J. Biosci., Vol. 7, Number 2, March 1985, pp. 161–173. © Printed in India.

Is there a role for antiestrogens (estrogen antagonists) in the regulation of fertility?

M. R. N. PRASAD

Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India

MS received 15 January 1985

Abstract. Estrogens secreted by the ovary during the pre/periimplantation period and/or by the blastocyst and acting locally on the endometrium are involved in the initiation of implantation. Estrogens induce a cascade of metabolic changes in the uterus and blastocyst prior to and soon after the attachment and implantation of the blastocysts. Antiestrogens either administered intraluminally into the uterus prior to implantation or washing free blastocysts with antiestrogens prior to transfer to uteri of progesterone treated hamsters leads to failure of implantation. A number of antiestrogens which inhibit fertility in the rats do not interfere with implantation in the hamster and monkey when administered post-coitally. However, Zuclomiphene administered during days 5–11 of the menstrual cycle inhibits implantation in the rhesus monkey. Antiestrogens are being evaluated in other non-human primates to confirm the above results and to determine the time in the menstrual cycle susceptible to modification and inhibition of implantation. Tamoxifen administered from days 18–30 of the cycle to mated bonnet monkeys inhibited implantation despite maintenance of high levels of circulating progesterone. Neutralization of the vitamin carrier proteins (by active immunization against these proteins) interferes with established pregnancy in the rat and perhaps in the bonnet monkey. Whether antiestrogens can reduce the levels of vitamin carrier proteins to a level which is not adequate for maintenance of early pregnancy is not clear. Compounds which show antiestrogenic and antiprogestational properties may have advantages in inhibiting implantation or disruption of early pregnancy. Critical experiments need to be carried out in non-human primates to delineate the effectiveness of antiestrogens, with particular emphasis on time, dose, duration and route of administration in inhibition of implantation. Centchroman, an antiestrogen with antiprogestational properties, has been found to provide pregnancy protection with minimal side effects. However, several concerns relating to safety in toxicological studies in monkeys and a dose which would provide acceptable rate of contraceptive efficacy without major effects on the menstrual cycle need to be clarified before considering the potential of centchroman as a possible oral contraceptive administered either post-coitally or once a week. Inhibition of implantation by administration of tamoxifen opens up new possibilities of use of antiestrogens for fertility regulation.

Keywords. Antiestrogens; clomiphene; tamoxifen; bonnet monkey; implantation; fertility regulation.

Introduction

The importance of the development of drugs which can be taken once-a-month or in cases where there is a delay in the onset of menstrual cycles by 7–10 days is well recognized. The availability of such technology would limit the exposure to fertility regulating agents only to such occasions when there is the possibility of a pregnancy (Prasad, 1983). Interruption of early pregnancy leading to the onset of menstrual

Abbreviation used, hCG, Human chorionic gonadotropin.

bleeding or menstrual regulation could be achieved by one or more of the following methods: blocking progesterone receptors and interference with the preparation of the uterus for implantation or deprivation of the endometrium of progesterone required for the maintenance of an established pregnancy in the immediate post-implantation period [*e.g.* antiprogestins like RU-38486 presently being evaluated by the World Health Organization (WHO), 1983]; induction of luteolysis leading to decreased progesterone levels and interruption of early pregnancy [*e.g.* human chorionic gonadotrophin (hCG) derivatives acting as hCG antagonists and blocking luteal receptors: developed and evaluated by the WHO (1982)]; termination of early pregnancy by prostaglandins (WHO, 1982, 1983); interference with the action of estrogens on the fallopian tube/uterus in the post-coital period by treatment with antiestrogens resulting either in the expulsion of the product of fertilization or inhibition of implantation (*e.g.* Centchroman: Kamboj *et al.* 1977; Anordrin: Lei and Hu, 1981).

Attempts at the development of antiestrogens (estrogen antagonists) for interference with implantation have not been successful so far. This review highlights the role of estrogens in the initiation of implantation in mammals and possibilities of interfering with implantation or termination of early pregnancy by estrogen antagonists which will be referred to in this review by the commonly accepted terminology as “antiestrogens”.

Need for estrogen in implantation

The uterus is prepared for attachment and nidation of the blastocyst by sequential action of estradiol secreted by the follicles before ovulation and by progesterone secreted by the corpus luteum. Ovarian progesterone is always essential for the preparation of the endometrium for the attachment of the blastocyst. Studies on the hormonal requirements for implantation have shown that estrogens (estradiol) are generally involved in the initiation of implantation in a number of mammalian species (Psychoyos, 1973).

Rat and mouse

In the intact rat elevated estradiol levels on day 4 post-coitum permit the attachment and implantation of the blastocyst (Psychoyos, 1973; Yoshinaga, 1976). In the rat ovariectomised on day 3 post-coitum and treated with progesterone to delay implantation the levels of endogenous progesterone are high; implantation occurs only after exogenous administration of estrone or estradiol (Psychoyos, 1973). A local action of estradiol administered to a small segment of the uterus permits implantation of the blastocysts (during delayed implantation) at the site of injection of estrogen (Yoshinaga, 1976). The role of ovarian estrogens in the initiation of implantation is similar in the mouse (McCormack and Greenwald, 1974).

Hamster and rabbit

Implantation occurs in the ovariectomized hamster only by the administration of progesterone (Prasad *et al.,* 1960; Harper *et al.,* 1969). Progesterone is capable of

inducing implantation in the post-coitally ovariectomized hamster by exerting its effects on the uterus primed by the action of estradiol secreted during the pre/periovulatory period (Prasad and Rath, 1974). There is an increase in plasma estradiol by day 3 post-coitum but its role in implantation is not clear (Joshi and Labhshetwar, 1972). While exogenous administration of estrogen is not essential for implantation in the ovariectomized-progesterone treated hamster, a combination of estrone or estradiol with progesterone increases the number of implantation sites (Orsini and Psychoyos, 1965; Kwun and Emmens, 1974; Evans and Kennedy, 1980).

In the rabbit, as in the case of hamster, implantation occurs in the post-coitally ovariectomized animals treated with progesterone only; however, the number of embryos implanting increase with the addition of estradiol (Kwun and Emmens, 1974).

Primates

A limited amount of information is available on endocrine changes associated with implantation in primates. The requirement of ovarian progesterone for implantation was demonstrated by the failure of implantation in pregnant monkeys after ovariectomy (Meyer *et al.,* 1969) or lutectomy on day 6 post-coitum (Bosu and Johnsson, 1975). Meyer *et al.* (1969) successfully maintained pregnancy in 7 of 10 rhesus monkeys ovariectomized on day 6 of gestation and treated with 5 or 50mg/kg/day of progesterone from the day of ovariectomy until parturition. While these studies demonstrate the requirement of progesterone for implantation in the rhesus monkey, the requirement of ovarian estrogen for implantation is less clear. The fact that pregnancy occurs in the ovariectomized-progesterone treated monkey indicates that implantation occurs in the absence of estrogens, in a manner similar to that in the hamster. It is likely that priming of the uterus by estrogens secreted during the periovulatory period is adequate to permit implantation in the progesterone treated monkeys. The issues that remain to be clarified relate to the role of estrogen in the preimplantation period (days 1–5 post-coitum). Patterns of circulating estradiol 17 b (E2) or total estrogen (E) during the periimplantation period (in the early luteal phase) have been described in five primate species. A gradual increase in the levels of E2 occurs in the preimplantation period in the fertile and infertile cycles of the rhesus monkey, bonnet monkey (Anandkumar *et al.,* 1980; Murthy *et al.,* 1980; Rao and Moudgal, 1984; Hendrickx and Enders, 1980); chimpanzee and stump tailed macaque (Hearn, J. P. personal communication), and marmoset (Hearn, 1978), with a profile similar to that seen in women (Thomas *et al.,* 1973). There is no proof for the involvement of estradiol in implantation in any non-human primate, neither is there any evidence to the contrary; tonic levels may be quite sufficient for implantation to occur as in the case of the hamster.

Role of estrogen derived from the blastocyst in implantation

Preimplantation blastocysts of several mammals synthesize estrogens *in vitro.* In the rat, mouse and hamster, steroidogenic capacity of the blastocyst was demonstrated by

the presence of 3*β*-17 *β*-OH steroid dehydrogenases before implantation (Dickman and Sen Gupta, 1974; Wu and Liu, 1982). Hamster blastocysts synthesize estradiol from steroid precursors (Sholl *et al.,* 1983). Dickman *et al.* (1977) proposed that estrogen secreted by the blastocysts may be necessary for embryonic differentiation and initiation of implantation and could play a role in maternal recognition of the blastocyst during the attachment phase of implantation (Dickman *et al.,* 1977). In species like the hamster which has been shown to be dependent on progesterone alone for implantation (Prasad *et al.,* 1960), blastocyst estrogen may also be involved in the initiation of implantation; injection of an estrogen antagonist (CI-628) at a dose of 5 μg into the uterine horns of intact ovariectomized females receiving progesterone post-coitally prevents implantation as does incubation of the blastocysts with the antiestrogen before their transfer to the uterus of ovariectomized hamsters treated with progesterone (Sen Gupta *et al.,* 1983). These results show that while preovulatory ovarian estradiol primes the uterus, estradiol produced by the blastocyst may also be involved in initiating implantation; this is further substantiated by the observation that progesterone alone permits implantation in hamsters ovariectomized and adrenalecto-mised post-coitum to eliminate any extraovarian source of estrogen (Evans and Kennedy, 1980). It is also likely that the endometrium may regulate the local concentration of estrogens by 17*β*-hydroxysteroid dehydrogenase permitting estradiol 17$\beta \rightarrow$ estrone conversion and by a sulphotransferase-sulphatase system which allows estrogen sulphate$\rightleftharpoons$estrogen interconversion (Kreitman and Bayard, 1981; Woman: Gurpide and Marks, 1980; Satyaswaroop *et al.,* 1982; Rhesus: Kreitman *et al.,* 1980; Utaaker and Stoa, 1980; hamster: Legault *et al.,* 1980; Tseng and Lin, 1981; Clark *et al.,* 1982). However, there are no data to relate the endometrial estrogen synthesis with events leading to the initiation and maintenance of implantation.

Role of estrogen in implantation: Mechanism of action

Estradiol was shown to be involved in one or more of the following events in implantation: embryonic development (Roy *et al.,* 1982); spacing of blastocysts in the uterine horns (Pope *et al.,* 1982); increase of blood flow and capillary permeability (Psychoyos, 1973); changes in uterine secretions (Aitken, 1977); regulation of prosta-glandin synthesis at the time of implantation (Psychoyos, 1984); induce nucleic acid and protein synthesis in the uterus and blastocyst (Mohla *et al.,* 1970; Mohla and Prasad, 1971); stimulate mitotic divisions in the epithelial cells in the ovariectomized-hormone starved uterus of the rat and hamster while estradiol initiates mitosis in the uterine stroma and gland cells after treatment with progesterone (Martin and Finn, 1971; Tachi *et al.,* 1972; Prasad and Rath, 1974); and induce increase in adenyl cyclase and cyclic AMP (Szego and Davis, 1967).

Most of the studies on the mechanism of action of estradiol were carried out in ovariectomized rat and mice treated with estrogen alone; this is not comparable to the condition in the intact animal at the time of implantation where estradiol modulates the activity of the progesterone primed uterus.

Antiestrogens

Chemistry

A number of steroidal and non-steroidal antiestrogens were studied for their antifertility action in laboratory rodents and primates (Giannina *et al.,* 1971; Prasad and Sankaran, 1975; Harper, 1982 for detailed review). Anti-estrogens are diverse in their chemical structure although all the compounds with the exception of U-11634 are basically estrogenic. They show marked variations in the degree of estrogenicity and antiestrogenicity.

Mechanism of action

The current concepts on the mode of action of antiestrogens may be summarized as follows. These generalizations are based on review of the literature cited by Prasad and Sankaran (1975) and some selected recent studies (Liberman *et al.,* 1983; Sutherland *et al.,* 1980; Sudo *et al.,* 1980; Eckert and Katzellenbogen, 1981; Katzellenbogen *et al.,* 1984; Murthy and Sutherland, 1981; Markaverich and Clark, 1981; Furr and Jordan 1984). The list of references on mechanism of action of antiestrogens is too long to be cited in full.

(a) Antiestrogens inhibit the action of estrogens either in their original molecular form or through their metabolites by influencing: (i) the metabolism, transport and eventual entry of estrogens to the target cells; (ii) the uptake of estrogens by the cytoplasmic hormone receptors; (iii) the transport of the cytoplasmic receptor-hormone complex to the nucleus as the nuclear receptor; (iv) the interaction between the nuclear receptor and nuclear acceptor; and (v) the transcriptional and translational steps in estrogen action.

(b) Estrogens and antiestrogens are mutully competitive for binding to saturable estrogen receptor binding sites in the target tissues. However, high affinity binding sites may have a role in regulating the effects of non-steroidal antiestrogens.

(c) Antiestrogens differ from one another in their dynamics of interaction with the estrogen receptors and in their ability to stimulate increase in cellular progesterone receptor. Apparently complex relationships exist between the number of receptors and the duration of the presence of the hormone receptor complexes in the nucleus.

(d) Cell growth and progesterone receptor induction may be modulated by antiestrogens.

(e) The antiestrogenicity of a compound is proportional to the affinity of binding to the cytoplasmic estrogen/antiestrogen receptor and also its intrinsic ability to transfer the receptor complex into the nucleus.

(f) Triphenylethylene compounds bind to a saturable binding site distinct from the estrogen receptor. The significance and subcellular role of this component needs to be defined before speculating on its function.

(g) Heterogeneity of nuclear estrogen receptors are implicated in the action of estrogens. Low abundance of nuclear Type II binding sites is a feature of antiestrogens but this hypothesis needs further verification.

(h) The degree of antiestrogenicity of a compound is generally proportional to its inherent estrogenicity.
(i) Some antiestrogens retain their antiestrogenicity for prolonged periods of time following a single administration. This reflects the long biological half life of the compound, high level of binding to plasma proteins and enterohepatic circulation.

Antifertility action

Antifertility action of a number of antiestrogens evaluated in several species is shown in table 1.

Table 1. Post-coital antifertility efficacy of nonsteroidal antiestrogens.

Antiestrogens	Species in which evaluated	Mode of action
Clomiphene	Rat (+), Rabbit (+), Rhesus (−), Human (−).	Interference with ovum development, tubal transport of ova and implantation due to the estrogenicity/antiestrogenicity of the compound.
MER-25	Rat (+), Rabbit (+).	-do-
MRL-37	Rat (+), Mouse (+), Rhesus (−).	-do-
U-11000A	Rat (+), Rabbit (+), Guinea pig (+), Hamster (−).	-do-
U-11555A	Rat (+), Rabbit (+), Hamster (−).	-do-
ORF-3858	Rodents (+), Rhesus (−), Human (−).	Inhibits implantation.
Tamoxifen	Rat (+), Mouse (+).	Interference with implantation and tubal transport of ova.
H-1067	Rodents (+), Rhesus (−).	-do-
66/179 and 67/20	Rodents (+), Rabbit (−), Dog (+), Rhesus (−), Human (?).	Inhibits implantation due to estrogenic/antiestrogenic antiprogestational action.
F6066	Rodents (+), Rhesus (+).	Interferes with fertility due to antigonadotrophic/antiimplantation action.
Diethyl Stilbesterol	Rodents (+), Rhesus (+), Human (+).	Highly estrogenic. Accelerates ovum transport.
DMS	Rat (+), Mice (+), Rabbit (+).	Interfere with tubal transport of ova/estrogenic/antiestrogenic
Anordrin	Rodents (+), Human (+).	Interfere with implantation due to estrogenic/antiestrogenicity

(+) Effective; (–) ineffective. For details of description of compounds and structures See Prasad and Sankaran (1975); anordrin (Lei and Hu, 1981).

The antiimplantation action of antiestrogens administered post-coitally may be due to one or more of the following mechanisms (Prasad and Sankaran, 1975): (i) the compounds may increase tubal motility due to their estrogenic activity resulting in the expulsion of the blastocysts (MER-25; DMS; Clomiphene; U-11100A U-11555A, DBF, Centchroman); (ii) the compounds may be cytotoxic and affect the viability of the

blastocysts (MER-25); (iii) they may inhibit the uptake of estrogens and subsequently the action of estrogen on the target organs (MER-25; Clomiphene; CN-55945-27; Centchroman); (iv) by their antihistaminic activity, they may inhibit decidual cell response by blocking estrogen-dependent enzyme activities (MER-25) or estrogen dependent protein synthesis prerequisite to the preparation of the uterus for implantation (U-11634); (v) the compounds may cause luteolysis through the stimulation of pituitary LH; the lowered progesterone level then intereferes with implanttation (CN-55945-27).

Although the mode of action of antiestrogens in interfering with implanation in the rat and mouse is clear, their action in other species like the hamster and non-human primates is as yet unclear. Antiestrogens which are effective in inhibiting in the rat are ineffective when administered post-coitally in the hamster (Duncan *et al.,* 1966) and rhesus monkey (Morris *et al.,* 1967; Segal *et al.,* 1972). It is to be noted that in both these species progesterone alone permits implantation which is possibly facilitated by the local action of estrogen produced by the blastocyst in the hamster. The failure to inhibit implantation in these species may be due either to the inappropriate timing and dose of the antiestrogens administered or their inability to reach the uterine milieu in adequate concentrations to interfere with the action of estrogen produced by the blastocyst and acting on the uterus locally at the site of implantation.

Critical phases in reproduction vulnerable to interference with antiestrogens

The critical and crucial events in reproduction in the female nonhuman primate that can lead to inhibition of implantation or disruption of an established implantation by administration of antiestrogens are: (a) Inhibition of luteal function, (b) Inhibition of estrogen mediated changes in the endometrium, the estrogen being derived either from the ovary, or by synthetic activity of the blastocyst and /or endometrium. There is no evidence to show that the monkey or human blastocyst produces estrogen, (c) Estrogen antagonism on progesterone production in early pregnancy. (d) Interfere with estrogen-induced synthesis of vitamin carrier proteins involved in the transplacental transfer of vitamins essential for the survival and growth of the foetus.

Inhibition of luteal function

In the rhesus monkey, the elevation in progesterone levels of the fertile cycle, termed as the ‘rescue’ of the corpus luteum is due to a luteotrophic stimulus originating in the implanting blastocyst. The chorionic gonadotrophin secreted by the blastocyst can be detected in the peripheral blood or urine earliest on day 9–10 of pregnancy in the rhesus or bonnet monkey (Atkinson *et al.,* 1975; Hendrickx and Enders, 1980; Murthy *er al.,* 1980). Treatment with antiestrogens which leads to interference with mechanisms rescuing the corpus luteum resulting in a decline in progesterone levels required for the initiation and maintenance of implantation would be an attractive approach to regulate fertility. This needs to be evaluated in different non-human primates.

Inhibition of implantation

Hendrickx and Sankaran, (personal communication) have reported inhibition of implantation in 4 out of 5 rhesus monkeys by administration of zuclomiphene (2 mg/kg/day) for seven days on days 5–11 of the menstrual cycle; the antiestrogen did not have any effect on the normal levels of hormones in the peripheral blood and ovulation at this dose. Through the mechanism/s of action of zuclomiphene in inhibiting implanation are not clear, it is possible that the compound, due to its antiestrogenic properties, might have inhibited the action of periovulatory estrogens resulting in subnormal progestational action and impairment of implantation of the blastocyst. These studies demonstrate, though not conclusively, the role of peri-ovulatory estrogens and offer an experimental model for studies on interactions of hormones during the periimplantation period of early pregnancy in non-human primates. Similar studies are being carried out in the bonnet monkey (Moudgal, N. R., personal communication). It would be of interest to determine if antiestrogens administered on specific days during the preovulatory period of the non-human primates would induce luteal insufficiency and other luteal defects (short luetal phase) in a manner similar to that caused by selective inhibition of follicle stimulating hormone during this period.

Moudgal (personal communication) has shown that administration of 3 mg/kg/day of Tamoxifen on days 18–30 to bonnet monkeys (following successful mating in an ovulatory cycle) resulted in interference with implantation and establishment of pregnancy in 9 of the 10 treated animals; menstrual cycles occurred in these monkeys on days 23–29 despite maintenance of high levels of progesterone comparable to those in pregnant monkeys. Concurrent administration of Depot Medroxy progesterone acetate did not reverse effects of tamoxifen. The mechanism of action of tamoxifen in interfering with implantation is not clear. The action of tamoxifen could be sought for in its possible direct effects on the uterus in inhibiting the action of progresterone on the uterus either by modifying the endometrial progesterone receptors or expression of metabolic responses to progesterone and preparation of the uterus for implantation. These results which demonstrate the antiimplantation effect of tamoxifen are of considerable interest and open up new possibilities of use of antiestrogens for fertility regulation. Clinical trials need to be carried out to assess the antiimplantation effect of tamoxifen by administering the drug at different time periods in the mestrual cycle. Since tamoxifen is used extensively in the treatment of breast cancer in women, there should be no difficulty in the initiation of clinical trials.

Effect of antiestrogen on progesterone production during pregnancy

Treatment of baboons with MER-25 in the last trimester of pregnancy results in a decline in plasma progesterone without any effects on the clearance of progesterone (Albrecht, 1980). Since there was no effect on progesterone levels during the luteal phase of the non-pregnant baboons, the effect on the pregnant animals was presumed to indicate a role for progesterone in placental progesterone production and its inhibition by the antiestrogen MER-25. Oral administration of MER-25 to pregnant baboons on

days 35–55 after conception results in a decline in the peripheral plasma levels of progesterone within a few days and persists for at least 20 days without any effects on plasma estradiol levels (Castracane *et al.,* 1983).

Administration of 15 mg/kg of MER-25 or enclomiphene citrate throughout the luteal phase of the menstrual cycle did not have any effects on the maintenance of the corpus luteum, length of the cycle, and levels of serum progesterone, 17 hydroxy progesterone or estradiol (Albrecht *et al.,* 1984). The decline in progesterone production in animals treated with MER-25 may reflect reduction in the function of the placenta but not of the corpus luteum. Even though the progesterone levels were decreased in these baboons treated with MER-25, pregnancy was maintained which may be due to the circulating levels of progesterone being still adequate to maintain pregnancy. Similar studies need to be carried out in other non-human primates. The marmosets which have very high levels of progesterone during pregnancy may not be suitable animal models for such studies.

Interference with estrogen-induced vitamin carrier proteins in pregnancy

Adiga and Murthy (1983) have shown that specific vitamin carrier proteins for riboflavin and thiamin in the rat are estrogen-induced gene products of hepatic origin. These proteins are essential for the transplacental transport of the vitamins essential for the maintenance of the viability of the foetus. Interference with the vitamin carrier proteins by active or passive immunization against these proteins leads to the termination of pregnancies without any deleterious effects on the vitamin status of the pregnant animal or its subsequent fertility. Sheshagiri *et al.* (1984) have also shown that maximum levels of the vitamin carrier proteins in circulation occur in the bonnet monkey on days 16–18 of the menstrual cycle, *i.e.* 3–4 days after the preovulatory surge of estrogen; similar proteins have been demonstrated in the bonnet monkeys and in human pregnancy serum in the cord blood. Immunological neutralization of the vitamin carrier proteins in the pregnant bonnet monkey led to the termination of pregnancy. Recent studies presented at the Indo-US Workshop on Blastocyst Research, Seshagiri *et al.* (1984) showed that in the female bonnet monkeys of proven fertility, active immunization with the chicken riboflavin and thiamin-carrier protein did not interfere with their general health, cyclicity, hormonal profile or vitamin status; when mated during the fertile cycles such actively immunized animals with high titres of antibody against the vitamin carrier proteins rejected their foetuses. These results are of interest and need further amplification in specifying (a) whether the vitamin carrier proteins are critical during the pre/periimplanatation period and (b) the role of antiestrogens in inhibition of induction of the vitamin carrier proteins by endogenous estrogens and consequent effects on pregnancy. These studies need to be carried out with other antiestrogens like Centchroman. Problems that are inherent in the design of a study to specify the role of antiestrogens in inhibiting implantation are: the choice of antiestrognes, dose, route of administration and duration of treatment; since the vitamin carrier proteins are essential for maintenance of pregnancy, termination of pregnancy resulting from antiestrogen treatment should be complete and 100% effective. Acceptance of the approach as a method of fertility regulation would depend

on its safety and efficacy being better than the currently available methods for the termination of early pregnancy.

Compounds with antiestrogenic and antiprogestational action

Recent studies have highlighted interest in an antiprogestational compound, RU-38486 which is being evaluated clinically for termination of very early pregnancy (WHO, 1983); the compound apparently acts by blocking endometrial progesterone receptors to disrupt early pregnancy; preliminary results seem to indicate that the drug, even in high doses, may not result in complete evacuation of the product of conception from the uterus. In the light of recent studies by Adiga and Murthy (1983) on the role of estrogen-induced vitamin carrier proteins in the maintenance of pregnancy, it is of interest to consider the possibility of evaluating compounds which show both antiestrogenic and antiprogestational activities.

Centchroman (trans-2,2-dimethyl-3-phenyl-1-4-(*p*-b-pyrrolidoneethoxyphenyl)-7-methoxy chroman hydrochloride) (see Prasad, 1983 for structure), developed by the Central Drug Research Institute, Lucknow, India, has weak estrogenic and anti-estrogenic activity; its antifertility activity in rats and monkeys may be due to its multiple attributes such as weak estrogenic, antiestrogenic and antiprogestational activities (Kamboj *et al.,* 1977). The activity profile of Centchroman is as follows: antiimplantation activity in rats, dogs and monkeys; weak estrogen and potent antiestrogen; interferes with progesterone action (in induction of deciduomata in rats, inhibition of progesterone induced delayed implantation in rats, Clauberg Assay, inhibition of exogenously administered progesterone); no effects on adrenal, thyroid and pituitary function; pharmacologically inert at contraceptive dose; no teratogenicity (mice-rabbit) or mutagenicity; safe in chronic toxicity evaluation in rat and rhesus monkey; good therapeutic ratio (> 1800); safe in Phase I clinical studies; dose range evaluation in weekly regimen completed; currently under Phase II clinical trial as a 30 mg/weekly oral pill; effective against 40 % of stage four breast cancer patients.

Phase II clinical trials have been carried out in post-coital (60 mg) and once-a-week treatment schedules at doses of 120, 60 and 45 mg. The results reported indicate that Centchroman provides good protection against pregnancy at all doses studied (Nityanand, personal communication); ovarian and uterine enlargement was observed in all treatment regimes, with recovery to normal size within 30 days of the withdrawl of drug treatment. Delay in menstruation of varying duration has also been reported. Since these high doses led to ovarian/uterine enlargement and irregular menstrual cycles, further clinical evaluation was carried out with 30 mg/week; data involving 368 subjects indicate acceptable pregnancy protection with minimal side effects and delay in the menstrual cycles of 10% of the treated subjects (Nityanand, personal communication). High priority in such studies is to determine a dose which will provide a high degree of protection against pregnancy without effects on the menstrual cycle and ovarian/uterine functions. Questions have also been raised by the drug regulatory agencies on the acceptability of some of the toxicology data. The Central Drug Research Institute has apparently carried out once again a one-year toxicity evaluation of Centchroman and has initiated life term studies. Should these new toxicology and

clinical data be considered satisfactory by the toxicology review panel of the Indian Council of Medical Research and the Drug Regulatory Authority in India, a case could be made for further clinical evaluation of Centchroman which has the advantage of possessing antiestrogenic and antiprogestational properties. A number of years have elapsed since claims were made that Centchroman, developed solely by an Indian laboratory would be available as a post-coital or weekly pill for regulation of fertility. It is time that concerted efforts are made to evaluate all the available clinical and toxicological data to determine the potential of Centchroman as a fertility regulating agent for introduction in the National Family Welfare Programme in India.

Anordrin

Anordrin has been used in the Peoples Republic of China as a post-coital pill at a dose of 7·5 mg (Lei and Hu, 1981). The drug apparently interferes with implantation by inhibiting luteal function and endometrial development. It is not clear from the Chinese studies if anordrin was ever used strictly as a post-coital pill. The antifertility action of Anordrin has been attributed to its antiestrogenic activity (Mehta *et al.,* 1981), or to its estrogenic acceleration of tubal transport or degeneration of eggs during tubal transport in the hamster (Gu *et al.,* 1975; Gu and Chang, 1979).

A number of attempts have been made to synthesize related compounds to dissociate estrogenic activity from antifertility effects. Crabbe *et al.* (1979) reported the synthesis and biological evaluation of 2α anordrin and its corresponding diproprionate, dinordrin I (2*α*, 17*α*-diethynyl-A-nor-5*α*-estrane-2*β*-17-*β*-diol) and the 2*β* epimer, dinordrin II (see Prasad, 1983 for structures of the two compounds). Dinordrin I was 20 times more potent than anordrin or dinordrin II. Both anordrin and dinordrin I showed antifertility effect in rats and were luteolytic in baboons; the antifertility activity generally paralleled uterotrophic activity (estrogenic activity). Chinese scientists have synthesized derivatives of anordrin and dinordrin and its epimers by total synthesis in an attempt to separate the estrogenic from antifertility activities (WHO, 1983).

A number of steroidal and non-steroidal compounds that showed promise as post-coital antifertility agents have been evaluated in animals in an attempt to identify an effective drug having little estrogenic activity but with enhanced antiestrogenic activity, but none has reached phase III or IV clinical trials (Giannina *et al.,* 1971; Prasad and Sankaran, 1975; Harper, 1982). Centchroman has been evaluated as a once-a-week pill and found to be effective. Inhibition of implantation by tamoxifen in bonnet monkey opens up new possibilities of use of another antiestrogen for fertility regulation. Clinical trials need to be carried out to assess the efficacy of tamoxifen in interfering with implantation in women.

References

Adiga, P. R. and Murthy, C. V. R. (1983) in *Molecular Biology of Egg Maturation;* Ciba Foundation Symposium. Number 98 (London: Pitman), p. 111.

Aitken, J. (1977) *J. Reprod. Fertil.,* **50,** 29.

Albrecht, E. D., Haskins, A. L., Hodgen, G. and Pepe, G. J. (1984) *Biol. Reprod.* (In Press).

Albrecht, E. D. (1980) *Am. J. Obstet. Gynecol.,* **136,** 569.
Anandkumar, T. C., David, J. F. X., Sharma, D. N., Puri, C. P., Puri, V., Dubey, A. R., Sehgal, A., Shankaranarayanan, A. and Pruthi, J. S. (1980) in *Non-Human Primate models for Study of Human Reproduction* (ed. T. C. Anandkumar), (Basel: Karger), p. 36.
Atkinson, L. E., Hotchkiss, J., Fritz, G. R., Surve, A. H., Neill, J. D. and Knobil, E. (1975) *Biol. Reprod.,* **12,** 335.
Bosu, W. W. K. and Johansson, E. D. B. (1975) *Acta Endocrinol.,* **79,** 598.
Castracane, V. D., Kuehl, T. J. and Goldzieher, J. W. (1983) *Am. J. Obstet. Gynecol.,* **145,** 725.
Clarke, C. L., Adams, J. B. and Wren, B. G. (1982) *J. Clin. Endocrinol. Metab.,* **55,** 75.
Crabbe, P., Fillion, H., Letourneaux, Y., Diczfalusy, E., Aedo, A. R., Goldzieher, J. W., Shaikh, A. A. and Castracane, V. V. (1979) *Steroids,* **33,** 85.
Dickman, Z. and Sen Gupta, J. (1974) *Dev. Biol.,* **40,** 196.
Dickman, Z., Sen Gupta, J. and Dey, S. K. (1977) *Science,* **195,** 687.
Duncan, G. W., Johnston, R. L. and Lyster, S. C. (1966) *J. Reprod. Fert.,* **11,** 85.
Eckert, R. L. and Katzellenbogen, B. S. (1981) *J. Biol. Chem.,* **257,** 8840.
Evans, C. A. and Kennedy, T. G. (1980) *Steroids,* **36,** 41.
Furr, B. J. A. and Jordan, V. C. (1984) *Pharmacol. Ther.,* **25,** 127.
Gianina, T., Butler, M., Popick, F. and Steinetz, B. (1971) *Contraception,* **3,** 347.
Gu, C. P., Zhu, M. K., Jiang, X. J., Zhan, S. Y., Pang, B. W. and Zhou, G. (1975) *Sci. Sin.,* **18,** 262.
Gu, Z. and Chang, M. C. (1979) *Contraception,* **20,** 549.
Gurpide, E. and Marks, C. (1980) *J. Clin. Endocrinol. Metab.,* **52,** 465.
Harper, M. J. K. (1982) *Fertil. Control Med. Res. Rev.,* **2,** 403.
Harper, M. J. K., Dowd, D. and Elliot, A. S. W. (1969) *Biol. Reprod.,* **1,** 253.
Hearn, J. P. (1978) in *Biology and Conservation of the Marmoset, Callithrix jacchus* (ed. Kleinmann) (Washington: Smithsonian Institution), p. 163.
Hendrickx, A. G. and Enders, A. C. (1980) in *Non-Human Primate Models for Study of Human reproduction* (ed. T. C. Anandkumar) (Basel: Karger), p. 109.
Joshi, S. H. and Labhshetwar, A. P. (1972) *J. Reprod. fertil.,* **31,** 297.
Kamboj, V. P., Setty, B. S., Harischandra, Roy, S. K. and (late) Kar, A. B. (1977) *Indian J. Exp. Biol.,* **15,**1144.
Katzellenbogen, B. S., Miller, M. A., Mullick, A. and Shen, Y. Y. (1984) *J. Steroid Biochem.,* **20,** 1629.
Kreitman, O. and Bayard, F. (1981) in *Cellular and Molecular Aspects of Implantation* (eds S. Glasser and D. W. Bullock) (New York: Plenum Press), p. 417.
Kreitman, O., Bayard, F. and Hodgen, G. (1980) *Steroids,* **36,** 365.
Kwun, J. K. and Emmens, C. W. (1974) *Aust. J. Biol. Sci.* **27,** 275.
Legault, Y., Bleau, G., Chapdelaine, A. and Roberts, K. D. (1980) *Biol. Reprod.,* **23,** 720.
Lei, H. P. and Hu, Z. (1981) in *Recent Advances in Fertility Regulation,* (eds C. Chang, D. Griffin and L. Woolman) (Geneva: Atar), p. 70.
Liberman, M. E., Gorski, J. and Jordan, U. C. (1983) *Nature* (*London*), **258,** 4741.
Markaverich, B. M. and Clark, J. H. (1981) *Endocrinology,* **105,** 1458.
Martin, L. and Finn, C. (1971) in *Basic Actions of Sex Steroids on Target Organs* (eds P. O. Hubinondt, F. Leroy and P. Galand) (Basel: Karger), p. 172
McCormack, J. T. and Greenwald, G. S. (1974) *J. Reprod. Fertil.,* **41,** 297.
Mehtha, R. R., Jenco, J. M. and Chatterton, R. T. Jr. (1981) *Steroids,* **38,** 679.
Meyer, R. K., Wolf, R. C. and Arslan, M. (1969) in *Recent Advances in Primatology,* (ed. Hoffer) (Basel: Karger) Vol. 2, p. 30.
Mohla, S., Prasad, M. R. N. and Dass, C. M. S. (1970) *Endocrinology,* **87,** 383.
Mohla, S. and Prasad, M. R. N. (1971) *J. Endocrinol.,* **47,** 87.
Morris, J. M., Van Wagenen, G., McCann, T. and Jacob, D. (1967) *Fertil. Steril.,* **18,** 18.
Murphy, C. L. and Sutherland, R. L. (1981) *J. Endocrinol.,* **9,** 155.
Murthy, G. S. R., Ramasarma, K., Mukku, V. R., Srinath, B. R. and Moudgal, N. R. (1980) in *Non-Human Primate Models for Study of Human Reproduction,* (ed. T. C. Anandkumar) (Basel: Karger) p. 50.
Orsini, M. W. and Psychoyos, A. (1965) *J. Reprod. Fertil.,* **10,** 300.
Pope, W, F., Maurer, R. R. and Stormshak, F. (1982) *Biol. Reprod.,* **27,** 575.
Prasad, M. R. N. (1983) in *International Symposium on Research in the Regulation of Human Fertility* (eds E. Diczfalusy and A. Diczfalusy) (Copenhagen: Scriptor) p. 678.

Prasad, M. R. N., Orsini, M. W. and Meyer, R. K. (1960) *Proc. Soc. Exp. Biol. Med.,* **104,** 48.
Prasad, M. R. N. and Sankaran, M. S. (1975) *J. Sci. Indust. Res.,* **34,** 336.
Prasad, M. R. N. and Rath, N. C. (1974) *Proc. Indian Nat. Sci. Acad.,* **B39,** 359.
Psychoyos, A. (1973) *Vit. Horm.,* **31,** 201.
Psychoyos, A. (1984) *J. Biosci.,* **6, (Suppl. 2),** 7.
Rao A. J. and Moudgal, N. R. (1984) in *Proc. Indo-US Symposium on Ovum Implantation,* Indian Councial of Medical Research, p. 54.
Roy, S. K., Sen Gupta, J., Paria, B. C. and Manchanda, S. K. (1982) *Acta Endocrinol.,* **99,** 129.
Sathyaswaroop, P. G., Wartell, D. J. and Mortel, R. (1982) *Endocrinology,* **111,** 743.
Segal, S. J., Atkinson, L., Brinson, A., Hertz, R., Hood, W., Kar, A. B., Southarn, A. L. and Sundaram, K. (1972) *Acta Endocrinol.,* **79,** 153.
Seshagiri, P. B., Sandhya, V. and Adiga, P. R. (1984) in *Proc. Indo-US Symposium on Ovum Implantation,* (Indian Council of Medical Research), p. 23.
Sen Gupta, J., Paria, B. C. and Manchanda, S. K. (1983) *J. Reprod. Fertil.* **62,** 437.
Sholl, S. A., Orsini, M. W. and Hitchins, D. J. (1983) *J. Steroid Biochem.,* **19,** 1153.
Sudo, K., Monsma, F. J. Jr. and Katzellenbogen, B. S. (1983) *Endocrinology,* **112,** 425.
Sutherland, R. L., Murthy, L. C., San-Foo, M., Green, M. D., Whyboune, A. M., and Krizowski, Z. S. (1980) *Nature* (*London*), **288,** 5788.
Szego, C. M. and Davis, J. S. (1967) *Proc. Natl. Acad. Sci. USA,* **58,** 1711.
Tachi, C., Tachi, S. and Lindner, H. R. (1972) *J. Reprod. Fertil.,* **31,** 59.
Thomas, K., DeHertogh, R., Puzzaro, M., Van Exeter, and Ferin, J. (1973) *Int. J. Fertil.,* **18,** 65.
Tseng, L. and Lin, M. C. (1981) *J. Clin. Endocrinol. Metab.,* **53,** 418.
Utaaker, E. and Stoa, K. F. (1980) *Horm. Res.,* **13,** 180.
World Health Organization (1982, 1983) *Special Programme of Research in Human Reproduction,* Annual Reports.
Wu, J. T. and Lin, G. M. (1982) *J. Exp. Zool.,* **220,** 121.
Yoshinaga, K. (1976) in *Implantation of the Ovum,* (eds K. Yoshinaga, R. K. Meyer and R. O. Greep) (Cambridge: Harvard University Press), p. 3.