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Post-coital agents and menses inducing drugs

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Abstract. The importance of developing of drugs which could be taken post-coitally or used once-a-month in the case of a delay in the onset of the menses is well recognized. The availability of such technology would limit exposure to fertility regulating agents to such occasions where there is coital exposure or possibility of pregnancy.

Methods of post-coital contraception used so far include IUD's inserted post-coitally, estrogens, and combinations of estrogens and gestagens. These are reserved primarily for emergency situations to protect women from unwanted pregnancy resulting from rape or unprotected coitus. Levonorgestrel has shown satisfactory results in terms of contraceptive efficacy and is being further evaluated clinically. A number of problems inherent in the development of post-coital contraception are discussed.

Menstrual regulation could be achieved by a number of approaches: (a) block progesterone receptors and interfere with the preparation of the endometrium for implantation; (b) luteolysis leading to decreased progesterone levels and interruption of implantation; and (c) termination of early pregnancy by prostaglandins. A number of progesterone antagonists have been evaluated. One of the compounds, RU38486 is being evaluated clinically for termination of very early pregnancy.

Deglycosylated derivatives of human chorionic gonadotropin have been shown to antagonize the action of human chorionic gonadotropin and interfere with established pregnancy in rats. Appropriate methods of delivery, immunogenicity and alternate methods for production of human chorionic gonadotropin need to be considered before evaluation of the derivatives for clinical use.

In vitro and in *vivo* models need to be developed for evaluation of the teratogenicity and embryotoxicity of post-coital and menses inducing agents.

There are a number of gaps in the knowledge of the processes regulating implantation which should be investigated in rodents and in different non-human primate species.

Keywords. Contraceptives; abortifacients; antiprogesterone; antiestrogens.

Introduction

The importance of the development of drugs which could be taken post-coitally, or used once-a-month in the case of a delay in the onset of menstrual cycles has been stressed by a number of countries (WHO Special Programme in Human Reproduction, Tenth Annual Report, 1981). The relative acceptance of these two approaches to birth control in different populations will, however, be influenced by cultural differences.

Post-coital agents

There is a felt but as yet unmet need for safe, effective and acceptable post-coital drugs. Methods of post-coital contraception used so far have been reserved primarily for

Abbreviations used: DES, Diethylstilbesterol; LH, luteinizing hormone; PC, post-coitum; hCG, human chorionic gonadotropin; HF, hydrogenfluoride; DG, deglycosylated.

'emergency situations' to protect women from unwanted pregnancy resulting from rape, a single act of unprotected coitus, failed barrier methods such as ruptured condoms or defective diaphragms, and for the many women who are unprotected because of infrequent coitus.

A 'post-coital pill', by definition, is a drug which is ingested after intercourse takes place (but not necessarily following each coital act on any one day). One of the primary objectives of the post-coital approach is to limit drug exposure, particularly for women who do not have intercourse daily or have a limited number of coital exposures in a month, as compared to the presently available methods of oral contraception in which a tablet containing a combination of a gestagen and estrogen is taken daily for 22 days, long-acting gestagens in which a compound is injected intramuscularly once every two or three months, or IUDs which are inserted by a physician. These methods involve the use of a contraceptive modality unrelated to the act of coitus. Probabilities that an act of intercourse will lead to conception (with establishment of a pregnancy of at least six weeks duration) can be calculated with reference to the estimated day of ovulation. Using self-observed vulvar changes in the characteristics of cervical mucus, a fertile period can be described, in which the last day of 'fertile-type mucus' designated as the 'peak day', has been shown to be correlated closely with the time of ovulation (Billings *et al*., 1972; Flynn and Lynch, 1976; Hilgers *et al*., 1978). The fertile period is defined as commencing on the first day of any recognizable mucus, and as ending on the evening of the fourth day after the peak day. In a multicentred study carried out in five centres from four continents, the WHO Task Force on Methods for the Determination of the Fertile Period (1983) has estimated that the probability of conception is low (0·004 per cycle) following the act of intercourse outside the fertile period. It rose to 0·024 on days of 'sticky' mucus four days or more prior to the peak day, to 0·5–0·55 in the three days prior to the peak. It was 0·667 on the peak day, and fell to 0·089 on the third day after the peak. The overall length of the fertile period in the study was 9·6 ± 2·6 (SD) days. It seems reasonable to aim for a post-coital pill that would be taken no more than a maximum of 10–12 days a month. The ultimate objective of a post-coital drug would be to interfere with implantation, irrespective of the day of the cycle when the drug would be taken. A drug effective in inhibiting implantation during the most fertile period is likely to be effective in other periods of the cycle also.

The desirable biological profile of a post-coital drug is difficult to define, since the physiological events that should be interefered with are varied in different stages of the menstrual cycle. No single drug is likely to affect all the physiological mechanisms during the different phases of the menstrual cycle. Some oral contraceptives (combinations of gestagen and estrogen) are effective by inhibiting ovulation. A post-coital drug is intended to act, among other ways, by inhibition of the processes crucial to the initiation and establishment of pregnancy *viz.* the implantation of the blastocyst in the uterus. It is clear that the post-coital pill would be needed most in the periovulatory period when the chances of conception are highest.

The problems concerning the use of the post-coital pill are the: (a) number of times the pill is taken during a cycle; (b) side effects depending on the number of days of coital exposure and tablets taken; (c) variation of side effects according to the stage of the cycle (follicular, ovulatory, or luteal phase) when the pills are ingested, *e.g.* a compound with gestagenic properties when taken a number of times during the luteal phase of the

menstrual cycle may delay the onset of menstruation and result in heavy bleeding, whereas if taken a few times during the follicular phase of the cycle it may lead to spotting or progesterone withdrawal bleeding.

Clinical studies

Two approaches to post-coital contraception, used as "emergency" measures involve: (a) insertion of a copper containing intrauterine device, the copper T, within five days of unprotected coital exposure; no pregnancies were reported in a series on 97 women treated in this way (Lippes *et al*., 1976); or (b) administration of synthetic, natural or conjugated estrogens. Diethylstilbesterol (DES), has been used at a dose of 50 mg daily in divided doses for five days with a failure rate from 0 to 2·4 %. The effectiveness of this method depends partly on its early administration, within 24–72 h after coitus (Kuchera, 1975). Ethinyl estradiol and other conjugated estrogens have also been evaluated with varying success. The action of these steroids appears to be due to changes in the endometrium rendering it hostile to implantation (Yuzpe, 1979). Synthetic estrogens like DES are no longer used and have been associated with adverse effects on the reproductive tract in male and female progeny (Stilman, 1982). One of the major handicaps in the development of post-coital drugs has been the difficulty of completely dissociating the oestrogenicity of available compounds from their anti-implantation effect. A number of non-steroidal compounds have been evaluated in animals in an effort to identify an effective drug having little estrogenic activity, but none has reached the stage of clinical trials (Harper, 1982; Prasad and Sankaran, 1975).

A combination of 200 μg of ethinyl estradiol and 2 mg of dl-norgestrel seems to be effective as an emergency post-coital contraceptive with several advantages over estrogens alone. The combination was administered either as a single dose (Van Santen and Haspels, 1982) or in two doses (12 h apart) after an unprotected coitus within the previous 72 h (Yuzpe *et al*., 1982). In a multicentred study involving 692 women the pregnancy rate was 1·6%; no serious side effects other than nausea/vomiting were encountered and menstrual bleeding occurred in more than 98 % of treated subjects within 21 days after treatment. Because of the potential failure rate and unpredictability of ensuring menstrual bleeding, Yuzpe *et al.* (1982) suggest that the method be reserved as an emergency post-coital contraceptive only.

Randomized double-blind studies are necessary to further compare the efficacy of this form of therapy with ethinyl estradiol or conjugated estrogen employed alone. It has been speculated that the combination acts either by: (a) suppressing ovulation: (b) disrupting luteal function by acting directly on the corpus luteum; or (c) interfering with appropriate endometrial responses to ovarian steroids.

Progestins

The two most frequently used compounds include d-norgestrel and quingestanol acetate (Mischler *et al*., 1974); the latter is no longer in use.

Post-coital administration of levonorgestrel has shown satisfactory results in terms of contraceptive efficacy, tolerance and acceptability. A dose-response relationship has been reported in a number of studies (Kesseru *et al*., 1974; Kovacs *et al*., 1979); the use of 150 μg dose was associated with a high failure rate, whereas 750 μg of levonorgestrel administered within 3 h after coital exposure was highly effective as a contraceptive

(Farkas, 1978); four pregnancies occurred in a two and a half year study involving 111 women treated during 999 cycles. In another study, when the drug was administered after coitus only during the periovulatory period in 50 women (150 cycles), there was no pregnancy (Kovacs *et al*., 1979). Although the study does not reflect on the real-life situation, *i.e.* restricting coitus only to the periovulatory period, it does indicate the efficacy of the drug when administered during the most fertile period. Levonorgestrel (Postinor) is marketed in Hungary as a post-coital drug, subject to a maximum intake of four tablets (each of 750 μg) a month, *i.e.* 3 mg/total dose/month. Depending on the dose employed, nausea and vomiting, headache, dizziness and breast tenderness have been reported. However, one of the most frequent problems is the alteration in the menstrual pattern produced by the progestin. Irregular menstrual bleeding does not appear to be acceptable to many cultures, and if amenorrhoea results, it is necessary to rule out the possibility of pregnancy before continued use of the progestin. The WHO is carrying out a multicentre study in eight countries to evaluate the post-coital efficacy of 750 μg of levonorgestrel when administered during the periovulatory or fertile period only. The other objectives of the study are (a) to assess the side-effects of the drug and to correlate these, if possible, with the dose of the drug administered, as well as the timing of its administration in relation to the menstrual cycle, and (b) determine the-acceptability of such a regime (WHO Special Programme of Research in Human Reproduction, Eleventh Annual Report, 1982).

Home visiting pill

Nine home visiting antifertility pills or vacation pills have been developed and used as post-coital or anti-implantation agents in the People's Republic of China (Lei and Hu, 1981); of these, anordrin (at a dose of 7·5 mg) apparently used as post-coital pill interferes with implantation by inhibition of luteal function and of endometrial development. It is not clear from the Chinese studies if anordrin was even used strictly as a post-coital pill. The antifertility activity of anordrin has been attributed to its antiestrogenic activity (Mehta *et al*., 1981), or to estrogenic acceleration of tubal transport or degeneration of eggs during tubal transport in the hamster (Gu and Chang, 1979).

A number of attempts have been made to synthesize related compounds to dissociate the estrogenic activity from antifertility activity but none had yet reached the stage of clinical evaluation.

Centchroman

The biological and antifertility effects of centchroman (3,4-trans-2, 2-dimethyl-3-phenyl-4 *p*-(β-pyrrolidinoethoxy)-phenyl 7-methoxychroman have been investigated (Kamboj *et al*., 1977); the compound has weak estrogenic and potent anti-estrogenic activity; it is devoid of progestational androgenic and anti-androgenic properties but antagonises progesterone in the rabbit. Its antifertility activity in rats, dogs and monkeys may be due to its multiple hormone attributes such as estrogenic, anti-estrogenic and antiprogestational activities.

Phase II clinical studies have been carried out in post-coital (60 mg) and once-a-week treatment schedules at doses of 120, 60, 30, 25, 20 and 15 mg. The results reported

indicate that centchroman provides good protection against pregnancy at 30 mg and other higher doses studied (Nitya Anand, personal communication). Ovarian and uterine enlargement was observed in both treatment regimes, with recovery to normal size within 30 days of withdrawal of the drug. Delay in menstruation of varying duration has also been reported. Since the doses used led to ovarian/uterine enlargement and irregular cycles, smaller doses (30 mg/week) are being evaluated clinically. As centchroman has been shown to induce release of luteinizing hormone (LH) in rats (Arabatti *et al*., 1977) the possibility of induction of ovulation as with clomiphene cannot be ruled out. The reported side effects on the ovary, uterus, and the irregular menstrual cycle caused by centchroman, call for further studies with much lower doses than used so far, before the hopes raised of the compound being used as a post-coital or once-a-week drug can be realized.

Evaluation of post-coital drugs

Animal models

A primary problem is the animal model for evaluation of post-coital drugs. By definition, such drugs should be evaluated for their efficacy by administration after mating. However, a post-coital drug can be tested in a rodent or non-human primate model only during the periovulatory period, when the females are sexually receptive and mate. Since mating does not occur at other times of the cycle, as is the case in the human, there is no possibility of testing the post-coital efficacy of drugs in any animal model by administration during periods of the menstrual/estrus cycle other than the periovulatory period. A possible, but tedious approach is to artificially inseminate laboratory rodents/non-human primates at other periods of the cycle and administer the drug to determine the side effects.

Toxicology

There are no standard procedures set out for the toxicological evaluation of post-coital drugs in animals. The questions that need to be answered are (a) how many times in a month should the drug be administered, and (b) what should be the frequency of administration? For example, for levonorgestrel which is administered as a post-coital agent (Postinor) in Hungary, the maximum suggested intake is 4 tablets/month, *i.e.* 750 μg/tablet, equivalent to a total dose of 3 mg/month. The question is, should the toxicology of such a drug be carried out by administering the test compound to animals (rodents or non-human primates) using the same schedule as would be applicable to the human, or should the drug be administered daily and, if so, for how long? This is true for evaluation of menses-inducing drugs also.

Problems associated with post-coital contraceptives

Yuzpe (1979) has summarized very well the problems associated with post-coital contraceptive studies, as follows.

Ideally, post-coital evaluation studies should be carried out in randomized, double-

blind fashion, with couples of proven fertility who will utilize the specific technique (or a placebo) once at mid-cycle only when the risk of pregnancy is at a maximum, and who will avoid coitus at all other times during that cycle. However, it is obvious that the ethical considerations of such studies make it difficult to obtain volunteers for this purpose. For this reason, studies are generally carried out upon women who are exposed at various times during the menstrual cycle. Thus, the number of women evaluated for a particular method must be sufficiently large to provide statistically significant data regarding efficacy.

Studies which utilize patient volunteers whose admission to only one single act of unprotected coitus is necessary for study inclusion are at a disadvantage. Reliance upon the patient to take the entire dosage of prescribed medication also has its obvious disadvantages, especially if nausea and vomiting are common occurrences.

Risks of post-coital contraception

In the light of potential failure of this technique, back-up abortion facilities should be available. The true potential of the adverse effects of treatment failures is, as yet, not fully understood. Furthermore, failure in such instances should be considered as a method failure similar to pregnancy resulting from the failure of an IUD or other barrier method.

Benefits of post-coital contraception

The major value of post-coital contraception in countries like North America seems to be in the area of emergency treatment. The woman at risk of pregnancy from sexual assault or a single, unprotected coital exposure may be spared the emotional and physical trauma of unwanted pregnancy.

Once the purpose has been achieved, the patient may be counselled, and her choice of contraception made from one of the available methods. This choice, of course, depends upon patient motivation, coital frequency, socioeconomic status, as well as other sociologic variables.

Easily available post-coital contraception also has the potential of reducing the use and acceptability of cyclic oral contraception, IUDs and barrier methods which have proved so effective for millions of women.

If a simple effective method of post-coital contraception is available, the woman may be spared the psychic trauma which is often associated with awaiting the passage of time in anticipation of the next expected menses.

Menses-inducing drugs

There is great demand from women and from national family planning programmes for a simple and safe method of birth control for use when menses are delayed for a few days. The availability of such technology would limit exposure to fertility regulating methods only to such occasions when there is a probability of pregnancy. Moreover, if fertilization had indeed, occurred, interruption would take place at the earliest stage of pregnancy, thereby reducing the excessive bleeding encountered with later termination of pregnancy.

The only currently available methods of termination of very early pregnancy are mechanical aspirators that in most instances require trained personnel. Experience with them indicates an efficacy of 95 % when used at five or six weeks since the last menstrual period. About 5% of patients have severe bleeding or retention of products of pregnancy requiring vacuum aspiration. Uterine perforation rates are approximately equivalent to those at seven to eight weeks vacuum aspiration.

Menstrual regulation could be achieved by any one or more of the following approaches:

—block progesterone receptors and interfere with the preparation of the endometrium for implantation;
—luteolysis leading to decreased progesterone levels and interruption of pregnancy;
—termination of early pregnancy by prostaglandins.

A study comparing the efficacy and safety of two prostaglandin E_2 analogues and vacuum aspiration for termination of very early pregnancy in women with a delay of menstruation of up to 14 days is ongoing at present in several countries (WHO Special Programme of Research in Human Reproduction, Eleventh Annual Report, 1982; 1983).

Progesterone receptor blockers —progesterone antagonists

Progesterone plays an indispensible role during the implantation phase and early pregnancy in animals and women; withdrawal of progesterone leads to breakdown of the secretory endometrium in the normal menstrual cycle. Likewise, the decidua which develops from the endometrium following implantation regresses and is shed in the absence of progesterone. There are several possible methods for interference with the secretion and action of progesterone. One of these is to block the action of progesterone on the endometrium.

It has been demonstrated that progesterone receptors are generated during the follicular phase of the menstrual cycle reaching a maximum just before ovulation, after which they decline markedly (Bayard *et al*., 1975). During the luteal phase of the menstrual cycle, despite the decrease in the total concentration of progesterone receptors in the endometrial cells, there is an increase in their concentration in the cellular nuclei (Bayard *et al*., 1979; Levy *et al*., 1980). Early pregnancy endometrium (8–10 weeks gestation) is characterized by a large concentration of progesterone receptors, exceeding those of any period in the menstrual cycle (Levy *et al*., 1980). A progesterone antagonist, or antiprogestin, taken orally during very early pregnancy or after missed menses could, by binding to endometrial progesterone receptors, interfere with the maintenance of the secretory endometrium essential for continuation of pregnancy. A number of compounds evaluated so far have shown either agonistic (progestational) and/or estrogenic activity.

The desired characteristics of a progesterone antagonist are that it should: (a) be orally active; (b) have a high affinity for progesterone receptors in the radio-receptor binding assay in competition with progesterone. There is no agreement on whether the ideal compound would bind rapidly and dissociate slowly or bind and dissociate rapidly; (c) have high antiprogestin and low progestin activity in the Clauberg test in

rabbits, in deciduoma test in rats, in pregnancy maintenance test in rats and in the diamine oxidase assay; (d) have no or markedly reduced estrogenicity; (e) show significant anti-implantation effect in rats/hamsters/rabbits and non-human primates; and (f) be free from toxicological and teratological effects.

Over the past decade, attention has been directed towards understanding the molecular basis for the biological activity of progesterone in order to permit the development of an anti-progestational agent. Most of these efforts have been empirical. Investigators have examined the steroids that have progestational activity in the hope of defining the molecular requirements for activity and structural features that could be manipulated to alter the degree of response. It is now clear that knowledge of the structures of the progestins alone will not permit the development of an antiprogestin.

The WHO Task Force on Post-coital and Once-a-Month Drugs has embarked on the synthesis of new progesterone receptor blockers based on the study of the structure of the uterine progesterone receptor. The human endometrial progesterone receptor has been purified (Smith *et al*., 1982). Three active sites of the receptor have been identified by affinity labelling with 21-, 16- and 11-O^{3}H bromo-acetoxyprogesterone; these three derivatives specifically bound to and displaced progesterone bound to the human endometrial progesterone receptor and migrated as a single protein band on Polyacrylamide gel electrophoresis of molecular weight 42,000 (Holmes *et al*., 1981).

A few questions which need to be answered are:

What does the binding site look like? Exactly what happens to the site when progesterone binds to it? Do compounds with vastly different progestational activity trigger different degrees of change in the receptor? To what extent is the steroid buried in the receptors? Does part of the steroid control binding and another part control activity? Is the steroid partially exposed when bound to the receptor? Does this exposed part control activity by interaction with another macromolecule, histones, non-histones or DNA? Can we design a molecule that will fulfil the structural requirements for binding but fail to elicit the activating response?

The logical way to obtain answers to these questions is to determine the X-ray crystal structure of the progesterone receptor. Once the receptor protein has been crystallized, a series of steroidal and non-steroidal compounds can be examined to determine how they fit into the receptor site.

Thus the scientific rationale for such an approach for development of antiprogestins is clear. However, it does require large amounts of financial investment.

Herrmann *et al.* (1982) have described the use of a 19-nor testosterone derivative RU38486, 17α-propynyl-ll*ß*-(4-dimethyl-aminophenyl)-, 9,10-dihydro-19-nor testosterone for induction of abortion and menses regulation. This compound has great affinity for the progesterone receptor without progestational, estrogenic or anti-estrogenic activity. It binds to glucocorticoid and human progesterone receptors. No toxic effects were seen during 30 days of administration to rats and monkeys except for those attributable to the antiglucocorticoid effects in high doses. When 200 mg/day of the compound was given, either in two or four divided doses to women who were 6–7·5 weeks pregnant, abortion occurred in 8 of the 11 subjects in 3–8 days after the initiation of treatment. Six women with normal menstrual cycles were administered 50 mg/day for four days between days 22–25 of the cycle and in all cases bleeding occurred within 48 h of treatment concurrent with a rapid fall in plasma progesterone and estradiol to

follicular phase levels. No side effects were reported in any of the eight treatment cycles and the subsequent menstrual cycles were apparently ovulatory as judged by basal body temperature. These results are highly encouraging in demonstrating clinical effectiveness of an antiprogestational agent. The WHO Special Programme in Human Reproduction is carrying out a dose finding study of the compound for termination of very early pregnancy (delayed menses) (WHO, 1983). Even as these clinical studies progress, attempts to synthesize even more potent and active progesterone antagonists continue based on a better understanding of the receptor binding sites of the human endometrial progesterone receptor.

The objective in the development of such compounds would be to use them regularly once-a-month for menstrual regulation or for termination of very early pregnancy.

Other compounds

A possible anti-implantation agent is α-difluoromethylornithine which inhibits implantation in rats when administered intraperitoneally at a dose of 200 mg/kg twice daily on day 4–7 of pregnancy (Reddy and Rukmini, 1981). It is also effective in rabbits (Fozzard *et al*., 1980). The mechanism of action of the compound is by inhibition of ornithine decarboxylase enzyme and consequently the synthesis of putrescine, which is essential for embryogenesis (Fozzard *et al*., 1980). The compound is active *in vivo* and apparently non-toxic (Prakash *et al*., 1978). In view of the possible consequences of systemic inhibition of a ubiquitous enzyme like ornithine decarboxylase, the potential of its use for fertility regulation remains to be determined.

An anti-progesterone monoclonal antibody, has been shown to block, the establishment of pregnancy in mice, probably by interfering with the transport of the fertilized ovum along the fallopian tube when administered at 32 h post-coitum (PC) and interrupted implantation when injected 109–130 h PC (Wright *et al*., 1982); these effects were possibly due to reduction by the antiprogesterone antibody of readily available progesterone in circulation by more than 85 % during the critical phase of implantation. The acceptability and safety of a passive immunization method needs to be established before its use for inhibition of implantation. Passive immunization even with monospecific antibodies runs the risk of sensitizing the recipient to the foreign antibody protein. Although in theory this risk could be reduced by 'typing' the recipients and the clone products, this is unlikely to prove practical or entirely feasible for wide-scale application. Another way in which the risk of sensitization can be reduced is by the use of $F(ab)_2$ fragments although this would add an expensive additional step in the preparation. Even if the intended use is as a monthly administration, or at less frequent intervals, the risk of sensitization would still remain.

Zoapatle

Zoapatle, is the common name for *Montanoa tomentosa,* which grows in Mexico. Ethnobotanical studies and the clinical use of a number of constituents extracted from the plant have been reviewed recently (Gallegos, 1983); extracts from zoapatle have been shown to be uterotonic (Gallegos, 1983); bleeding occurred in four out of six subjects when administered at a dose of 100 g/day during early pregnancy (Landgren *et al.,*1979). Zoapatanol, however, which is obtained by total synthesis, has apparently no

uterotonic effect (Smith *et al*., 1981). This discrepancy between the plant extract and the pure synthetic compound needs to be resolved. A number of other chemical entities extracted from zoapatle such as kauradienoic acid, kaurenoic acid, zoapatlin and monoginoic acid need to be evaluated for their biological activity, in addition to continuation of studies with pure zoapatanol (Gallegos, 1983). The question that needs to be considered is whether optimal effects are produced by combination of one or more of the above constituents.

Interference with luteal function

Normal corpus luteum function and secretion of progesterone are needed for implantation to occur and pregnancy to be established. Its function is directly under the control of pituitary gonadotropins and in a non-pregnant menstrual cycle the corpus luteum regresses within 10—12 days after ovulation. Human chorionic gonadotropin (hCG) secreted by the trophoblast of the developing and implanting blastocyst, rescues the corpus luteum from regression and maintains it.

hCG derivatives

Attempts have been made to prepare derivatives of hCG which could act as antagonists of hCG for inhibition of luteal function. Such an hCG antagonist when administered after missed menses could competitively displace hCG bound to the corpus luteum leading to luteal regression, decreased progesterone levels, interruption of pregnancy and induction of menstrual-like bleeding (WHO Special Programme of Research in Human Reproduction, Annual Reports, 1979–1983). A number of derivatives of hCG have been prepared and their physico-chemical characteristics and biological activities evaluated *in vitro* and *in vivo*. One of the derivatives, periodate oxidized–reduced derivative, designated PORA-hCG which had lost all its galactosyl and 40 % of mannosyl residues, following sequential treatment with specific exoglycosidases has been evaluated (Bahl and Moyle, 1978). This derivative bound to the ovarian receptors with a 2·4 times higher affinity than hCG, caused a dose-dependent inhibition of the action of hCG in production of cAMP by corpus luteal cells *in vitro,* had 2–5 % of agonistic activity in stimulating progesterone production (Kalyan *et al*., 1982; Channing and Bahl, 1978a,b); PORA-hCG inhibited implantation in rats when administered subcutaneously at doses greater than 0·25 μg/day from days 1–5 PC (Kalyan *et al*., 1981). However, one of the principal disadvantages is that the derivative is stable only at – 20°C in the lyophylized or solution form (Kalyan *et al*., 1982).

A different approach for the production of hCG antagonists has been reported by Manjanath and Sairam (1982) who have shown that treatment of purified hCG with anhydrous hydrogenfluoride (HF) at 0°C for 60 min removed 75% carbohydrate without affecting the peptide moiety. The O-glycosidically-linked glycopeptide part in the C-terminal peptide of the α-subunit was least affected by treatment. De-glycosylation reduced heterogenicity that is inherent to native hCG. Its solubility was not affected. The product was obtained in good yield in a stable and lyophilized form. Even the most drastic conditions could not remove all of the carbohydrate; this may even lead to reduction of receptor binding and antagonistic activities. In cell receptor

binding assays, *viz*. rat testis, pseudopregnant rat ovary, porcine granulosa cell, rhesus monkey testis, deglycosylated (DG) hCG showed approximately 200% activity as compared to native hCG. There was no reduction in immunological activity of DG hCG as tested by radioimmunoassay using [^{125}I]-hCG and an antibody to native hCG. As against full agonistic activity in receptor binding, DG hCG almost completely lost its activity in responsive cells incubated *in vitro*. Both cyclic AMP accumulation and testosterone synthesis caused by native hCG were inhibited by the concomitant presence of DG hCG (Sairam, 1982).

DG hCG is heat stable. Significant receptor binding, immunological and hormonal antagonistic properties are retained by DG hCG after exposure in a boiling water bath. This may contribute to the long shelf life of the antagonist in aqueous solutions; it is stable in the lyophilized form at 4°C for up to 12–16 months (Sairam and Manjanath, 1983). DG hCG has been shown to inhibit implantation when administered subcutaneously at a dose of 50 μg/day from days 1–5 PC; similar doses administered between days 8–11 of pregnancy resulted in complete termination of pregnancy and foetal resorption accompanied by a fall in serum progesterone levels. The derivative had little effect on pregnancy, foetal growth or parturition when administered from days 13–16 (Kato *et al*., 1983).

A number of issues that need to be studied before these derivatives may be considered for use in termination of early pregnancy are: (a) immunogenicity of DG hCG; (b) antifertility effects in non-human primates; (c) alternate methods of derivative production; and (d) delivery system and mode and route of administration.

Evaluation of menses-inducing drugs

The efficacy of menses-inducing drugs (anti-progestins or luteolytic agents) can be evaluated in non-human primates in which pregnancy can be determined accurately by timed mating. Reproductive profiles and standard criteria for the choice of females for such studies have been reported for the rhesus monkey, bonnet monkey and the baboon (Anand Kumar *et al*., 1980; Hendrickx and Enders, 1980; Murthy *et al*., 1980). The test drugs are administered on days 22–24 of the menstrual cycle in animals in which pregnancy is established by monitoring levels of chorionic gonadotropin and the effects evaluated by termination of pregnancy, onset of menstrual bleeding and decrease in serum levels of chorionic gonadotropin to non-detectable levels.

The luteolytic activity of compounds can also be evaluated in monkeys by administration of the compound during the luteal phase of non-pregnant rhesus monkeys (Ricardo *et al*., 1982). One of the principal disadvantages in these studies is the short luteal phase. If the functional life of the corpus luteum of a non-pregnant cycle can be prolonged for 10–15 days by administration of hCG, it should provide a suitable model to evaluate the efficacy of luteolytic agents. Such studies are in progress in the bonnet monkey (WHO 1982).

Research on implantation in non-human primates

Development of primate models: There are a number of gaps in the knowledge of the processes regulating implantation which need to be investigated. Some of these are:

—hormonal profiles during the periimplantation period

—changes in the concentration of progesterone and estrogen receptors during the periimplantation and post-implantation periods;

—hormonal requirements for ovum implantation;

—changes in histochemistry of the endometrium during implantation: whether there are any enzymes which are crucial to the initiation of implantation;

—role of the blastocyst in initiating metabolic and cellular changes in the endometrium at the time of implantation;

—whether the functional life of the corpus luteum of a non-pregnant menstrual cycle can be prolonged by hCG administration, if so, for how long? Such a model would be useful for screening luteolytic agents.

These studies could provide new leads for evaluation and development of new anti-implantation agents which could be used either PC or for menses-induction. There is no agreement on the appropriate primate species to be used for thses studies. Such studies should be carried out in a range of primate species and not concentrated on the few on which data is currently available, since it is most unlikely that any one species will prove ideal as a model for all aspects of implantation in the human. There are also other factors on the availability, cost, ease of management, breeding performance in captivity. That might affect their choice for achieving different goals in research on implantation.

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