18 of the cycle. Since these monkeys ovulate between days 11–12 of cycle, fertilization should have occurred between days 11–13 of cycle. The first group of 6 monkeys (control group) did not receive any treatment. The second group of 10 monkeys were treated with tamoxifen orally, 3 mg/kg body weight daily from day 18 of cycle for 13 days. The third group of 16 monkeys also received tamoxifen between days 18–30; however, 5 of them were supplemented with intramuscular injections of 10 mg of medroxyprogesterone acetate (MPA) while 11 were given 10 mg progesterone/monkey/day from day 18–30. By providing a gap of 4–7 days between fertilization and start of tamoxifen therapy we have ensured that its effect is confined to events beyond tubal development of the fertilized ovum. Blood samples collected by femoral venipuncture at regular intervals till the termination of cycle were assayed for serum estradiol, progesterone and monkey chorionic gonadotropin (mCG) by appropriate radioimmunoassays standardised in this laboratory (Rao *et al.,* 1984). Serum samples collected between days 28–35 of cycle, from those mated monkeys which did not show premature break of cycle, were used for mCG assay. The present study was undertaken during the months of August-December when the animals are most fertile.

Results and discussion

It is evident from table 1 that tamoxifen is effective in the inhibition of initiation/establishment of pregnancy in 22 out of 26 monkeys. All the 32 monkeys used in the present study were proven fertile females. Based on the colony performance for the last 4 years and from the data included for controls in the present study, 60–70 % of the monkeys conceive and go through pregnancy when exposure to the male is restricted to one cycle only.

Out of 6 monkeys in the control group (group I), 4 became pregnant and continued to term.

Table 1. Effect of tamoxifen administration during postcoital phase on conception in bonnet monkeys.

Group	Treatment[a]	Number pregnant/ Number mated	Peak estradiol (pg/ml) levels during follicular phase (mean ± SEM)	Progesterone (ng/ml) levels on day 18 of cycle (mean ± SEM)	pregnant (%)	protection[c] (%)
I	Nil	4/6	388 ± 74·9	5·00 ± 2.8	66	—
II	Tamoxifen	1/10	388 ± 58·4	6·28 ± 0·9	10	84
III	Tamoxifen + progestin[b]	3/16	720 ± 120·9[d]	3·43 ± 0·6	18	70

[a] All monkeys were mated with proven fertile males between days 9–14 of cycle. Excepting those in group I the rest of the monkeys were administered tamoxifen at 3 mg/kg body weight per monkey per day from days 18–30 of cycle.

[b] In the group III, 11 monkeys received progesterone at 2 mg/kg body weight per monkey per day and 5 monkeys received MPA at 2 mg/kg body weight per monkey per day from days 18–30 of cycle.

[c] Per cent protection was calculated assuming that even in the Experimentals % conception in the absence of treatment would have been 66 % like in controls.

[d] Five animals showed values ranging from 800–1200 pg/ml while 11 monkeys showed values ranging from 250–380 pg/ml.

In group II fed tamoxifen alone, out of 10 monkeys, one became pregnant and continued to term; 5 monkeys bled prematurely on day 24 itself and of the other 4 monkeys which were mCG positive on day 28 of cycle, 3 bled on day 29 and one on day 56. Even in the monkey that bled on day 56, pregnancy termination must have occurred much earlier as no fetal remnants could be recovered in the menstrual blood; this is to be expected if the monkey had aborted this late in pregnancy due to some other reason. The present observation that tamoxifen can block conception to the extent of 84% over the control is statistically significant at 1 % level according to the test of significance.

The specificity of the effect of tamoxifen is evident from the fact that neither progesterone nor MPA, a long acting progestin, could reverse the effect significantly. Out of 16 animals supplemented with progestin, 3 continued with pregnancy, indicating a drop in percentage protection from 84 % to 70 % over the control value. But the overall protection rate was 85 % considering both tamoxifen and tamoxifen + progestin treated groups.

The observation that 5 of the tamoxifen treated monkeys showed premature bleeding (earlier than day 28) is of significance and may imply an interference with the implantation process *per se*. In the tamoxifen + progestin supplemented group only one showed such an effect and this is perhaps due to the protective effect of the supplemented progestin against endometrial bleeding. In our experience administration of tamoxifen at 3 mg/kg dose to cycling but nonmated monkeys results in an extension of the cycle length from a normal 28 ± 2 to 48·6 ± 3·9 days. Following tamoxifen treatment, the luteal phase of the cycle, based on the progesterone levels was not curtailed but extended from a normal of 18 ±2·1 to 38·6 ± 4·0 days.

The present study thus provides direct evidence for the involvement of estrogen in maintenance of early pregnancy in the primate. Since tamoxifen exerts its effect even in the presence of normal serum estrogen titers, it is perhaps acting by blocking uterine

estrogen receptors and also interfering with the turnover of both estrogen and progesterone receptors. The observation that tamoxifen administered during the post-ovulatory phase is effective in blocking implantation indicates that the low level of estrogen present during the early luteal phase may have a role in the initiation of implantation. Since the half-life of tamoxifen is long (~ 7 days) (Furr *et alb.,* 1979), it may be possible to reduce the dosage and duration of administration without impairing its contraceptive efficacy. The per cent protection against conception can perhaps be further increased by initiating the treatment on day 16 of the cycle myself. Currently experiments are underway to explore this possibility. It may also be of interest to note that monkeys treated with tamoxifen are capable of achieving normal conception in subsequent cycles. This study may portend the use of an antiestrogen as an effective post-ovulatory, once-a-month contraceptive agent for regulation of fertility in the human female.

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