MENSTRUAL INDUCTION:
METHODS AND MECHANISMS OF ACTION

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Declaration

The contents of this thesis have not been submitted elsewhere for any other degree, diploma or professional qualification.

The thesis has been composed by myself, and I have been responsible for patient recruitment, clinical management and laboratory studies, unless otherwise acknowledged.

Jane Norman

February 1992.

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Abbreviations:

The following abbreviations have been used :-

CI	confidence interval
hCG	human chorionic gonadotrophin
GAG	glycosaminoglycan
PLA_2	phospholipase A_2
PG	prostaglandin
WHO	World Health Organization
NPL	neutrophil polymorphonuclear leucocyte

Abstract

One hundred and ninety five thousand abortions were performed in Britain in 1989, a rate of 13 per 1000 women of reproductive age. Data from England and Wales indicates that 35% of women undergoing abortion in 1989 were at less than nine weeks gestation, a stage when menstrual induction can readily be performed. The method of abortion in almost all these women was vacuum aspiration, but there is increasing interest in menstrual induction, whereby early pregnancy can be terminated medically without the need for surgical intervention. The development of the progesterone antagonist mifepristone offers the possibility of a safe method of menstrual induction (in combination with a prostaglandin), which is effective and has minimal side effects. The minimum effective dose, and the mechanism of action of mifepristone are however unknown. The work in this thesis has investigated these two questions further.

When the efficacy and side effects of three different doses of mifepristone: 200 mg, 400 mg and 600 mg followed 48 hours later by 1 mg gemeprost in women of ≤ 56 days amenorrhoea were compared, all three regimes were equally effective, with a mean complete abortion rate of 98%. There were no differences in the incidence of side effects between the three groups. In contrast, a lower complete abortion rate of 87% was seen in women at the same gestation following gemeprost alone (1 mg 6 hourly to a total of 3 mg) ($p<0.01$). In a randomised comparison between the two methods, there was a significantly greater requirement for analgesia following the use of gemeprost alone ($p<0.02$).

The effects of a new prostaglandin (misoprostol) which could be used in combination with mifepristone were also studied. A significant increase in uterine tone was observed 30-120 minutes after 200-600 μg misoprostol alone ($p<0.01$); in women pretreated 48 hours earlier with 200mg mifepristone, an increase in uterine contractility measured in Montevideo units was observed 30 minutes after 200-600 μg misoprostol ($p<0.01$). Eighteen out of twenty one women aborted using 200 mg mifepristone followed 48 hours later by 200-1000 μg misoprostol.

In the second part of this thesis, the mechanism of action of mifepristone has been investigated. In vitro studies have already shown that the addition of mifepristone to culture medium results in an increased ability of decidua to generate $PGF_{2\alpha}$ in culture. In the studies described here, decidua harvested from women pretreated in vivo with

mifepristone showed a similar increase in ability to generate $PGF_{2\alpha}$. This increase was inhibited by co-administration of indomethacin in vivo. Uterine activity measurements showed an increase in uterine activity following mifepristone in vivo, but co-administration of indomethacin failed to inhibit this increase. The increase in uterine activity seen following mifepristone is likely, therefore, to be due to mechanisms other than an increase in decidual prostaglandin production.

Finally, when the effects of mifepristone on the cervix were studied in primigravidae of eight to twelve weeks gestation, it was found that mifepristone significantly reduced the median total force required to dilate the cervix from 4 mm to 10 mm, from 93 Newtons in a control group to 47 Newtons in a group of women pretreated with 200 mg mifepristone 36 hours earlier ($p<0.0005$). There was no difference in the ability of the cervix to generate PGE_2 and $PGF_{2\alpha}$ in tissue culture between the groups.

In summary therefore, the minimum effective dose of mifepristone to induce abortion in combination with prostaglandin is still to be established. The mechanism of action of mifepristone is similarly unclear, but appears to involve mechanisms other than an increase in decidual or cervical prostaglandin production.

Chapter 1:

Literature review

> " the world needs a Fifth Freedom - freedom from the tyranny of civilised man's excessive fertility". (Baird, 1965).

> " No developed country has brought down its birth rate without a considerable recourse to abortion and it appears unlikely that developing countries can ever hope to see any decline in their fertility without massive resort to induced abortion". (Potts et al, 1977).

Innovations in methods of abortion have implications not only for individual women, but also for the world's population as a whole. In the United Kingdom, since the 1967 Abortion Act allowed induced abortion under certain specified circumstances, the number of abortions performed has increased steadily each year. In Scotland in 1990, 10,000 abortions were performed, giving an abortion rate (the number of abortions performed per 1000 women of reproductive age) of 9.1 (Scottish Home and Health Department, 1991). In England and Wales the abortion rate is higher at 13.4 (Office of Population Censuses and Surveys, 1990). Worldwide it has been estimated that some 40-60 million abortions are performed each year (Tietze and Henshaw, 1986), with an estimated maternal mortality of 200 000 per year from induced abortion alone (Mahler, 1987). In the U.K, the mortality from induced abortion is low, with only two maternal deaths being recorded following induced abortion (legal or illegal) during the triennium 1985-1987, the most recent period for which the Report on Confidential Enquiries into Maternal Mortality is available (Department of Health et al, 1991). This gives a rate of 4.2 deaths per million legal abortions. The method of abortion used depends on the gestation. Data collected on all abortions performed in NHS hospitals in England and Wales in 1985 indicates that medical abortifacients (mainly prostaglandins) were used in 56% of cases at 13-19 weeks gestation, whereas for abortions performed on women of less than 9 weeks gestation, surgical methods were used in 98% of cases (Office of Population Censuses and Surveys, 1986). The unpopularity of medical abortion (or menstrual induction) in the early first trimester relates primarily to the side effects of the agents used, which are thought to be excessive in comparison with the relatively straightforward procedure of vacuum aspiration under general or local anaesthetic. However, with the development of an effective antiprogesterone (mifepristone), interest has been rekindled in menstrual induction and medical abortion.

Part 1:

Methods of menstrual induction.

The unpopularity of medical abortion in early pregnancy amongst providers of abortion services is not shared by women using these services. In a study performed in Sweden, women of up to 49 days amenorrhoea requesting legal termination of pregnancy were offered a choice between vacuum aspiration, and medical abortion (using vaginal prostaglandins) at home or in hospital. The majority (43/51) opted for medical abortion, despite an awareness that this would probably be more painful than vacuum aspiration (Rosén et al, 1984). In another study using intramuscular sulprostone as an abortifacient in women with a mean menstrual delay of 17 days, 89% of the women who had previously also undergone surgical termination of pregnancy stated a preference for medical abortion (Csapo et al, 1980). Similar data has been obtained following the use of antiprogesterone and prostaglandin. In the U.K, 95% of women seeking abortion at less than 56 days of amenorrhoea, who were offered a choice of either medical abortion using mifepristone and gemeprost or vacuum aspiration opted for medical abortion. Following the procedure 89% found the procedure acceptable and 10 out of the 13 women who had previously also undergone surgical abortion stated a preference for medical abortion should a future abortion be required (Urquhart and Templeton, 1988).

Menstrual induction (medical abortion within a few weeks of the first missed menses) is potentially more straightforward than medical termination at a later gestation. Spontaneous abortions before the eighth week of pregnancy are often complete, probably due to a lack of anchoring villi which develop later in pregnancy, suggesting that menstrual induction at this early stage of pregnancy is unlikely to be complicated by incomplete abortion. Where blood loss following medical termination has been measured, it is proportional to gestation (Rodger and Baird, 1989). The work in this thesis concentrates on the methods and mechanisms of action of medical abortion in women of up to 56 days amenorrhoea. In the first three chapters, the use of prostaglandins for menstrual induction, either alone or in combination with the antiprogesterone mifepristone, is investigated. The literature on the use of prostaglandins and the antiprogesterones for early medical abortion is reviewed below.

The uterotonic effects of prostaglandins have been apparent since an active substance in the seminal plasma (Kurzrok and Lieb, 1930), later termed "prostaglandin" (Von Euler, 1935) was first discovered, but it was not until after the prostaglandins were isolated and their structure delineated that this effect could be exploited for fertility control. The use of natural $PGF_{2\alpha}$ as an abortifacient was first described in the early 1970s in women in the late first and early second trimester; complete abortion was achieved in 13/15 women following intravenous administration of $PGF_{2\alpha}$ (50 μg/min) (Karim and Filshie, 1970), and in 3/11 women following intravenous PGE_2 (1-10mcg/min) or $PGF_{2\alpha}$ (10-50 μg/min), or subcutaneous $PGF_{2\alpha}$ (5 mg 3 hourly) (Roth Brandel et al 1970). The first description on the use of prostaglandins for menstrual induction was the use of $PGF_{2\alpha}$ as a continuous intravenous infusion (up to 300 μg/min) for 7 hours to twelve women of up to 17 weeks gestation (Wiqvist and Bygdeman, 1970). Abortion occurred in all seven women of up to nine weeks gestation within 24 hours, and in three of the other five women after a second course of treatment the following day. The apparent increased effectiveness in the earlier stage of pregnancy was attributed to decreased cervical resistance and a more vulnerable vascular connection between the conceptus and the uterus at this early stage of pregnancy. This complete abortion rate of 100% in early pregnancy was not achieved by other workers. In another study, thirteen women reporting menstrual delay of between 6-18 days (34-47 days of amenorrhoea) were given $PGF_{2\alpha}$ intravenously (50 μg/min) for 8 hours 20 minutes (Wentz and Jones, 1973). Nine out of the thirteen women were subsequently found to have been pregnant (by measurement of hCG by radioimmunoassay, with a sensitivity to 5 IU/l). Five patients aborted completely, but four continued to have a positive immunological pregnancy test following treatment. Histological evaluation of products of conception obtained at uterine evacuation from these latter four women showed severe trophoblastic damage. The authors concluded that caution should be exercised in the use of $PGF_{2\alpha}$ as a post conceptive agent for fertility control, because of lack of reliability of the method and because of concern about adverse effects on the pregnancy should the pregnancy continue. In addition, abortion was not an inevitable consequence of vaginal bleeding, so that follow up was necessary to determine outcome.

One of the advantages of menstrual induction over surgical termination of pregnancy is that the potential for self medication exists. In an attempt to find a method which would allow this, other routes of administration of prostaglandin have been explored. Vaginal $PGF_{2\alpha}$ in THAM solution was administered to nine women of between 34-40 days

amenorrhoea every 2-4 hours for 24 hours, up to a total dose of 1100 mg $PGF_{2\alpha}$ (Corlett et al, 1972). Three out of nine women aborted completely. Evidence of adequate absorption into the systemic circulation was confirmed by finding a two to eight fold increase in serum prostaglandin levels during treatment. Although there was a fall in the concentration of 17-hydroxyprogesterone, a marker of luteal function, following treatment, this occurred only after a fall in serum hCG, suggesting that prostaglandins did not induce luteolysis directly. Variable rates of success of between 50 and 80% have been achieved by other groups, using vaginal prostaglandins within a few weeks of the first missed period (Sato et al, 1973; Bolognese and Corson, 1973; Tredway and Mishell, 1973; Jones et al, 1974), although gastrointestinal side effects were noted to be a problem in most of these studies. Again, the lack of efficacy and poor correlation between vaginal bleeding and eventual abortion meant that follow up was necessary.

A modification of the use of prostaglandins for early pregnancy termination was suggested by Csapo, who proposed that the mechanism of action of prostaglandins in inducing abortion was biphasic. The initial effect was to promote uterine activity, resulting in suppression of the endocrine function of the fetoplacental unit, and withdrawal of progesterone, which in turn increased spontaneous uterine activity (Csapo and Pulkinnen, 1979a). The success of prostaglandin in abortion therefore depended on the initial "prostaglandin impact" (Csapo et al, 1972). Although this was initially described in women of nine weeks gestation (i.e after the luteoplacental shift), it also appeared to operate in early pregnancy, with the insult to the endocrine function of the fetoplacental unit leading to luteolysis. From these observations, it seemed sensible to administer prostaglandins as a bolus. Thereafter the above mechanism would ensure the development of uterine activity without further doses of prostaglandin being given. Clinical studies used intrauterine administration of prostaglandins, so that a large dose of the drug could be given directly to the target organ. Complete abortion rates of 91% (in twenty two women at a mean of 11 days of missed menses following a single intrauterine injection of 5 mg $PGF_{2\alpha)}$ (Csapo et al, 1973a), and 100% (65 women with a mean of eleven days of missed menses given either 5 mg $PGF_{2\alpha}$ [n=50] or 1mg PGE_2 [n=15]) (Moscary and Csapo, 1973) were achieved. Similar success rates were found following intrauterine prostaglandin therapy by other investigators (Karim 1973; Ylikorkala et al, 1974). An American group had a lower complete abortion rate of 65%, using a similar protocol at a slightly later gestation of 38-47 days amenorrhoea and felt that the incidence of side effects (up to 35%), including hypertension and haemorrhage, and septic abortion in those who

failed to abort completely made the method unsuitable for routine use (Lichtman et al, 1974). Despite the apparent success of extraovular prostaglandin for menstrual induction, some disadvantages of the regime were becoming obvious. In all the above studies - with the exception of one (Karim, 1973), premedication was required and the route of administration precluded self administration. Side effects were greater than experienced with vacuum aspiration. In a comparison between intrauterine $PGF_{2\alpha}$ (5 mg) and vacuum aspiration under intramuscular analgesia alone, in 200 women of up to 56 days amenorrhoea the incidence of vomiting in the prostaglandin treated group was 30%, significantly greater than in the group undergoing vacuum aspiration (9%), although the rate of complete abortion was high in each group, and only one patient (in the vacuum aspiration group) failed to abort completely (Ragab and Edelman, 1976).

When prostaglandins are administered via a route which involves systemic absorption, repeated high doses have to be given because of the short half life of the natural prostaglandins in the circulation. The development of a synthetic prostaglandin, with an alkyl group at C15 such that oxidation at C15 did not occur, and thus had increased activity (Bundy et al, 1971) offered a prostaglandin which could be given once only. The effects of 15 methyl $PGF_{2\alpha}$ on uterine contractility were found to be 100-400 times greater than that of the natural compound, depending on the route of administration (Karim and Sharma, 1972). The abortifacient effects of 15 methyl $PGF_{2\alpha}$ and PGE_2 were initially demonstrated in the late first, and second trimesters, with a complete abortion rate of 91% (Lauersen and Wilson, 1975a; Lauersen and Wilson, 1975b). When 15-methyl $PGF_{2\alpha}$ was used for menstrual induction, complete abortion was achieved in 8/9 women (Lauersen and Wilson, 1976) and 32/34 women (Fylling and Jerve, 1977), of up to 60 days gestation following multiple or single intramuscular injections respectively. The latter study also investigated the effects of vaginal therapy with the synthetic prostaglandin, this having the advantage that it could be given at home. Thirty women were given the first dose of prostaglandin in hospital, and subsequently self administered further doses at home. All aborted completely. Many other investigators also reported their results with vaginal 15-methyl $PGF_{2\alpha}$ therapy, and initially promising success rates were achieved with complete abortion without further therapy, of between 70-94% in women up to 61 days from the last menstrual period (Bygdeman et al, 1976, Ylikorkala et al, 1976; Zoremthangi et al, 1976; Hamberger et al, 1978; Lauersen and Wilson, 1980). However, 15-methyl $PGF_{2\alpha}$, did not constitute an ideal abortion method, since repeated doses were necessary, making non compliance a problem. Gastrointestinal side effects were still a

significant feature, occurring in up to 50% of patients, and premedication was still required in all but two (Zoremthangi et al, 1976; Lauersen and Wilson, 1980) of the above studies. A long acting suppository of 15 methyl $PGF_{2\alpha}$ has been developed which gives a sustained release of prostaglandins (Spilman et al, 1976). In women of less than 50 days amenorrhoea, the complete abortion rate following a single vaginal pessary was dose dependent. 120 out of 128 women aborted following a single 3 mg dose (Gréen et al 1978). Although compliance was not a problem with this regime, gastrointestinal side effects were still excessive, and the development of a regime giving consistent results remained elusive. In addition, objective measurement of blood loss showed a mean blood loss during abortion of 131 ml, and a mean blood loss during menstruation 2-4 months later at the upper limit of normal, perhaps reflecting continued presence of trophoblast (Hamberger et al, 1978). Notwithstanding these problems, vaginal 15-methyl $PGF_{2\alpha}$ has become the method of choice for early abortion in some countries (Population Information Program, 1980).

Introduction of methyl groups at C16 has generated prostaglandins which are more resistant to oxidation of the 15 hydroxy group, thus increasing in vivo activity. These compounds are called the "third generation" prostaglandins. Four have been extensively investigated for their use in pregnancy termination: 16 phenoxy-tetranor PGE_2 methyl sulphonylamide (sulprostone); 16, 16 dimethyl PGE_2; 16, 16 dimethyl PGE_1 methyl ester (gemeprost); and 9-deoxo 16,16 dimethyl 9 methylene PGE_2 (9 methylene PGE_2).

These compounds were first administered as extraovular injections, and a complete abortion rate of 95% achieved in a study of 240 women of up to 6-14 days menstrual delay following a single 50 μg dose of sulprostone into the uterine cavity (Karim et al, 1977). Intramuscular administration of sulprostone induced complete abortion in 94-100% of women of less than 49 days amenorrhoea (Csapo and Pulkkinen, 1979b; Csapo et al, 1980, Fleicher et al, 1982; Bygdeman et al, 1983). In the largest of these studies, 90 women with a mean menstrual delay of 17 days were given repeated intramuscular injections of 500 μg sulprostone every 4 hours to a maximum of 2 mg. The complete abortion rate was 96% and the incidence of vomiting and diarrhoea was 26% and 10% respectively (Csapo et al, 1980). 16, 16 dimethyl PGE_2 has been administered as a vaginal suppository; abortion rates of 87-100% have been achieved following up to 4 mg in divided doses in women of less than 56 days amenorrhoea (Lundström et al, 1977; MacKenzie et al, 1978). Gastrointestinal side effects were reported by up to 50% of patients. The advantages of

the newer prostaglandins were indicated by the greater efficacy and lower incidence of gastrointestinal side effects in women of a similar gestation treated with vaginal 16, 16, dimethyl PGE_2 compared to vaginal 15-methyl $PGF_{2\alpha}$ although a randomised comparison was not performed (MacKenzie et al, 1978). Vaginal administration of 16, 16, dimethyl PGE_1 (1 mg every 1-3 hours; up to 5 mg) induced abortion in 87% of patients with up to 49 days of amenorrhoea (Smith and Baird, 1980). Finally, 9 methylene PGE_2 has also been administered vaginally, complete abortion occurring in 92-98% of women, depending on the dose (Bygdeman et al, 1983).

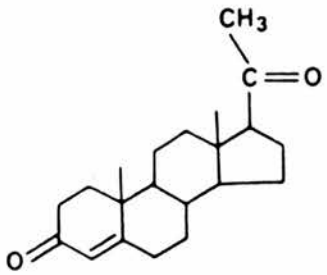
Several trials have compared menstrual induction using one of the third generation prostaglandins to vacuum aspiration under local or general anaesthesia. Menstrual induction using vaginal administration of up to 3.6 mg of 16, 16 dimethyl PGE_2 (Lundström et al, 1977), up to 5 mg of 16, 16 dimethyl PGE_1 (Smith and Baird, 1980), up to 120 mg 9-methylene PGE_2 (Rosén et al, 1984) or up to 1.5 mg intramuscular sulprostone (World Health Organization Task Force, 1987) was shown to be as effective as vacuum aspiration either under local anaesthesia or under premedication only. Premedication before menstrual induction was not given routinely in any of these studies, but between 12% and 30% of women undergoing menstrual induction required an injection of opiate analgesia, and most investigators highlighted the greater incidence of gastrointestinal side effects in these women, compared to those undergoing vacuum aspiration. In the largest of these studies, involving 473 women, mean duration of bleeding was 9 days in the prostaglandin treated group compared with 4 days in the vacuum aspiration group ($p<0.001$) (World Health Organization Task Force 1987). Similar trends were observed in the other three studies. Objective measurement of blood loss shows a mean loss of 70-85 ml in women treated with prostaglandin, vacuum aspiration under local anaesthesia or vacuum aspiration under general anaesthesia, with no significant differences between the groups (Smith and Baird, 1980).

Progesterone is essential for the maintenance of early pregnancy in humans. In a study on removal of the corpus luteum (the main source of progesterone in early pregnancy prior to the luteoplacental shift) uterine activity developed in all eleven women who underwent lute-ectomy at a mean of 50 days gestation, and seven out of eleven women aborted completely (Csapo et al, 1973b). In another group of seven women who also underwent lute-ectomy, but received progesterone replacement (200 mg intramuscular per day), the pregnancy continued apparently unaffected in all seven. In contrast, oestradiol replacement therapy failed to prevent abortion following

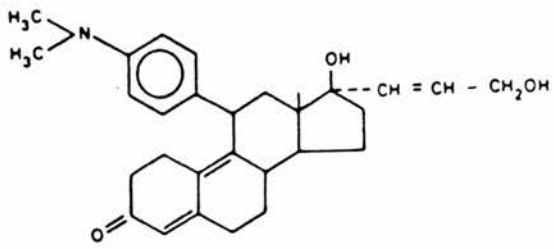
lute-ectomy (Csapo et al, 1973c), demonstrating that the luteal hormone which inhibits uterine activity and maintains pregnancy is progesterone rather than oestradiol.

Compounds which reduce levels of endogenous progesterone have long been sought as abortifacients. The synthesis of progesterone can be blocked by a variety of synthetic gestagens and indeed by progesterone itself in vitro (Shinada et al, 1978). However, this effect cannot be exploited therapeutically because of the intrinsic progestational activity of these compounds. The group of 3β hydroxysteroid dehydrogenase inhibitors, epostane, trilostane, azastene and cyanoketone (Sterling Winthrop) inhibit progesterone production but have no agonistic activity themselves; several studies have investigated the use of epostane for menstrual induction. Despite initially promising results (Birgerson et al, 1987; Crooij et al, 1988) this work has not been continued, partly because of the controversy surrounding medical abortifacients. An alternative approach is to block the action of progesterone at the receptor, using an "antiprogesterone" with a chemical structure sufficiently similar to the natural hormone for adequate binding to the receptor, but with some modification which confers antagonistic activity. R2323 (gestrinone) is a trienic steroid which binds to the progesterone receptor and exerts a pronounced antiprogestinic effect. When given to women after ovulation but before implantation it also results in a decrease in serum progesterone and oestradiol 17β (Mora et al, 1975). When given for three days around the time of ovulation it can be used as a contraceptive, with a Pearl index of 9.6% including user failures (Sakiz et al, 1974) although its contraceptive effect in the luteal phase is very weak (Mora et al, 1975). However, decrease in progesterone and oestradiol levels is inhibited by the presence of hCG, and thus it is ineffective as an abortifacient after implantation. Other antiprogestational compounds, ORF 9371 and anordrin, have also been investigated as abortifacients, but are not useful clinically either because of low efficacy in vivo, or because of androgenic side effects (Healy,1985). The discovery that some 11β aryl steroids have antigluocorticoid and antiprogestogenic activity has generated a series of compounds which can be used as abortifacients, including mifepristone (Roussel Uclaf), ZK 987 34 and ZK 98 299 (Schering AG) (Teutsch, 1985) (Figure 1.1). A single report into the use of ZK 98734 as an abortifacient in women has been published (Kovacs et al, 1989). The data on the use (of ZK 98 734) of mifepristone is more extensive, and is reviewed below.

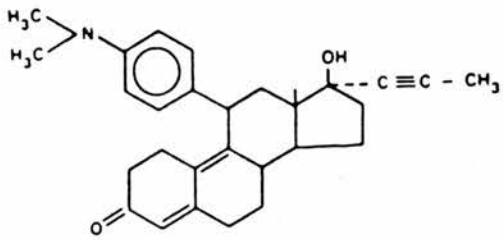
Figure 1.1.



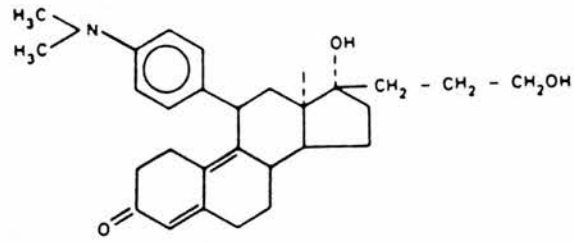
Progesterone



ZK 98 734



RU486



ZK 98 299

Structure of progesterone and antiprogesterone.

In the first clinical study of mifepristone in human pregnancy, eleven women of less than 56 days amenorrhoea requesting legal termination of pregnancy were given mifepristone 200 mg/day for 4 days (Herrmann et al, 1982). Eight women aborted completely, there was one incomplete abortion and two ongoing pregnancies. Although the overall success rate with mifepristone alone seemed promising, the patient with an incomplete abortion bled heavily and required a blood transfusion. Fortunately this has been an infrequent occurrence in subsequent trials. Further trials have confirmed similar success rates, with complete abortion occurring in around 60% in women of up to eight weeks gestation (Sitruk-Ware et al, 1985; Vervest and Haspels, 1985; Shoupe et al, 1986; Cameron et al, 1986; Mishell et al, 1987; Birgerson and Odlind, 1988). Modification of the regime of administration, and of the total dose given does not improve efficacy. However when treatment is restricted to women within 14 days of the first missed menses, a higher complete abortion rate of 84-85% can be achieved (Couzinet et al, 1986; Ulmann, 1987). The inverse relationship between gestation and complete abortion rate is also illustrated in a study including women of 56-70 days amenorrhoea, treated with 200 mg mifepristone daily for a total of 4 days; only 3 out of 9 women at this later gestation aborted completely

following treatment (Vervest and Haspels, 1985). Within each study, there appeared to be few differences between women who abort completely and those who do not. Plasma levels of mifepristone were the same in groups of responders and non responders (Couzinet et al, 1986; Mishell et al, 1987), although in a group of women of less than 49 days amenorrhoea, low βhCG levels (<15,000 IU/l) and small gestational sac diameters (<10 mm) were found to correlate best with a successful outcome (Sitruk-Ware et al, 1990).

The abortion rate following mifepristone alone in the first trimester is therefore insufficient for routine clinical use. Earlier studies on lute-ectomy have shown that progesterone withdrawal not only induces uterine activity, but also sensitises the uterus to the action of oxytocic agents (Csapo et al, 1973b). Following progesterone inhibition with mifepristone, an increase in uterine activity and an increased sensitivity to exogenous prostaglandins can be demonstrated (Bygdeman and Swahn, 1985; Swahn and Bygdeman, 1988). This effect can be exploited to improve on the complete abortion rate achieved using mifepristone alone and was initially demonstrated in a study of 34 women of up to 49 days amenorrhoea treated with mifepristone 25 mg b.d for 4 days, and 0.25 mg sulprostone intramuscularly on the fourth day, 32 of whom aborted completely (Bygdeman and Swahn, 1985). The complete abortion rate of 94% is similar to the success rate seen using sulprostone alone for menstrual induction (Bygdeman et al, 1980), but pretreatment with mifepristone allows one sixth of the dose of sulprostone to be used. The improved abortion rate when mifepristone is used in combination with prostaglandins, compared to the use of mifepristone alone, has been confirmed in other studies using a combination of mifepristone and sulprostone, with abortion rates of 89-95% achieved in women of less than 50 days amenorrhoea (Swahn and Bygdeman, 1989; World Health Organization Task Force, 1989). Pretreatment with mifepristone for 3 days prior to treatment with sulprostone appears to be as effective as pretreatment for 4-6 days. Other prostaglandin analogues have also been used in combination with mifepristone. Abortion rates of 94-100% (Cameron et al, 1986; Rodger and Baird, 1987; Dubois et al, 1988; UK Multicentre Trial, 1990), can be achieved in women given 0.5-1.0 mg gemeprost 48-72 hours after mifepristone. There was no significant difference in abortion rates between the smaller and larger dose of gemeprost used (0.5mg or 1.0mg) (Rodger et al, 1989).

The use of a prostaglandin administered orally in combination with mifepristone has potential advantages. Firstly, the oral route of administration is more acceptable than vaginal or intramuscular routes. Secondly, it allows for the possibility of self

administration. However, the combination of oral PGE_2 (1-2mg) and mifepristone (25 mg bd for four days) was initially no more successful than that of mifepristone and placebo (Swahn et al, 1989), with an overall complete abortion rate of 59%. One of the newer prostaglandins, 9 methylene PGE_2 seems to be more active orally, with an abortion rate of 95% achieved using 600 mg mifepristone followed 3 and 4 days later by 10 mg 9-methylene PGE_2 (Swahn et al 1990). Another oral prostaglandin (misoprostol) marketed for the treatment of peptic ulcer has also been used following pretreatment with 600 mg mifepristone, and an overall complete abortion rate of 95% achieved, in women of less than 49 days amenorrhoea (Aubeny and Baulieu, 1991).

Since the combination of mifepristone and prostaglandin allows a smaller total dose of prostaglandin be given compared to the use of prostaglandin alone for menstrual induction, prostaglandin related side effects are reduced. Mifepristone alone appears to cause few side effects other than vaginal bleeding. Following large doses of mifepristone (400 mg/day for four days) headache occurs in up to 30% of women (Shoupe et al, 1986) and nausea and vomiting in up to 80%. However, these latter are common pregnancy symptoms, so that only a small proportion of these symptoms may be attributable to mifepristone (Cameron et al, 1986; World Health Organization Task Force, 1989; World Health Organization Task Force, 1990). Whilst the stimulatory effects of mifepristone on uterine activity may induce abdominal cramps, opiate analgesia is not required. When mifepristone is followed by a prostaglandin, prostaglandin related gastrointestinal side effects and abdominal pain (presumably due to increased uterine activity) become a problem. In a study of over 500 women given mifepristone and gemeprost, 26% and 13% of patients reported vomiting and diarrhoea respectively as a new symptom in the 4 hours following prostaglandin treatment, and 28% required opiate analgesia (UK Multicentre Trial, 1990). In a large French trial of over 2000 women, the reported incidence of vomiting and diarrhoea was 15% and 7% respectively, and the overall requirement for opiate analgesia was 1% (Silvestre et al, 1990). However, some of these women were given premedication and so these data are likely to underestimate the side effects of mifepristone. The requirement for opiate analgesia is related to prostaglandin dosage (Silvestre et al, 1990), and since smaller doses of prostaglandins are as effective as the larger doses used in these studies (Rodger et al, 1989; Silvestre et al, 1990), this offers a method of reducing side effects without compromising efficacy. Median blood loss during abortion using mifepristone and gemeprost up to 56 days amenorrhoea has been found to be 81 ml in a series of 13 women (range 32-222 ml) (Cameron et al, 1986) and 71 ml in a series of

206 women (range 14-512 ml) (Rodger and Baird, 1989). Duration of amenorrhoea has a major influence on blood loss, such that the median blood loss in 7 women of between 56-63 days amenorrhoea was 154 ml (range 84-341 ml) (Rodger and Baird 1989). The requirement for blood transfusion was 0.1% in the first 10 000 women treated in France from October 1988 (Aubeny, 1990).

If menstrual induction is to be more widely adopted, it should be shown to be at least as safe as vacuum aspiration. Few studies have compared the long term effects of menstrual induction and vacuum aspiration. In an analysis of 132 women in their second pregnancy who had previously undergone menstrual induction with intrauterine prostaglandin, the prematurity rate was 8% (Moscary and Csapo, 1978). The authors compare this to Hungarian data showing a greater prematurity rate (of 13-20%) in women who have previously undergone therapeutic abortion, mostly by dilation and curettage (Csapo, 1973). However, a randomised comparison was not performed, and accepted data fails to demonstrate an increased prematurity rate following termination of pregnancy in the first trimester (World Health Organization Task Force, 1979). There is no data on the long term effects of menstrual induction using a regime of prostaglandins and mifepristone.

Increasing experience with the use of mifepristone and a prostaglandin for menstrual induction has shown the combination to be safe and effective. However, the minimum effective dose of mifepristone in combination with prostaglandin is unknown. In Chapter 2, the results of a double blind randomised trial comparing a single dose of 200 mg, 400 mg or 600 mg mifepristone in combination with a single 1mg dose of gemeprost are reported. Where mifepristone is unavailable, or contraindicated, there is a need for another effective agent for menstrual induction. Chapter 3 describes a study to determine the efficacy and side effects of a new regime of gemeprost (a PGE_1 analogue) for menstrual induction, and the efficacy of this compared to menstrual induction with 200-600 mg mifepristone and 1 mg gemeprost. In Chapter 4, a study to quantify the effect of the orally active prostaglandin misoprostol on uterine activity in early pregnancy is reported, and the efficacy of misoprostol for early abortion, either alone or in combination with mifepristone, investigated.

Part 2:

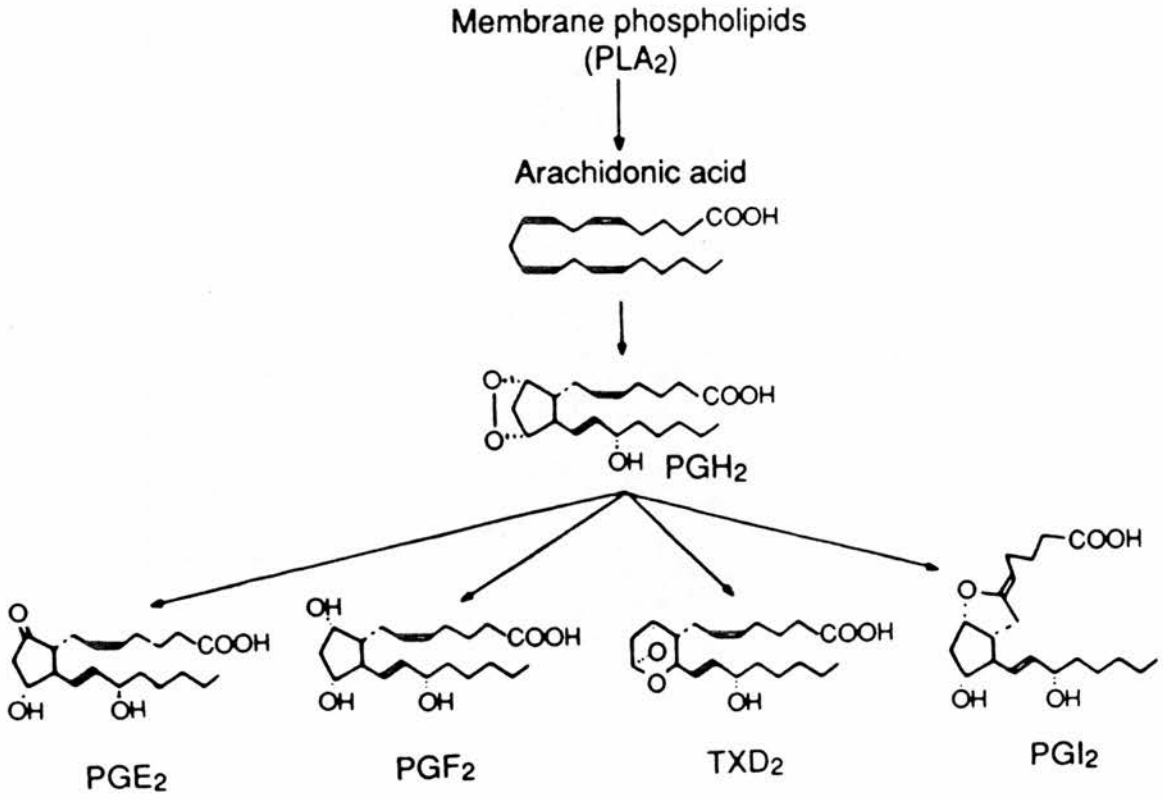
Mechanism of action of mifepristone in menstrual induction.

Although the importance of progesterone in maintaining early pregnancy, and the development of uterine activity often sufficient to induce abortion when progesterone is removed (as in lute-ectomy) is clear, the mechanism of action of mifepristone in inducing abortion is unknown. At least three mechanisms may operate, either alone or in combination. Firstly, there is evidence that mifepristone causes ultrastructural changes in the endothelium of decidual capillaries (Schindler et al, 1985). Baulieu has proposed that this is evidence of vascular damage induced by mifepristone, leading to bleeding, and detachment of the conceptus (Baulieu, 1985). Secondly, administration of mifepristone results in the generation of spontaneous uterine activity (Bygdeman and Swahn, 1985; Swahn and Bygdeman, 1988), and an increased sensitivity to exogenous oxytocic agents. This latter effect accounts for the increase in complete abortion rate when mifepristone is followed by a small dose of prostaglandin which would be ineffective when used alone. Thirdly, mifepristone provokes cervical dilation and ripening, so that a reduced force is necessary to dilate the cervix following treatment with mifepristone compared with placebo (Rådestad et al, 1988). In vivo these effects could act in combination, so that mifepristone would induce decidual damage and separation of the conceptus, and expulsion of the products of conception would be engendered by spontaneous uterine activity and reduced cervical resistance. A further mechanism has been proposed, arising from observations that mifepristone inhibits trophoblastic production of βhCG and progesterone in vitro (Bischof et al, 1986, Das and Catt, 1987), and from evidence that mifepristone inhibits progesterone production from granulosa cells in vitro (DiMattina et al 1986). However, during abortion induced by mifepristone and prostaglandin, hCG and progesterone levels fail to decline until prostaglandin is given and the conceptus is expelled (World Health Organization Task Force, 1989), suggesting that mifepristone does not have any direct effect on production of these hormones in vivo.

It is unclear whether the effects of mifepristone in early pregnancy described above are direct effects, or whether they operate via an intermediate mechanism. Mifepristone has been shown to stimulate decidual prostaglandin production in vitro (Smith and Kelly, 1987) and if mifepristone also stimulates uterine prostaglandin production in vivo, this would explain many of the observed effects of mifepristone in vivo. In Chapter 5, the effects of mifepristone on decidual prostaglandin production in vivo have been

investigated. Firstly, it is appropriate to review the synthesis and metabolism of the prostaglandins, and the known effects of progesterone and the antiprogesterone mifepristone on these pathways.

Figure 1.2



Synthesis of prostaglandins from membrane phospholipids.

Prostaglandin synthesis and metabolism is illustrated in Figure 1.2. The first step in prostaglandin production is the liberation of arachidonic acid mainly from cell membrane phospholipids, this reaction being catalysed primarily by phospholipase A_2 (PLA_2). Free arachidonic acid is then converted (under the influence of the cyclo-oxygenase enzyme system) to one of the cyclic endoperoxides (PGH_2 and PGG_2), these are then quickly converted into the primary prostaglandins $PGF_{2\alpha}$ and PGE_2. Metabolism of $PGF_{2\alpha}$ and PGE_2 proceeds via oxidation at C15 under the action of 15-hydroxyprostaglandin dehydrogenase, followed by reduction of the double bond between C13 and C14 under the action of D^{13} reductase, to give a 15 keto, 13 14 dihydro derivative (Gréen 1986). Progesterone may influence prostaglandin production at a number of sites in this pathway. There is evidence that progesterone

has a priming effect on prostaglandin production by laying down stores of arachidonic acid which can be used as a precursor for prostaglandin production. In vitro pretreatment with progesterone and arachidonic acid results in a subsequently greater ability of the tissue to generate $PGF_{2\alpha}$ and PGE_2 compared with pretreatment with arachidonic acid alone (Kelly et al, 1986a). The evidence that this effect also operates in vivo can be inferred from studies on persistent proliferative endometrium (taken from women with heavy irregular menses, suggesting the presence of anovulatory cycles, and a lack of exposure to progesterone) which has a lower endogenous concentration of $PGF_{2\alpha}$ than normal secretory endometrium (Smith et al, 1982), although when persistent proliferative endometrium was incubated with arachidonic acid, production of $PGF_{2\alpha}$ and PGE_2 increased to that of normal secretory endometrium. The authors concluded that persistent proliferative endometrium has the same synthetic capacity as secretory endometrium, but lacks endogenous arachidonic acid required as a precursor for prostaglandin synthesis. Paradoxically, progesterone has a direct inhibitory effect on prostaglandin synthesis. $PGF_{2\alpha}$ and PGE_2 production from both secretory and proliferative endometrium in organ culture is decreased by the addition of 5-500 ng/ml progesterone to the culture medium (Abel and Baird, 1980). Similar effects of progesterone on $PGF_{2\alpha}$ production were seen using isolated fragments of proliferative endometria in tissue culture (Kelly and Smith, 1987a). The inhibitory effect of progesterone was not overcome by the addition of arachidonic acid suggesting that progesterone inhibits prostaglandin synthetic activity. Similar mechanisms appear to operate in the myometrium (Jeremy and Dandona, 1986). Progesterone may also inhibit prostaglandin production via an increase in lysosomal membrane stability (Dawood, 1981), thus reducing availability of PLA_2. The inhibitory effect of progesterone on prostaglandin production which has been demonstrated in vitro also appears to operate in vivo during pregnancy, with a decrease in decidual prostaglandin production in both intrauterine (Maathuis and Kelly, 1978) and ectopic pregnancy (Abel et al, 1980), although a similar inhibitory effect of progesterone does not appear to operate in the second half of the menstrual cycle (Maathius and Kelly, 1978).

Prostaglandin synthesis and metabolism may be linked such that pathophysiological processes resulting in increased prostaglandin production also result in decreased prostaglandin metabolism, and vice versa (Hoult and Moore, 1979). In vitro, progesterone not only inhibits prostaglandin synthesis, but also increases prostaglandin metabolism: pretreatment of stromal cells in tissue culture with progesterone increases the conversion of PGE_2 and $PGF_{2\alpha}$ to their 13,14 dihydro

15 keto metabolites (Kelly et al, 1986a). The stimulatory effect of progesterone on prostaglandin metabolism also operates in vivo. The activity of endometrial 15-hydroxyprostaglandin dehydrogenase is highest during the secretory phase of the menstrual cycle when progesterone dominates, and low in anovulatory cycles, where progesterone is lacking (Casey et al, 1980). In pregnancy between 5 and 14 weeks gestation, there is a close correlation between placental 15-hydroxy prostaglandin dehydrogenase activity and the placental progesterone concentration (Falkay and Sas, 1978).

Several studies have investigated the effects of the antiprogesterone mifepristone on prostaglandin production in vitro. Mifepristone stimulated $PGF_{2\alpha}$ and PGE_2 production from endometrial stromal cells in tissue culture (Kelly et al, 1986a) and this increase was competitively inhibited by progesterone. When separated decidual glandular cells from pregnant women in the first trimester were cultured in vitro with mifepristone, an increase in $PGF_{2\alpha}$ production compared with control was also seen (Smith and Kelly, 1987). This occurred both in the presence and absence of arachidonic acid, suggesting an effect of mifepristone on the cyclooxygenase enzyme. In contrast to these findings, and the findings expected by extrapolation from the action of progesterone in vitro, incubation of fragments of secretory endometrium with mifepristone alone resulted in a significant decrease in $PGF_{2\alpha}$ and PGE_2 production, suggesting that mifepristone acted as a progesterone agonist in this situation (Kelly and Smith, 1987b). The reasons for these apparently contradictory findings are not clear.

Few studies on the effects of mifepristone administered in vivo on prostaglandin production have been published. In recent studies on the effect of oral mifepristone in pregnant women, there was no increase in serum levels of prostaglandin metabolites 24-48 hours after mifepristone administration, either in the first (Hill et al, 1990a) or in the second (Hill et al, 1990b) trimester. The authors conclude that mifepristone in vivo has no effect on decidual prostaglandin production. However, since mifepristone inhibits prostaglandin metabolism in addition to stimulating prostaglandin production in vitro, a mifepristone-induced increase in decidual prostaglandin production may not result in increased prostaglandin metabolite levels in the serum. Data using the guinea-pig model, thought by some to be a good model for the action of mifepristone in the human (Elger et al, 1986), supports in vitro work suggesting an inhibitory effect of mifepristone on prostaglandin metabolism in vivo. A recent study using this model shows that a single 10 mg dose of mifepristone in vivo inhibited the subsequent ability of the myometrium / decidua and of the chorion to

metabolise prostaglandins in vitro (Kelly and Bukman, 1990), although there was no significant effect of mifepristone pretreatment on the capacity of these tissues to generate prostaglandins in tissue culture.

In general, therefore, progesterone inhibits decidual prostaglandin production, and mifepristone stimulates prostaglandin production in vitro. In vivo effects of mifepristone on prostaglandin production are yet to be fully determined, although there was no increase in peripheral prostaglandin metabolite levels following treatment with mifepristone. In Chapter 5, the results of a study to determine the effects of mifepristone in vivo by measuring the ability of decidua harvested from women pretreated with mifepristone to generate prostaglandins in culture are reported.

Mifepristone also stimulates uterine activity, and in Chapter 6 the mechanism of action of this effect is investigated further. Firstly, it is appropriate to consider the physiology of uterine contractility, and the mechanism of action of progesterone and the prostaglandins on the inhibition and stimulation respectively of uterine activity. The stimulation of uterine activity during pregnancy depends on the transformation of the uterus from a quiescent to an actively contracting organ. The functional units of uterine activity are the billions of small muscle cells which make up the myometrium. Individual cells are 2-10 μm wide and 50-800 μm long, and are arranged as bundles of 10-50 cells, oriented along the long axis of the bundle (Finn and Porter, 1975). These compose the myometrium, with its two distinct layers of smooth muscle, the inner circular layer and the outer longitudinal layer. Communication between individual smooth muscle cells is achieved through "gap junctions". These intercellular channels allow the passage of inorganic ions and small molecules of less than 1.6-2.0 nm diameter (Schwarzmann et al, 1981).

The stimulation of uterine activity involves the generation of an action potential in an individual muscle cell, and the conversion of this action potential into a contractile force. In addition, for uterine activity to be effective in dilating the cervix and expelling uterine contents, the myometrium has to function as a syncitium. Propagation of the action potential into other muscle cells, and the orderly spread of uterine activity throughout the uterus is also required, so that a wave of contraction can impart directional force to the uterine contents. Uterine activity is controlled through myogenic, neurogenic and hormonal mechanisms. The intrinsic properties of the uterine smooth muscle are such that the uterus is able to contract spontaneously in the absence of any other input. Although the myometrium is innervated by post

ganglionic fibres from the autonomic nervous system, neurogenic mechanisms are thought to play little if any part in the control of uterine activity. However, the observation that adrenergic nerves disappear near term during human pregnancy (Thorbert et al, 1979), suggests that neurogenic mechanisms may be more important than previously thought. The predominant controlling factors in the regulation of uterine activity appear to be hormonal. In general, progesterone and relaxin inhibit uterine activity, whereas oestrogen and oxytocin stimulate uterine activity.

The prostaglandins are a heterogeneous group, some of which ($PGF_{2\alpha}$, PGE_2) stimulate and others (PGI_2) inhibit uterine activity (Challis and Lye, 1986). The evidence for the suppressive effect of progesterone on uterine activity has been discussed in Part 1 of this chapter (Csapo et al, 1973b). The mechanism by which progesterone may control uterine activity can be inferred from in vitro studies. Progesterone causes hyperpolarisation of smooth muscle cells in vitro, inhibiting uterine activity by increasing the change in membrane potential required before an action potential can be stimulated (Marshall, 1959). Since there is decreased spontaneous electrical activity in areas of the myometrium overlying the placenta (Csapo 1969), these effects may also operate in vivo. Progesterone may not only inhibit action potential formation but also inhibit the conversion of these action potentials to active contractions by affecting availability of calcium (Carsten, 1979). Progesterone also appears to inhibit electrical coupling in the myometrium, as evidenced by an increase in junctional resistance in conditions of progesterone dominance (Ichikawa and Bortoff, 1970; Bortoff and Gilloteaux, 1980), presumably by inhibition of gap junction formation. Thus the propagation of action potentials into other smooth muscle cells, and the orderly spread of uterine activity throughout the uterus is inhibited. In addition progesterone alters uterine activity via effects on other oxytocic agents; it inhibits prostaglandin synthesis as discussed in Part 1, and inhibits the replenishment of oestrogen receptors, thus inhibiting the stimulatory effects of oestrogens on uterine activity (Clark and Peck, 1979).

The effect of prostaglandins on uterine contractility are mediated by mechanisms controlling intracellular calcium levels. Prostaglandins stimulate intracellular calcium release (Grosset and Mirroneau, 1977) and inhibit active calcium extrusion from the cell (Popescu et al, 1984) both of which effects increase the resting tension of the smooth muscle cell.

Chapter 6 describes a study to determine whether the mechanism of action of the antiprogesterone mifepristone in stimulating uterine activity depends mainly on an increase in uterine prostaglandin production, or on other mechanisms, by using indomethacin to inhibit endogenous prostaglandin production in women treated with mifepristone.

In addition to its effects on the myometrium and endometrium, mifepristone also induces cervical softening and dilation (World Health Organization Task Force, 1990; Cohn and Stewart 1991), an action which will contribute to its abortifacient effect. For abortion to occur, the cervix has to efface and dilate under the influence of uterine contractions. "Ripening" of the cervix reduces the force required for this, either when generated in vivo from uterine activity or artificially with cervical dilators. Either in early pregnancy to facilitate surgical abortion, or in late pregnancy to induce labour, cervical ripening can be induced pharmacologically by agents such as prostaglandins (Calder et al, 1977), oestrogen (Gordon and Calder, 1977), relaxin (MacLennan et al, 1980) or laminaria tents (Johnson, 1989). The physiological mechanisms underlying cervical ripening are not clear, but may involve changes in collagen and glycosaminoglycan concentrations in the cervix.

The human cervix is composed of collagen fibres, elastin, a matrix of proteoglycans containing glycosaminoglycan (GAG) chains extending out from a protein core, cellular elements dominated by smooth muscle cells and fibroblasts, and water (Dobson, 1988). The relative proportions of these substances change during pregnancy, with an increase in water content with increasing gestation (Uldbjerg et al, 1983a), and a greater collagen content (expressed as a proportion of the dry weight of the cervix) in pregnant compared with non - pregnant women (Danforth et al, 1974; Granström et al, 1989). Both these effects reduce the mechanical strength of the tissue. During cervical ripening, histological changes in cervical collagen can be observed, with a lower proportion of intact collagen fibres in dilated cervices, using those from non pregnant women as a control (Junqueira et al, 1980). The mechanism of collagen breakdown during cervical ripening is not certain, but may involve collagenolysis induced by collagenase, generated by fibroblast and leucocytes; and leucocyte elastase, from polymorphonuclear leucocytes (Uldbjerg et al 1983b). Leucocyte elastase increases steadily during pregnancy and is at a maximum post partum (Uldbjerg et al, 1983b). In addition, the ability of the cervix to breakdown a synthetic DNP-peptide having the same amino acid composition as the collagenase-sensitive part of the tropocollagen molecule increases during pregnancy,

suggesting increasing collagenase activity (Uldbjerg et al, 1983b). Although direct evidence is lacking, it seems likely that cervical ripening is associated with activity of proteolytic enzymes particularly that of collagenase, leading to collagen breakdown and, therefore, a decrease in mechanical strength of the cervix. Changes in the glycosaminoglycan concentration of the cervix may also contribute to cervical ripening, with an increase in keratan sulphate (associated with a decrease in diameter of collagen fibrils, [Borcherding et al, 1975]), an increase in hyaluronate concentrations (associated with an increase in tissue compliance, [Bryant et al, 1968]), and a fall in chondroitin sulphate (associated with a reduction in rigidity of cervical tissue, [Bryant et al, 1968]) being observed during pregnancy (Von Maillot et al, 1979).

Biochemical changes in the cervix are also observed during pharmacologically induced cervical ripening. Prostaglandin E_2 increases DNP-peptide hydrolytic activity, and therefore by inference collagenase activity (Uldbjerg et al, 1983b), and alters the proportions of GAGs in cervical tissue (Norström, 1984). Intravenous administration of the oestradiol precursor dehydroepiandrosterone sulphate increases cervical compliance. This change is associated with an increase in collagenase activity in vivo (Mochizuki and Tojo, 1980), although in vitro, oestradiol appears to inhibit collagenase activity (Wallis and Hillier, 1981). The reasons for this difference are unclear, but may be due in part to infiltration of leucocytes containing collagenase in vivo (Basset, 1962) an effect which cannot occur in vitro. In the sheep, progesterone concentrations correlate with cervical resistance (Fitzpatrick and Dobson, 1979). Possible mechanisms are via a reduction in collagenase activity (Jeffrey and Koob, 1980) or via leucoytic migration (Dobson, 1988). Lastly, the stimulatory effects of relaxin on cervical softening (MacLennan et al, 1980) may be mediated via changes in fibroblast activity, since relaxin appears to stimulate mitosis of fibroblasts in culture (McMurtry et al, 1980).

Although the ripening effect of mifepristone on the cervix is well established, few studies have investigated the mechanism of this. Extrapolating from the effects of mifepristone on the decidua (Chapter 5) and myometrium (Jeremy and Dandona, 1986), it is tempting to speculate that mifepristone stimulates cervical prostaglandin production. This would account for the known effects of mifepristone on the cervix. The limited evidence available, however, fails to show an increased bioconversion of radiolabelled arachidonic acid in vitro, by cervical biopsies taken from women pretreated with mifepristone in vivo (Rådestad et al, 1990).

In Chapter 7 of this thesis, the results of a quantitative study to compare the effect of mifepristone versus placebo on the force required for cervical dilation, and studies to determine the role of cervical production of prostaglandins, and collagen and glycosaminoacid breakdown are described.

In summary, the work in this thesis aims to investigate further the methods and mechanisms of action of agents used for menstrual induction, principally those of the antiprogesterone mifepristone.

Chapter 2:

The use of mifepristone (three different doses) and gemeprost to induce abortion in women of up to eight weeks amenorrhoea.

Introduction

The use of mifepristone in combination with gemeprost provides an effective agent for menstrual induction. Since mifepristone was made available in France in October 1988, over 80,000 women of up to 49 days amenorrhoea have been treated, in a protocol using a single 600 mg dose of mifepristone followed 48 hours later by a prostaglandin (usually sulprostone). Mifepristone was granted a product licence in the UK by the Committee of Safety of Medicines in the summer of 1991, for medical termination of pregnancy up to a maximum gestation of 63 days. The data sheet recommends a dose of 600 mg mifepristone followed 36-48 hours later by 1 mg gemeprost. Experience in the UK shows a complete abortion rate of 94% can be achieved with this latter protocol (UK Multicentre Trial, 1990). The minimum effective dose of mifepristone in combination with gemeprost is, however, unknown. Previous studies have shown no differences in efficacy between 400 mg, 500 mg or 600 mg mifepristone (Rodger and Baird, 1987); and a WHO supported trial has shown that small doses of mifepristone (5 x 25 mg) were as effective as a single 600 mg dose when followed by 0.5 - 1 mg gemeprost (World Health Organization Task Force, 1991). Although mifepristone itself is reported to induce few side effects, the use of an unnecessarily large dose of mifepristone has both cost and toxicological implications. In this chapter, the effects of 200 mg, 400 mg or 600 mg mifepristone each followed 48 hours later by 1 mg of gemeprost, are compared. This study was part of a multicentre trial under the auspices of the WHO, the results of which have not yet been published.

Methods

One hundred and fifty women of ≤ 56 days amenorrhoea undergoing legal termination of pregnancy were randomised to receive either 200 mg, 400 mg or 600 mg oral mifepristone (Roussel Uclaf) followed forty eight hours later by 1 mg of vaginal gemeprost (a PGE_1 analogue). Women were followed up one, two, six and ten weeks after mifepristone or until the next menstrual period. Completeness of abortion was the

main outcome measure. In cases of incomplete abortion or ongoing pregnancy, the uterus was evacuated surgically. The protocol was approved by the local Reproductive Medicine Ethical Subcommittee.

Recruitment.

General Practitioners and Family Planning Clinics in the area were asked to refer pregnant women of ≤ 56 days of amenorrhoea requesting therapeutic abortion, who had grounds for termination of pregnancy under the 1967 Abortion Act. Patients were initially seen in the gynaecology outpatient clinic. Their reasons for requesting termination of pregnancy were ascertained, and they were counselled in the usual manner. A full history was taken and general physical and pelvic examination performed. A cervical smear was taken if appropriate, a high vaginal swab taken for bacteriological culture and endocervical swab taken for culture for chlamydia. Once the decision was made to terminate the pregnancy, women who met the study criteria were offered medical termination of pregnancy using mifepristone and gemeprost. Details of the study were given both verbally and in the form of an information sheet. Written consent was obtained and patients were informed of their right to withdraw from the study without prejudice to their future medical care. An admission date was scheduled for mifepristone administration at the patient's convenience, usually on the same day or the day following the clinic visit.

Mifepristone administration.

On admission (day 1), details of the patient's menstrual cycle, the date of the last menstrual period, and previous obstetric and medical history were recorded on a data sheet. Women were specifically asked if they had had any one of six common pregnancy or prostaglandin related side effects since their last menstrual period (nausea, vomiting, dizziness, fatigue, lower abdominal pain and breast tenderness) for comparison before and after mifepristone and after prostaglandin. Blood pressure, pulse rate, height and weight together with details of physical examination were recorded. A pelvic ultrasound examination was performed, mean gestational sac diameter, fetal crown rump length (if present) and the presence or absence of the fetal heart was noted. Blood was taken for typing of the patient's blood group, haemoglobin and serum human chorionic gonadotrophin estimation.

Exclusion criteria for the study were :

- age less than sixteen
- recent irregular menstrual cycles
- menstrual delay of less than seven or more than twenty-eight days
- ectopic pregnancy
- chronic disease affecting the respiratory, gastrointestinal, endocrine, genitourinary, neurological or cardiovascular system
- contraindications to mifepristone or prostaglandins
- history of thromboembolism
- regular use of prescription drugs or the oral contraceptive pill in the cycle before conception or the conception cycle

A single oral dose of 200 mg, 400 mg or 600 mg of mifepristone was given. The mifepristone dosage was allocated in a randomised fashion, randomisation being performed by the World Health Organization in Geneva. The patients and investigator were blind to the randomisation. Patients were allocated to numbered bottles of tablets each containing a mixture of placebo tablets and tablets containing 200 mg mifepristone in order of recruitment into the study. The bottles of tablets were made up by the WHO in Geneva, or with total dose of 200 · mg, 400 mg or 600 mg mifepristone randomly distributed between them. Fifty bottles of each type were prepared and the tablets were swallowed with water after the patient had fasted for at least one hour. Women were allowed home immediately after taking the tablets, given a menstrual diary card to record the pattern of vaginal bleeding and asked to avoid the use of non steroidal anti-inflammatory drugs.

Prostaglandin administration

On readmission forty eight hours after mifepristone administration (day 3), details of vaginal bleeding, blood pressure and pulse rate were recorded, and blood taken for measurement of haemoglobin and serum human chorionic gonadotrophin The pregnancy / prostaglandin related symptom questionnaire was repeated. A single gemeprost pessary (1 mg) was inserted into the posterior vaginal fornix. Patients were asked to remain in bed for one hour after prostaglandin administration, and were then encouraged to mobilise or to sit up as they wished.

Following prostaglandin administration, blood pressure, pulse rate and temperature were recorded hourly. The presence or absence over the preceding hour of the series of

pregnancy / prostaglandin related symptoms was recorded. Analgesia was given as required, either oral paracetamol, oral or intramuscular dihydrocodeine, or intramuscular diamorphine (with cyclizine) as appropriate. Most patients who requested pain relief were given a mild analgesic initially, and then opiates if necessary. A few patients in more severe pain were given diamorphine as an initial analgesic. Non steroidal anti-inflammatory drugs were avoided because of theoretical inhibitory effects on the action of mifepristone and gemeprost via effects on prostaglandin production and metabolism. Many patients were aware when abortion occurred, or passed products of conception into a bedpan. Otherwise, patients were examined at the end of four hours to determine whether abortion had occurred, from examination of uterine size and of the vagina for products of conception. Patients were then allowed home if well and accompanied by an adult, some patients, especially those who had been given narcotic analgesia, stayed in hospital for a further few hours. Immunoprophylaxis (250 IU IgG to the rhesus D antigen) was given to rhesus negative patients. A follow up appointment was given for day 8 (ie one week after mifepristone administration). Women were asked to use non-hormonal methods of contraception until the first menstrual period after treatment.

Follow up.

At the follow up examination (day 8), the menstrual diary card was reviewed and the incidence of complications and requirement for medical attention or drug therapy noted. Blood pressure and pulse rate were recorded and pelvic examination performed. Blood was taken for measurement of haemoglobin and serum human chorionic gonadotrophin. The patient was given a further follow up appointment for one week later.

At the second follow up appointment (day 15), a similar protocol was employed, and in addition, a pelvic ultrasound scan was performed. Those patients with an ongoing pregnancy (rising serum human chorionic gonadotrophin and a gestational sac of increasing diameter on pelvic ultrasound scan with or without an active fetal heart), or a missed abortion (near constant level of serum human chorionic gonadotrophin, a gestational sac on ultrasound scan and no fetal heart activity) underwent surgical uterine evacuation. Those few patients with an incomplete abortion (slowly falling serum human chorionic gonadotrophin and products of conception visible in the uterus on ultrasound scan) were managed conservatively or underwent surgical termination of pregnancy, depending on their clinical condition.

Patients were seen again on day 43 and 71 until abortion was complete, and menstruation had occurred. The duration of bleeding and date of menstruation were noted. Once bleeding had stopped, patients were asked to compare the whole bleeding episode compared with their normal menses.

Blood samples.

Haemoglobin and serum hCG concentrations were measured by the Haematology Laboratory of the Royal Infirmary of Edinburgh, and the Reproductive Endocrine Laboratory in the Centre for Reproductive Biology respectively.

Data calculation

When follow up on all the patients was complete, data was entered into the "Excel" programme on an Apple Macintosh computer. The randomisation code was broken. Data on the fifty patients in each group were compared with each of the other two groups using "CLR anova" and "Statview 512".

Statistics.

Normally distributed data (weight, height, duration of amenorrhoea, and haemoglobin) were compared using analysis of variance. Kruskal Wallis testing was used for continuous data which were not normally distributed (interval to vaginal bleeding following mifepristone and before prostaglandin, time to abortion and change in haemoglobin and human chorionic gonadotrophin levels). Categorical data was analysed using the Chi squared test. Continuous data were plotted prior to analysis to determine the type of distribution of the data. In some instances (age, sac diameter, duration of vaginal bleeding and interval to next menses) the data were log transformed before analysis to generate a normal distribution. Serial data on the same patients have been compared using a paired t test for normally distributed data (haemoglobin) or the Wilcoxon Rank Sum test for data which were not normally distributed (human chorionic gonadotrophin). For normally distributed data, the mean value and the 95% confidence intervals have been quoted. For data analysed using non parametric methods, the median and the range of values have been quoted.

Results.

Patient characteristics

There were no significant differences between the three treatment groups in terms of patients characteristics on admission to the study (Table 2.1a and b).

Table 2.1a

Characteristic	200 mg	400 mg	600 mg
Age (years)	27.3 (25.7 - 28.9)	29.2 (25.8 - 30.2)	25.8 (24.0 - 27.6)
Height (cm)	164 (162 - 166)	164 (163 - 166)	165 (164 - 167)
Weight (kg)	61.1 (58.7 - 63.4)	60.0 (58.0 - 62.1)	61.6 (59.0 - 64.2)
Gestation (days)	46.7 (45.3 - 48.2)	47.3 (45.9 - 48.8)	47.8 (46.3 - 49.2)
Parous	40%	58%	48%
Previous live birth	32%	52%	38%

Characteristics of patients in each of the three treatment groups - 200, 400 or 600 mg mifepristone - mean (95% C.I), or percentage.

Table 2.1b

Characteristic	200 mg	400 mg	600 mg
Sac diameter (mm)	18.3 (16.6 - 20.1)	20.0 (17.6 - 22.7)	21.5 (19.5 - 23.8)
Fetal heart seen	66%	66%	55%
Hb (g/dl)	13.1 (12.9 - 13.3)	13.3 (13.0 - 13.5)	13.1 (12.8 - 13.3)
hCG (IU/l).	40,360 (1,038 - 125,700)	44,670 (2,194 - 191,000)	48,700 (2,103 - 154,900)

Results of investigations on patients in each of the three treatment groups, 200 , 400 and 600 mg mifepristone; mean sac diameter (95% C.I), presence of fetal heart on ultrasound scan (% age); mean haemoglobin (95% C.I) and median human chorionic gonadotrophin level (range) at initial visit.

Effect of mifepristone

There were no significant differences in the effect of the three mifepristone doses on vaginal bleeding, change in haemoglobin or on human chorionic gonadotrophin concentrations (Table 2.2), nor in the effect on pregnancy or prostaglandin related symptoms (Table 2.5). Fifty-eight percent of women had some vaginal bleeding in the 48 hours interval between mifepristone administration and prostaglandin administration. Median time (range) to bleeding in those women who did bleed was 29 hours (3-47.5). Serum human chorionic gonadotrophin levels increased significantly from before to 48 hours after mifepristone ($p<0.005$).

Table 2.2

	200 mg	400 mg	600 mg
Bleeding	56%	58%	60%
Time to bleed (hrs)	32 (12 - 46)	30 (3 - 47)	27 (16 - 47)
Change in Hb (g/dl)	0.1 (-0.8 - +1.3)	0.0 (-1.6 - +1.4)	0.3 (-0.7 - +1.9)
hCG as (% of original value)	124% (53 - 218)	106% (9 - 207)	106% (22 - 245)

Percentage of patients who bled in the forty eight hours after mifepristone, median interval to bleeding (range) in those who did; median change in haemoglobin concentration (range) over 48 hours following mifepristone and human chorionic gonadotrophin 48 hours after mifepristone, expressed as a percentage of the original value.

There was a significant increase in the percentage of patients who had noted abdominal pain during the forty-eight hours after mifepristone compared with at any time from the last menstrual period to mifepristone administration ($p < 0.02$) There was no significant change in any of the other pregnancy or prostaglandin related symptoms during this period (Table 2.5). There were no differences between the three treatment groups in terms of change of symptoms.

Effect of gemeprost and mifepristone.

One hundred and forty seven patients (98%) aborted completely without further intervention following mifepristone and gemeprost. There were no differences in abortion rates between any of the treatment groups. There was one ongoing pregnancy

(in a woman who had been given 400 mg mifepristone followed by 1 mg gemeprost). This was terminated surgically three weeks after mifepristone administration. One patient (in the 400 mg mifepristone group) underwent surgical uterine evacuation four weeks following mifepristone administration after being referred to another gynaecologist with prolonged vaginal bleeding; serial hCG levels had fallen to 39 IU/l and an ultrasound examination had shown an empty uterus. Products of conception were not obtained at curettage. A third patient (in the 600 mg group) underwent diagnostic curettage and sterilisation after 55 days of vaginal bleeding. Histology of the curettings showed "marked endometritis, occasional foci of decidua but no trophoblastic villi." Eighty three percent of patients aborted an identifiable gestational sac within four hours of prostaglandin administration, at a median time (in those patients who aborted) of 180 minutes after prostaglandin administration (Table 2.3). Analgesia was required by 55% of patients, and 21% of all patients were given diamorphine. There were no significant differences between the treatment groups in terms of products passed or analgesic requirements (Tables 2.3 and 2.4).

Table 2.3

	200 mg	400 mg	600 mg
P.O.C. identified	82%	82%	86%
Time to abortion (mins)	180 (99 - 255)	180 (80 - 240)	200 (60 - 240)

Percentage of women in each treatment group in whom products of conception were passed within four hours of prostaglandin administration, and median (range) time to abortion.

Table 2.4

	200 mg	400 mg	600 mg
None	38%	52%	44%
Paracetamol	18%	12%	16%
Codeine	26%	14%	16%
Diamorphine	18%	22%	24%

Analgesia: percentage of women requiring no analgesia (none), paracetamol alone (paracetamol), codeine ± paracetamol (codeine), diamorphine ± codeine ± paracetamol (diamorpine) in each of the three treatment groups.

There was a significant increase in the incidence of abdominal pain before to four hours after prostaglandin administration ($p < 0.002$) (Table 2.5) . The incidence of

breast tenderness decreased from before to after prostaglandin administration, this reached statistical significance when symptoms before mifepristone and after prostaglandin were compared ($p < 0.005$).

Table 2.5

	Before mifepristone	After mifepristone	After gemeprost
Nausea	64%	67%	66%
Vomiting	23%	24%	33%
Dizziness	27%	27%	35%
Fatigue	72%	69%	75%
Lower abdominal pain	47% [a]	61% [b]	95% [c]
Breast tenderness	67% [d]	57%	51% [e]

Percentage of patients with pregnancy or prostaglandin related side effects from the last menstrual period to immediately prior to mifepristone administration (before mifepristone), from mifepristone administration to immediately prior to prostaglandin administration (after mifepristone), and during the firt four hours after prostaglandin administration (after prostaglandin).
($a < b$, $p < 0.02$; $a < c$, $p < 0.0002$ $b < c$, $p < 0.002$; $d > e$, $p < 0.005$).

Of the one hundred and forty one patients who returned for follow up from day 7 to day 11 inclusive, there was a significant decrease in haemoglobin and in hCG concentration ($p < 0.0001$) compared with concentrations at initial visit. There were no significant differences between the treatment groups in this regard (Table 2.6).

Table 2.6

	200 mg	400 mg	600 mg
Change in Hb (g/dl)	-0.6 (-1.9 - +1.2)	-0.6 (-2.9 - +1.6)	-0.4 (-2.3 - +0.8)
hCG (as %age of original value)	3.36 (0.06 - 10.41)	3.95 (0.03 - 152.63)	2.9 (0.31 - 13.18)

Median (range) change in haemoglobin concentration from prior to mifepristone to one week after mifepristone, median (range) change in serum human chorionic gonadotrophin concentration over the same period, expressed as a percentage of the original value.

Bleeding.

Data on duration of bleeding following mifepristone and prostaglandin have been obtained from 142 patients. Data on three patients who underwent uterine curettage (including the patient with an ongoing pregnancy) have not been included in this analysis, nor have data on a further five patients who defaulted from follow up before bleeding had ceased (although two patients in this latter group were known to have prolonged bleeding). Mean (95% C.I) duration of bleeding in the remaining 142 patients was 13.9 days (12.8-15.2). There were no significant differences in bleeding duration between the treatment groups. Data on interval from mifepristone to the next menses were obtained in 137 patients. Eight patients defaulted from follow up before the scheduled six week visit, one patient attended the six week visit but had not yet menstruated, and failed to attend thereafter. Data from three patients who underwent surgical uterine evacuation were not included in the analysis. One patient conceived again shortly after abortion, prior to menstruation. Mean (95% C.I) interval from mifepristone to menstruation was 35.8 days (34.5-37.1) with no significant differences between the treatment groups (Table 2.7).

Table 2.7

	200 mg	400 mg	600 mg
Duration of bleeding (days)	13.2 (11.2 - 15.6)	14.2 (12.6 - 16.1)	14.4 (12.33 - 16.8)
Interval to next menses (days)	36.2 (34.2 - 38.5)	37.2 (34.7 - 39.8)	34.1 (32.3 - 36.1)

Mean (95% C.I) duration of bleeding and interval from mifepristone administration to the next menses.

Once the bleeding had ceased, patients were asked how the total amount of bleeding compared with their normal menstruation. Data was obtained from 144 patients (three patients who defaulted before the bleeding had ceased, and three patients undergoing surgical uterine evacuation were not included in the analysis). Seventy eight percent of patients described the bleeding as heavier or much heavier than their normal menses. There were no significant differences between any of the treatment groups in this regard (Table 2.8). No patient required a blood transfusion.

Table 2.8

	200 mg	400 mg	600 mg
Lighter	6.4%	4.3%	2.1%
Same	14.9%	17.0%	19.2%
Heavier	40.4%	44.7%	53.2%
Much heavier	38.3%	34.0%	25.5%

Comparison of bleeding with normal menstruation, percentage of each treatment group.

Discussion

The overall complete abortion rate of 98% in this study is similar to other that of other large studies on the use of mifepristone and gemeprost in women of up to 49-63 days amenorrhoea (Rodger and Baird, 1987; Silvestre et al, 1990; UK Multicentre Trial, 1990). In this study the complete abortion rate was similar in each of the three treatment groups, suggesting that pretreatment with 200, 400 and 600 mg mifepristone is equally effective, when followed 48 hours later by 1 mg gemeprost. The similarity in efficacy between the three doses of mifepristone correlates with data showing similar plasma levels of the drug 48 hours after a single dose of between 100 mg and 800 mg in non pregnant women (Heikinheimo et al, 1987), and only a very minor small differences in plasma concentration of mifepristone in pregnant women after 50 mg mifepristone compared with 25 mg mifepristone (Swahn et al, 1986). However, plasma levels of the metabolites of mifepristone are likely to be greater with the higher dose, and since these metabolites have been shown (at least in rats) to have biological activity of up to 30% that of the parent molecule (Deraedt et al, 1985), one would expect greater total antiprogestational activity following the higher dose. In practice, the effectiveness of mifepristone alone as an abortifacient is similar following different dose regimes (Van Look and Bygdeman, 1989) with duration of amenorrhoea being the main predictor of success. (Although the regime of mifepristone used was reported to be the strongest predictor of success in a recent study of 271 women, each regime included a variable proportion of women given either prostaglandin or methylergonovine maleate, as a confounding variable [Grimes et al, 1990]). An increase in uterine activity following mifepristone can be seen with doses as divergent as 25 mg mifepristone b.d and a single dose of 600 mg mifepristone, the change in uterine activity having a greater dependence on the time after treatment, than on the dose of mifepristone used (Swahn and Bygdeman, 1988; Chapter 6). The group of patients studied here was too small to determine minor

differences in success rates between the different dose regimes, at least one hundred and thirty women being needed in each treatment group to show (with 90% power) that either 200 or 400 mg was less effective than 600 mg mifepristone (assuming a success rate in the 600 mg group of around 98% or greater, and a success rate in the other groups of 90% or less). Smaller differences in complete abortion rate between the groups would require even greater numbers to demonstrate any difference. The complete abortion rates in each of the three groups (200 mg, 400 mg and 600 mg mifepristone) were 100%, 96% and 98% respectively. If data from the other participating centres shows similar results, the equivalent efficacy of 200, 400 and 600 mg mifepristone in combination with 1 mg gemeprost can then be claimed more confidently.

Analgesic requirements seen here were similar to those reported in a recent UK trial (UK Multicentre Trial, 1990) using 600 mg mifepristone and 1 mg gemeprost, but higher than those previously reported from this Centre (Rodger and Baird, 1987). However, this latter study included women given only 0.5 mg gemeprost, which results in a lower requirement for analgesia than a 1 mg dose (Rodger et al, 1989). Although the total requirement for analgesia in this study was apparently greater than the largest published French study (55% compared to 21%), the comparison is not valid since in the French study, not only were women of 50-56 days amenorrhoea excluded, but also some women received premedication. Nulliparous women required a significantly greater amount of analgesia than parous women, the requirement for opiate analgesia in each group being 33.8% and 8.2% respectively ($p<0.0002$). This agrees with previously reported data (UK Multicentre Trial, 1990).

Although the incidence of side effects following mifepristone appears high, when side effects before and after treatment were compared, only abdominal pain was shown to increase. An increase in abdominal pain following mifepristone and following mifepristone and prostaglandin has been seen in other studies (Swahn et al, 1989; Cameron et al, 1986), and presumably relates (at least in part) to increased uterine activity. The decrease in breast tenderness following prostaglandin and abortion is likely to be a response to the fall in the levels of hCG, progesterone and oestradiol which is seen following this treatment (World Health Organization Task Force, 1989). Unfortunately, the presence or absence of symptoms was compared over different time periods. The period of up to 56 days prior to mifepristone was compared with the 48 hour period after mifepristone, and the four hour period after prostaglandin

administration. Since patients were asked about the incidence of symptoms, this leads to a bias towards no effect of mifepristone or prostaglandin.

Haemoglobin concentrations decreased significantly by one week after the start of treatment. None of the 150 patients studied here required a blood transfusion, correlating with the known low requirement for blood transfusion (0.1% in the first 10,000 women treated in France from October 1988, [Aubeny 1990]). The duration of bleeding in this study was similar to that previously published (Rodger and Baird, 1987), and appears to be less when gemeprost is used (as in this study), compared to the use of mifepristone and sulprostone (Silvestre et al, 1990). The increase in human chorionic gonadotrophin seen here following mifepristone correlates with results of other workers (World Health Organization Task Force, 1989; World Health Organization Task Force, 1991). Other studies have failed to show a significant rise in human chorionic gonadotrophin 48 hours after mifepristone (Swahn et al, 1989; Kovacs et al, 1984). Since the natural progression of human chorionic gonadotrophin is to increase up to eight weeks gestation (Johnson and Everitt, 1988), a control group of untreated women given placebo would be required for comparison, to determine the effects of mifepristone in isolation.

The mean interval from mifepristone to next menstruation of 36 days seen here is similar to the interval previously reported following menstrual induction either with mifepristone and prostaglandin (Cameron et al, 1986; Swahn and Bygdeman, 1989), or with prostaglandin alone (World Health Organization Task Force, 1987). Studies on women after vacuum aspiration or prostaglandin termination of pregnancy in the first trimester indicate that most ovulate prior to the first menses (Cameron, 1987). Equivalent data is not available following the combination of mifepristone and a prostaglandin. However, one subject conceived prior to the first menstrual period after treatment, indicating that this cycle must have been ovulatory.

In summary, this study has failed to demonstrate any differences in the efficacy or side effects between 200, 400 or 600 mg mifepristone in combination with gemeprost 1 mg for early therapeutic abortion. Assuming these findings are confirmed when data from other centres is analysed, the minimum effective dose of mifepristone as an abortifacient in combination with 1 mg gemeprost is yet to be established. Since mifepristone and prostaglandin appear to act synergistically, it may be that the dose of mifepristone can be reduced further only at the expense of an increase in

prostaglandin dose, a poor exchange, since analgesic requirement is proportional to the prostaglandin dose.

Chapter 3:

The use of gemeprost for menstrual induction

Introduction.

The use of prostaglandins to induce menstruation has been reviewed in Chapter 1. Despite obvious advantages in terms of patient acceptability, menstrual induction has not become a popular method of abortion in the UK, since the side effects induced by the prostaglandins are thought to preclude routine use. The "third generation prostaglandins" have fewer side effects compared to the natural compounds, and appear to be as effective as vacuum aspiration under local anaesthesia in early pregnancy (World Health Task Force, 1987). 16, 16, dimethyl PGE_1 is the only third generation prostaglandin available in the UK. It is currently licensed for softening and dilatation of the cervix prior to transcervical intrauterine procedures in the first trimester, and for therapeutic abortion in the second trimester. The use of this compound for menstrual induction has previously been described in regimes using 1 mg doses at three hourly intervals, to a maximum of 5 mg (Smith and Baird, 1980, World Health Organization Task Force, 1982; Cameron and Baird, 1988), and complete abortion rates of 86-97% achieved. However, the incidence of gastrointestinal side effects is reported to be as high as 57% (Smith and Baird, 1980) and up to 53% of patients require opiate analgesia (Cameron and Baird, 1988). The use of a three hourly regime of gemeprost administration may be inappropriate however, since plasma levels of gemeprost are maintained for up to six hours after vaginal administration (Dimov et al, 1983). In an attempt to reduce the side effects associated with menstrual induction using gemeprost, and to capitalise on the "prostaglandin impact" which induces endogenous uterine activity and prostaglandin production following an initial dose of exogenous prostaglandins (Csapo et al, 1972; Csapo and Pulkkinen, 1979a), a protocol using six-hourly pessary administration was employed, the results of which are reported in Part 1 below.

Mifepristone is now marketed in France, China and the UK for menstrual induction. In combination with a prostaglandin it is associated with a high rate of complete abortion (Chapter 2) and is rapidly becoming the "gold standard" by which other methods of medical abortion should be judged. When gemeprost is used alone, a larger total dose of prostaglandin is required to induce abortion, compared with the use of mifepristone and gemeprost in combination, resulting in greater side effects, and often associated with lower efficacy. Whilst this is apparent from data on the two

methods published separately, only one small study has compared the two methods directly, and failed to find any difference in the rate of complete abortion (Cameron and Baird, 1988). In the second half of this Chapter, the efficacy and the side effects of the use of mifepristone (200 - 600 mg) in combination with gemeprost 1 mg, and those of gemeprost alone (1 mg 6 hrly x 3) are compared.

Part 1: The efficacy and side effects of a new regime of gemeprost for early therapeutic abortion.

Methods.

One hundred and fifty one women of ≤ fifty-six days amenorrhoea, were recruited to investigate the efficacy of vaginal gemeprost (1 mg six hourly to a total of three pessaries) for therapeutic abortion. The incidence of complete abortion was the main outcome measure. The protocol was granted ethical approval by the local Reproductive Medicine Ethical Subcommittee.

Recruitment.

Women were recruited from those referred by their general practitioner for legal termination of pregnancy, as described in Chapter 2. Exclusion criteria from the study were:

- gestation more advanced than 56 days as dated from menstrual history, pelvic examination or ultrasound scan;
- a history of asthma;
- an inability to stay in hospital overnight or to attend for follow up visits.

After the usual history, physical examination and counselling, details of the study were given verbally, and written consent obtained from each participating patient.

Admission and prostaglandin administration.

On the day of admission (day 1), patients were admitted to hospital and details of the history and physical examination were noted on a data sheet as described in Chapter 2. A more detailed symptom questionnaire was used than described in Chapter 2, and the point prevalence rather than the cumulative incidence of symptoms noted. Blood was taken for blood grouping, estimation of haemoglobin and of human chorionic gonadotrophin concentrations, and pelvic ultrasound performed. A single pessary of gemeprost (1 mg) was inserted into the posterior vaginal fornix. Patients were instructed to remain in bed for the first hour after pessary insertion, and then

encouraged to sit up or mobilise. Blood pressure, pulse rate and temperature were recorded every three hours. The symptom questionnaire was repeated six hours after the first pessary and a further 1 mg gemeprost was inserted into the posterior vaginal fornix, unless the patient had complained of severe abdominal pain, or abortion had occurred. The process was repeated six hours later and a third pessary given if necessary. The time at which bleeding and abdominal pain started and the number of vomiting and diarrhoeal episodes were noted. Analgesia was available as required; paracetamol, codeine and diamorphine were used in order for increasing severity of pain. Those patients who required a third prostaglandin pessary were encouraged to stay in hospital overnight.

Blood was taken 24 hours after prostaglandin administration for repeat serum human chorionic gonadotrophin estimation. The sanitary protection used by the patients during their stay in hospital was collected so that an accurate estimate of blood loss could be made and, in addition, patients were asked to bring the sanitary pads used during the remainder of the bleeding episode to their follow up appointments. Prior to discharge, anti D was given to rhesus negative women. A menstrual diary card was given to record the pattern of vaginal bleeding and a follow up appointment arranged for one week later.

Follow up.

At follow up (day 8) the patient's clinical condition was reviewed, details of vaginal bleeding and of any adverse effects were noted, and a physical examination performed. A pelvic ultrasound scan was carried out, and blood taken for haemoglobin and serum human chorionic gonadotrophin estimation. This procedure was repeated on day 15 and day 29, and the total duration of bleeding noted. Patients with an ongoing pregnancy underwent surgical termination after the two week follow up visit unless they requested earlier surgery. Women in whom abortion was incomplete by the second follow up visit (day 15) underwent surgical uterine evacuation. Four women with an ongoing pregnancy had a further course of pessaries.

Laboratory analysis.

Analysis of blood samples was performed by the appropriate laboratories, as described in Chapter 2. The calculation of the total amount of blood lost during abortion was performed from assessment of soiled sanitary protection (Hallberg and Nilsson, 1964).

Statistical methods.

Data were plotted before analysis to determine the distribution. For data which were normally distributed, or in which log transformation could achieve normality, the mean and 95% confidence intervals have been quoted, and analysis of variance, or a paired t test for paired samples from the same patient used to look for differences between the groups. Otherwise the median and range have been quoted, and non parametric tests (Mann Whitney, or Wilcoxon Rank Sum for paired data) used to explore the statistical significance of differences between the groups.

Results.

Patient details.

Characteristics of the patients and results of investigations on the first day of treatment are shown below (Table 3.1a and 3.1b).

Table 3.1a

	Mean (95% C.I)
Age (years)	24.5 (24.0 - 24.8)
Height (cm)	163.8 (162.8 - 164.7)
Weight (kg)	60.9 (59.5 - 62.3)
Gestation(days)	47.4 (46.5 - 48.2)
Parous	38%
Previous live birth	32%

Characteristics of patients on admission day; mean (95% C.I) or percentage.

Table 3.1b

Sac diameter (mm)	18.7	(17.1 - 20.6)
Haemoglobin (g/dl)	13.1	(12.9 - 13.2)
hCG (MU/l)*	38,510	(339 - 214,900)

Results of investigations, mean (95%CI) or * = median and range.

Abortion rate.

One hundred and thirty two of the one hundred and fifty one patients (87%) aborted completely by the second follow up visit. Nineteen patients (13%) required further intervention, including four patients who opted for a further course of pessaries. Three of these four women aborted completely. Sixteen patients, therefore, required uterine evacuation. Only nine patients had an ongoing pregnancy at the time of further intervention, the others had a diagnosis either of missed abortion (seven patients) or incomplete abortion (three patients). Eighty six patients (57%) were given all three pessaries, 43 (28%) were given two pessaries and twenty one patients (14%) were given one pessary. One patient became transiently hypotensive but failed to abort after one gemeprost pessary. A further half pessary was given and abortion occurred shortly thereafter without incident.

Side effects.

All patients bled following prostaglandin treatment, at a median time of 300 minutes, (range 90-765 minutes) after the first gemeprost pessary. All but five patients experienced abdominal pain prior to abortion, at a median time of 195 minutes (range 0-540 minutes) after prostaglandin administration. Fifty eight (38%) patients vomited following prostaglandin treatment, and sixty one (40%) had diarrhoea, with a median of 0 (range 0-5) and 0 (range 0-6) episodes respectively. There was a significant increase in abdominal and pelvic pain following the first prostaglandin pessary, and a significant decrease in breast tenderness (Table 3.2).

Fifty four patients (36%) required opiate analgesia, 50 (33%) were given codeine ± paracetamol, and 11 (7%) received paracetamol alone. Thirty six (24%) patients did not require analgesia. There were no significant differences in analgesic requirements between parous and nulliparous women. Data on duration of bleeding was available on 106 patients who aborted following the prostaglandin pessary course. (The first twenty four patients were treated as a pilot group and followed up only until abortion was found to be complete. Of the 127 patients remaining, 112 aborted completely, but six defaulted from follow up before the bleeding had ceased.) Mean (95% C.I) duration of bleeding in these women was 13.6 days (12.3-15.0). Median blood loss as assessed from measurement from the sanitary pads was 28 ml (range 2-237 ml).

Table 3.2

Time in relation to first pessary	0	6 hrs	12 hrs	Significance
Headache	7%	5%	4%	ns
Drowsiness	15%	29% *	31% **	* p <0.02 ** p <0.001
Nausea	32%	37%	18% **	** p <0.02
Vomiting	9%	22%	6%	ns
Indigestion	7%	5%	5%	ns
Abdominal distension	10%	22% *	21% **	* p <0.02 ** p <0.02
Abdominal pain	10%	73% *	57% **	* p <0.001 ** p <0.002
Pelvic pain	6%	29% *	26%**	*p <0.0002 **p <0.0002
Loss of appetite	23%	27%	19%	ns
Diarrhoea	3%	21% *	9%	*p <0.0002
Constipation	9%	5%	3%	ns
Breast pain	36%	22% *	16% **	* p <0.02 ** p <0.001
Weakness	13%	26% *	25% **	* p <0.02 ** p <0.02
Faintness	8%	10%	6%	ns
Tiredness	43%	46%	49%	ns
Dysuria	3%	4%	3%	ns
Hot flushes	1%	15% *	10% **	p <0.002 p <0.002

Point prevalence of pregnancy / prostaglandin related symptoms at time 0, 6 and 12 hours after the first prostaglandin pessary, statistical significance of the difference compared with time 0 are shown in the right hand column.

To determine whether there were any factors which might predict outcome, characteristics of "successful" patients (those who aborted completely without further intervention) and "unsuccessful" patients (those who required either a further pessary course or surgical uterine evacuation) have been compared (Table 3.3). Successful patients were significantly taller and lighter than those who failed to abort ($p<0.05$), but there were no other significant differences on recruitment.

Table 3.3

	"Successful patients"	"Unsuccessful patients"	Significance
Age (yrs)	25.6 (24.2 - 27.1)	24.3 (22.3 - 26.42)	ns
Height (cm)	163.9 (162.8 - 164.9)	157.87 (146.3 - 169.5)	$p < 0.05$
Weight (kg)	61.3 (59.3 - 63.3)	68.1 (57.5 - 78.7)	$p < 0.05$
Gestation (d)	47.7 (46.8 - 48.6)	45.0 (42.7 - 47.4)	ns
Parity	45%	32%	ns
Initial hCG (IU/l).	38,960 (339 - 214,000)	31,150 (706 - 136,500)	ns

Data at recruitment, mean (95%C.I) except for parity (percentage) and initial hCG (median and range)

When the response to treatment is compared, successful patients were found to bleed significantly earlier following the prostaglandin pessary ($p<0.05$) and to require stronger analgesia during abortion ($p<0.003$) than unsuccessful patients (Tables 3.4 and 3.5).

Table 3.4

	"Successful patients"	"Unsuccessful patients"	Significance
Vomiting episodes	0 (0 - 5)	0 (0 - 3)	ns
Diarrhoeal episodes	0 (0 - 6)	0 (0 - 4)	ns
Minutes to bleeding	300 (90 - 720)	360 (165 - 765)	p <0.05
Minutes to abdominal pain	195 (20 - 540)	180 (110 - 420)	ns

Median (range) number of vomiting and diarrhoeal episodes in patients who required further intervention to achieve complete abortion, compared with those who did not; interval from insertion of the first prostaglandin pessary to abdominal pain and vaginal bleeding.

Table 3.5

Analgesia	"Successful" patients	"Unsuccessful" patients
None	19%	58%
Paracetamol	8%	0%
Codeine ± paracetamol	35%	21%
Diamorphine ± codeine ± paracetamol	38%	21%

Analgesic requirements in patients who aborted following the prostaglandin course compared with those who did not (p<0.003 between successful and unsuccessful patients).

Follow up data shows a greater decrease in hCG concentration (expressed as a percentage of the original hCG concentration) both at 24 hours and at seven days after prostaglandin administration in women who aborted completely following treatment (Table 3.6). Blood loss as assessed from menstrual pads was significantly greater in women who aborted following therapy (although since collection of menstrual pads ceased once the patient underwent surgical curettage this is not an accurate assessment of total blood lost by these women).

Table 3.6

	"Successful" patients"	"Unsuccessful" patients	Significance
hCG after 24 hours	34.1 (7.8 -126.7)	58.9 (28.5 - 103.2)	p <0.0001
hCG after 7 days	1.7 (0.5 - 113.6)	90.7 (0.9 - 277.0)	p <0.0001
Change in Hb	-0.3 (-1.6 - +1.0)	-0.5 (-1.4 - +0.2)	ns
Blood loss (mls) from pads	34 (2 - 237)	10 (5 - 69)	p <0.01

Median (range) change in haemoglobin and serum hCG concentrations at 24 hours and or 7 days after prostaglandin treatment, and blood loss during abortion as assessed from the menstrual pads. hCG concentrations are expressed as a percentage of the original value.

Part 2: A randomised comparison between the use of mifepristone and gemeprost and gemeprost alone in early therapeutic abortion.

Methods.

One hundred and seventy women of ≤ 56 days gestation requesting therapeutic abortion were randomised to treatment either with mifepristone (200-600 mg) in combination with gemeprost (1 mg); or gemeprost alone (1 mg 6 hrly to a total of five pessaries) in order that the efficacy of the methods could be compared The main outcome measure was the number of complete abortions. The protocol was approved by the local Reproductive Medicine Ethical Subcommittee. Eighty five women were treated in each group. Their details have also been reported separately (in Chapter 2, and in Part 1 of this Chapter) as part of larger studies into the use of mifepristone in combination with gemeprost, and gemeprost alone. They are reported again here in order to compare the efficacy and the side-effects of the two methods.

Women were recruited from those referred by their general practitioner, as described in Chapter 2. Only women who fitted the inclusion criteria for both the study described in Chapter 2, and the study described in Chapter 3, Part 1 were included. Details of the study, and of the plan to randomise patients to one or other treatment were discussed with the patient, and consent obtained. Treatment was allocated by opening a sealed envelope containing directions as to one or other protocol. The envelopes were prepared in advance, so that an equal number of patients were recruited to each group over the course of the study. Once allocated to a therapeutic group, treatment was followed as described for each of the groups separately. Follow up on women treated

with prostaglandin pessaries alone was extended until the date of the next menstrual period following prostaglandin administration.

Results

Characteristics of patients in each group at recruitment are shown in Tables 3.7a and 3.7b. There were no significant differences in the groups for any of the parameters shown.

Table 3.7a

	Mifepristone and gemeprost	Gemeprost alone
Age (years)	25.9 (24.7 - 27.2)	27.0 (25.6 - 28.5)
Height (cm)	164.4 (163.1 - 165.7)	163.8 (162.5 - 165.0)
Weight (kg)	60.0 (58.4 - 61.5)	61.6 (59.7 - 63.5)
Gestation (days)	46.9 (45.8 - 48.1)	48.1 (47.1 - 49.2)
Parity:		
Parous	46%	40%
Previous live birth (≥28/40)	40%	29%
Previous abortion	29%	25%

Characteristics of patients in each of the two treatment groups on the first days of treatment, mean (95%C.I), or percentage. No significant differences between the groups.

Table 3.7b.

	Mifepristone and gemeprost	Gemeprost alone
Haemoglobin (g/dl)	13.1	13.1
	(12.9 - 13.2)	(12.9 - 13.3)
hCG (IU)	43,870	38,510
	(1,038 - 191,000)	(1,389 - 210,700)
Sac diameter on scan (mm)	19.6	19.5
	(17.9 - 21.5)	(17.8 - 21.5)

Results of investigations on the first day of treatment - median (range).

The combination of mifepristone and gemeprost was significantly more effective in inducing a complete abortion than the use of gemeprost alone, with complete abortion rates of 83/85 (98%) and 74/85 (87%) respectively ($p<0.01$). Details of the failures in each group have been described in Chapter 2, and Part 1 of this Chapter. Analgesic requirements were significantly greater in the group of women treated with gemeprost alone ($p<0.02$), (Table 3.8). Total duration of bleeding and interval to the next menses were similar in both groups (Table 3.9). The patient's subjective assessment of the amount of bleeding was greater in women treated with mifepristone and gemeprost compared to women treated with gemeprost alone ($p<0.01$), (Table 3.9). Levels of hCG (expressed as a percentage of the original value) were greater on day 8 in the group of women treated with the combination of mifepristone and gemeprost, compared with the group of women treated with gemeprost alone ($p<0.0001$). There was no difference in the change in haemoglobin in women treated with mifepristone and gemeprost, compared to women treated with gemeprost alone (Table 3.10).

Table 3.8

Analgesia	Mifepristone and gemeprost	Gemeprost alone
None	41%	22%
Paracetamol only	11%	7%
Codeine ± paracetamol	22%	26%
Diamorphine ± codeine ± paracetamol	25%	45%

Difference in analgesic requirements in the two treatment groups,

($p <0.02$ between the two groups).

Table 3.9

	Mifepristone and gemeprost	Gemeprost alone
Duration of bleeding (days)	13.7 (12.2 - 15.6)	13.1 (11.7 - 14.8)
Interval to menses	35.5 (33.8 - 37.2)	34.2 (30.4 - 38.5)
bleeding compared with menses:**		
Lighter	4%	8%
Same	26%	27%
Heavier	48%	61%
Much heavier	22%	4%

Effect of different treatments on duration of bleeding, interval to menses and amount of bleeding compared with menstruation.(** = $p<0.01$ between the treatment groups).

Table 3.10

	Mifepristone and gemeprost	Gemeprost alone
hCG (% of original value)	2.9% (0.0 - 42.5)***	1.8% (0.5 - 157.7)***
Change in Hb (g/dl)	-0.6 (-2.3 - +1.6)	-0.3 (-1.4 - +2.8)

Change in hCG and haemoglobin on day 7-11 median and range; (*** = $p<0.0001$).

Discussion

The complete abortion rate seen here is similar to that reported by other groups using a three hourly regime of prostaglandin administration for menstrual induction, where abortion rates of 86% in a study of 358 women (World Health Organization Task Force, 1982), and 87% in a study of 30 women (Smith and Baird, 1980) were achieved. A higher complete abortion rate of 97% has been achieved in a small sample of thirty women at the same gestation (Cameron and Baird, 1988). Although a comparison was not made here, the complete abortion rate of 87% seen in this study compares poorly to the complete abortion rate of 99.3% in a study of over 15,000 women undergoing suction termination of pregnancy at less than eight weeks gestation (Tietze and Lewit, 1972). In a previous study comparing gemeprost for menstrual induction with vacuum aspiration under general anaesthesia, the latter method is associated with a significantly higher rate of complete abortions (Smith and Baird, 1980).

The rationale behind a vaginal route of administration of prostaglandins is to reduce unwanted side effects, experienced when prostaglandins are given via other routes. However, this approach is not entirely successful, as the increase in nausea, abdominal distension, flushing and diarrhoea following the first prostaglandin pessary indicates. The use of a smaller dose of prostaglandin in this study has not resulted in a reduction in the incidence of vomiting and diarrhoea compared with other studies (Smith and Baird, 1980; World Health Organization Task Force, 1982; Cameron and Baird, 1988) although the analgesic requirement seen here was lower than in women of the same gestation given gemeprost three hourly (Cameron and Baird, 1988). Analgesic requirements in parous women were not significantly different from those in nulliparous women, in contrast to the data on women undergoing therapeutic abortion using mifepristone and prostaglandin (Chapter 2). The reasons for the different effect of parity on analgesic use in the two groups are not clear, but may represent a greater contribution of endogenous prostaglandin production to uterine activity and pain in women following mifepristone and prostaglandin, compared to women treated with prostaglandin alone. Median blood loss, as assessed from sanitary protection, was less than previously reported (Smith and Baird, 1980). Any measurement of blood loss using this method is an underestimate of the actual blood loss unless every pad has been included. However no patient in this study bled sufficiently heavily to require a blood transfusion.

The lower mean weight of women who aborted following treatment suggests that the dose/kg and/or the plasma profile of gemeprost is important, perhaps resulting in a greater "prostaglandin impact" in women who aborted successfully following gemeprost (Csapo et al, 1972). Despite a greater mean height in women who aborted following gemeprost alone, there was no significant difference in body mass index (weight/height^2, Quetelet's index). This contrasts with published data on the predictors of failed attempted abortion with the antiprogestin mifepristone, with or without prostaglandin (Grimes et al, 1990). Surprisingly, there was no significant difference in mean gestation in women who aborted compared with those who did not. The earlier interval to bleeding and the increased analgesic requirement in women who aborted following prostaglandin therapy suggests a greater sensitivity to prostaglandin in this group.

The randomised comparison between treatments shows mifepristone and gemeprost to be more effective in inducing complete abortion than gemeprost alone. These results

contrast to a non randomised study of 49 patients, where no difference in abortion rate was seen following either mifepristone 150 mg daily for four days with 1 mg gemeprost on the third treatment day, or gemeprost 1 mg three hourly to a total of five pessaries (Cameron and Baird, 1988). There are other advantages of the mifepristone and prostaglandin combination compared to prostaglandin treatment alone. Most women undergoing termination of pregnancy using gemeprost alone who required all three pessaries had an overnight stay in hospital. This was not a feature of termination of pregnancy using mifepristone followed by gemeprost. Staying in hospital overnight is not only inconvenient for many women, but has obvious cost implications. In addition, the requirement for analgesia (which correlates with the painfulness of the procedure) is significantly less following mifepristone and prostaglandin than prostaglandin alone.

A lower hCG concentration (expressed as a percentage of the original value) was seen seven days after the start of treatment in the gemeprost alone group, compared to the mifepristone and gemeprost group, despite the fact that the former group included a greater proportion of women with an ongoing pregnancy or missed abortion. This reflects the time of abortion in the two groups. In women treated with gemeprost alone, a significant decrease in hCG concentrations can be seen twenty-four hours after treatment, whereas in women given mifepristone and prostaglandin, hCG levels are greater forty-eight hours after mifepristone than at the time of mifepristone administration. It is not until prostaglandin is given and abortion occurs that hCG levels decline in this latter group. The change in hCG concentration seven days after prostaglandin administration in each group would, in retrospect, have been a more appropriate comparison.

Amongst women who aborted following treatment, there were no significant differences between the groups in terms of mean duration of bleeding or the interval to the next menses following mifepristone and gemeprost, compared with following gemeprost alone. The median change in haemoglobin concentration seven days after the start of treatment was similar in each group. In contrast, the patients' subjective report of bleeding compared with their normal menstruation (in women who aborted completely) indicates a significant trend towards heavier bleeding in the mifepristone and gemeprost group ($p<0.01$). However, the main difference lies in the fairly subtle distinction between "heavier bleeding" and "much heavier bleeding" compared to normal menstruation, and may therefore be artefactual. Indeed, when the data were grouped into two categories, bleeding heavier than menstruation, or bleeding the same

or lighter than menstruation, there were no significant differences between the two treatment groups.

In summary, the combination of mifepristone and prostaglandin is more effective than the use of prostaglandin alone in termination of early pregnancy, and results in fewer side effects. The duration of bleeding and interval to next menses in women who do abort is similar, suggesting an analogous process of abortion in each case.

Chapter 4 :

The effects of misoprostol, an orally active prostaglandin on uterine activity and early pregnancy, both alone and in combination with mifepristone.

Introduction

The comparison of the use of mifepristone and gemeprost for menstrual induction with the use of gemeprost alone (Chapter 3) shows clearly that the mifepristone-gemeprost combination has advantages both in terms of increased efficacy, and reduced side effects. Mifepristone has been used in combination with both vaginal (Rodger and Baird, 1987) and intramuscular (Bygdeman and Swahn, 1985) prostaglandins. However, neither of the commonly used prostaglandins are ideal. The recent reports of cardiovascular compromise and even death from myocardial infarction following the use of mifepristone in combination with intramuscular sulprostone (Anonymous,1991) are probably related to high peak levels of sulprostone in blood following injection. The formulation of gemeprost in a vaginal pessary requires it to be refridgerated prior to use, and absorption from the vagina is variable between individuals. In addition, gemeprost has been reported to induce cardiovascular complications (Kalra et al, 1989). Studies on the available oral prostaglandins have shown natural PGE_2 (1-2 mg) to be no more effective than placebo, in combination with mifepristone for menstrual induction (Swahn et al, 1989), and although 9-methylene PGE_2 is more active orally, it has to be given in solution, a formulation which is unsuitable for routine use (Swahn et al, 1990). Recently, misoprostol (Cytotec™, G.D.Searle), an orally active prostaglandin ([15-S]-15-methyl-PGE_2 methyl ester) marketed for the treatment of peptic ulcer has been used in combination with mifepristone in women of less than 49 days amenorrhoea (Aubeny and Baulieu, 1991), and an abortion rate of 95% achieved. It was claimed that the structure of misoprostol results in fewer gastrointestinal side and increased oral activity compared with other prostaglandins (Dajani et al, 1975; Collins et al, 1985). In this Chapter, the results of a study undertaken to determine the effects of misoprostol alone on uterine tone and contractility in pregnant women at an early gestation ($\leq$ 56 days amenorrhoea) are reported. Misoprostol (200-1000 μg) was also given to women pretreated with mifepristone (200 mg), to determine whether enhanced sensitivity to misoprostol could be seen following mifepristone. The clinical effects of the combination therapy as an abortifacient were also assessed.

Methods

Patients

Seventy-three women of ≤ 56 days amenorrhoea scheduled for legal termination of pregnancy were recruited, as described in Chapter 2. A full history and physical examination were performed, and blood taken for analysis of blood group and haemoglobin estimation prior to recruitment. An ultrasound scan was performed to confirm gestation. Women in whom the gestation was uncertain, or who were suspected of having an ectopic pregnancy were not recruited. The protocol was approved by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board, and all women gave informed consent to participation. Women were recruited into one of three groups, and treated as described below.

Group 1

Twelve women scheduled for suction termination of pregnancy were recruited to determine the effect of differing doses of misoprostol on uterine activity. Women were admitted in the morning for operation in the afternoon. On admission, an intrauterine pressure transducer was inserted into the uterus, via the cervix. Details of the equipment used, and of the method of transducer insertion are given below. After a ten minute period of equilibration, a thirty minute baseline recording was made of uterine activity. Thereafter, a single dose of misoprostol was given orally to each patient, and intrauterine pressure recorded over the following three hours. Three women were given one 200 μg misoprostol tablet, six women received two misoprostol tablets (total dose 400 μg), and the remaining three women received three misoprostol tablets (total dose 600 μg). At the end of the monitoring period, the pressure transducer was removed, and the patient transferred to theatre for suction termination of pregnancy under general anaesthesia in the usual manner. Post operatively, the patient's condition was reviewed, she was given an injection of anti D if necessary and discharged on the evening of admission if well, with contraceptive advice.

Group 2

A further forty women who had opted for surgical termination of pregnancy were recruited to determine whether misoprostol alone was sufficient to induce abortion. On admission to the study, blood was taken for measurement of serum human chorionic gonadotrophin, and details of the size of the gestational sac, and of the presence or absence of fetal heart activity on ultrasound scan were noted. A single oral dose of misoprostol (400 μg) was given, and following this the women were allowed home. Seven days later, the patient was readmitted to hospital. Clinical assessment, an ultrasound scan and measurement of serum human chorionic gonadotrophin levels

were used to determine whether there was an ongoing pregnancy, an incomplete or a complete abortion. The pregnancy was then terminated by suction termination under general anaesthesia in the usual manner.

Group 3

Twenty one women were recruited to determine the effect of mifepristone and misoprostol on uterine contractility in early pregnancy (≤56 days amenorrhoea) and to assess the clinical outcome of this therapy. On the first day of the study, a single oral dose of mifepristone (200 mg) was administered. Forty-eight hours later, the patient was readmitted. A pressure transducer was inserted into the uterus via the cervix, as described below. After a ten minute equilibration period, baseline intrauterine pressure was recorded over thirty minutes, to determine the effect of mifepristone alone. A single oral dose of misoprostol was then given. Four women received 200 μg misoprostol (one tablet), ten received 400 μg misoprostol (two tablets) and seven received 600 μg misoprostol (three tablets). Intrauterine pressure monitoring was continued either for a further three hours or until abortion occurred, whichever was the shorter time period. Patients were examined three hours after misoprostol administration to determine whether abortion had taken place. In three of the women receiving 600 μg misoprostol, a further dose of 400 μg misoprostol was given following examination since no products of conception had been passed vaginally. Surgical termination of pregnancy was not performed at the end of the recording period for any of the women in group 3 but they were allowed home and a follow up appointment was scheduled for one and two weeks later, to ensure that abortion was complete. Complete abortion was defined as an empty uterus on ultrasound scan and falling concentrations of human chorionic gonadotrophin. Women in whom abortion was not complete by the second follow up were scheduled for surgical termination of pregnancy. Women in each of the groups completed a short symptom questionnaire before and three hours after misoprostol administration.

Intrauterine pressure recordings.

Equipment

Intrauterine pressure was measured with a flexible catheter tipped with a pressure transducer (Gaeltec Ltd, Dunvegan, Isle of Skye, Scotland) (pressure range 0 - 300 mmHg). This was connected to an amplifier and thence to a pen recorder.

Transducer insertion

Under sterile conditions, the cervix was visualised with the aid of a vaginal speculum, the catheter was grasped with a pair of sponge forceps and passed through the cervical canal into the uterus. When resistance to further insertion was felt at a depth of around 8-10 cm, the catheter was withdrawn 1-2 cm so that it would be lying free in the uterine cavity. The other end of the catheter was then connected via an amplifier to a pen recorder. A vaginal tampon was inserted to hold the catheter in place, and the catheter taped to the patient's leg. A mark was made on the paper when the intravenous prostaglandin was administered.

Calibration

Prior to each insertion the transducer and amplifier were calibrated as follows. The system was zeroed at atmospheric pressure, i.e when the transducer was free in air, a pressure of 0 was recorded on both the amplification system and the pen recorder. The transducer was then placed at the bottom of a column of water at a depth of 43 cm (equivalent to a pressure of 31.6 mmHg). The gain on the amplifier was adjusted so that this pressure resulted in a deflection of 31.6 mm on the pen recorder. When high intrauterine pressures were experienced, the sensitivity of the system was halved by halving the sensitivity of the pen recording system so that the records were kept on one page. The paper was allowed to run at a speed of 5 mm/min. The pressure transducer was sterilised prior to insertion into the patient by immersing in 2% glutaraldehyde solution ("Cidex") for 10 minutes.

Calculation of uterine activity

The measurement of uterine activity is the subject of much debate. In abortion, as in labour at term, regular contractions of the uterus result in cervical dilation and expulsion of uterine contents. With the development of pressure transducers small enough to be inserted into the uterus, the measurement of intrauterine pressure is now relatively straightforward (Steer et al, 1978), although some authors feel that insertion into a fluid filled cavity ie the intra-amniotic rather that the extra-amniotic space is necessary (Gibb, 1989). The conversion of intrauterine pressure into units to quantify uterine activity is more complex. Following much work on the relationship between uterine activity in different states of pregnancy and in labour, a group of workers in Uruguay introduced the Montevideo Unit (Caldeyro Barcia et al, 1957). A Montevideo unit is the mean active intrauterine pressure (ie. the mean increase in uterine pressure over baseline pressure) multiplied by the number of contractions in a ten minute period. From a series of recordings they have produced reference values for normal labour at term. Alexandria units (El-Sahwi et al, 1967) are a modification

of this measurement, but also take into account the mean duration of the contraction, so that one Alexandria Unit is the average amplitude of uterine contractions, multiplied by the duration in minutes and the frequency of contractions in ten minutes. This modification has not been found to be helpful in clinical practice (Gibb, 1989). An alternative approach to the calculation of uterine activity is to calculate the area bounded by the curve of intrauterine pressure and the line of zero pressure, since this area is proportional to uterine work (Bourne and Burn, 1927). Uterine Activity Units are based on this approach, one Uterine Activity Unit being the area bounded by a uterine pressure of 1 mmHg for 1 minute (Hon and Paul, 1973).

No single method of quantifying uterine activity is ideal. The units described above are primarily designed to measure uterine activity in labour at term, and not during abortion in early pregnancy. For the studies described in this thesis, a method of quantifying the effects of the prostaglandins and of mifepristone ± prostaglandins on uterine activity in early pregnancy was required. Small doses of prostaglandins result in an increase in uterine tone (and therefore an increase in Uterine Activity Units), but do not result in discrete uterine contractions (and therefore no increase in uterine activity measured in Montevideo units), or abortion. In contrast, mifepristone results in the development of discrete uterine contractions, which lead to abortion in a proportion of women. The measurement of uterine contractility in Montevideo units discriminates best between the pattern of uterine activity seen before and after mifepristone. In addition, it may be that discrete uterine contractions are more important in overcoming cervical resistance and expelling the uterine contents than a general increase in uterine tone, and that pressures below basal pressure may not play such an active part (Gibb, 1989).

For these reasons, a different method of calculating uterine activity has been employed to determine the effects of mifepristone with or without prostaglandins, and the effects of small doses of prostaglandins alone on uterine activity. No attempt has been made to compare uterine activity following mifepristone ± prostaglandins with that following prostaglandins alone on a quantitative basis. For women treated with prostaglandins only uterine tone at varying times after prostaglandin administration has been calculated. For women pretreated with mifepristone ± prostaglandins, uterine activity has been calculated in Montevideo units. A review of the literature indicates that other workers have used a combination of Montevideo units and Total Pressure Area (a modification of uterine activity units: 1 TPA = area bounded by an intrauterine pressure of 1 mmHg over 10 minutes) to measure uterine activity following mifepristone in the first trimester (Swahn and Bygdeman, 1988) and Uterine Activity

Units have been used to measure uterine activity following mifepristone in the second trimester (Hill et al, 1990b). Montevideo units have also been used to quantify uterine activity in the first trimester following epostane, a progesterone synthetase inhibitor (Webster et al, 1985a), although following epostane in the second trimester, Uterine Activity Units were used (Selinger, 1988). Despite the assertion that a pressure tip transducer is unsuitable for measurement of extra-amniotic pressure, both studies to measure uterine activity following mifepristone quoted above have used this method. Both an increase in TPA and Montevideo Units has been observed following mifepristone in the first trimester (Swahn and Bygdeman, 1988). Having decided to use Montevideo Units to quantify uterine activity following mifepristone, no reports of an objective scoring system for discriminating between single contractions, the noise of the recording system and an increase in baseline intrauterine pressure when calculating uterine activity from actual pressure traces were found. The following system was therefore devised to reduce observer bias:

1. Any increase in uterine activity lasting for more than one minute was considered to be a change in baseline tone rather than a single contraction. These changes in baseline tone were not included in the calculation of Montevideo Units.

2. To distinguish a contraction from electrical "noise" in the system, we considered an increase in tone as a contraction, only if the amplitude of the increase was more than twice that of the variability around the baseline, and if the increase in tone lasted for one minute or less.

3. For an episode of increased uterine pressure where the pressure dipped towards the baseline but did not reach it before rising again to a further peak, we considered this as two separate contractions only if the difference between the pressure trough and the baseline was < 20% of the difference between the smaller peak and the baseline. Otherwise this was considered as one contraction provided it lasted less than one minute) with the greatest peak of intrauterine pressure taken as the amplitude of contraction.

In practice all significant increases in intrauterine pressure which appeared to be contractions were included by this system. It is possible that contractions of low amplitude would not have been included under this system, but the contribution of these contractions to the total score in Montevideo units would not have been great. Certainly, the score in Montevideo units correlated with a naked eye impression of uterine activity.

Statistics.

The effects of the dose of misoprostol, and of time after misoprostol administration on intrauterine tone or on uterine activity in women pretreated with mifepristone were determined by analysis of variance using Newman-Keuls procedure using the "CLR Anova programme" on an Apple Macintosh Computer. The data were log transformed before analysis. The geometric mean and 95% CI (or median and range) of the data have been quoted, the Statview programme was used to calculate these parameters.

Results

The characteristics of the women recruited to each group are shown in Table 4.1.

Table 4.1.

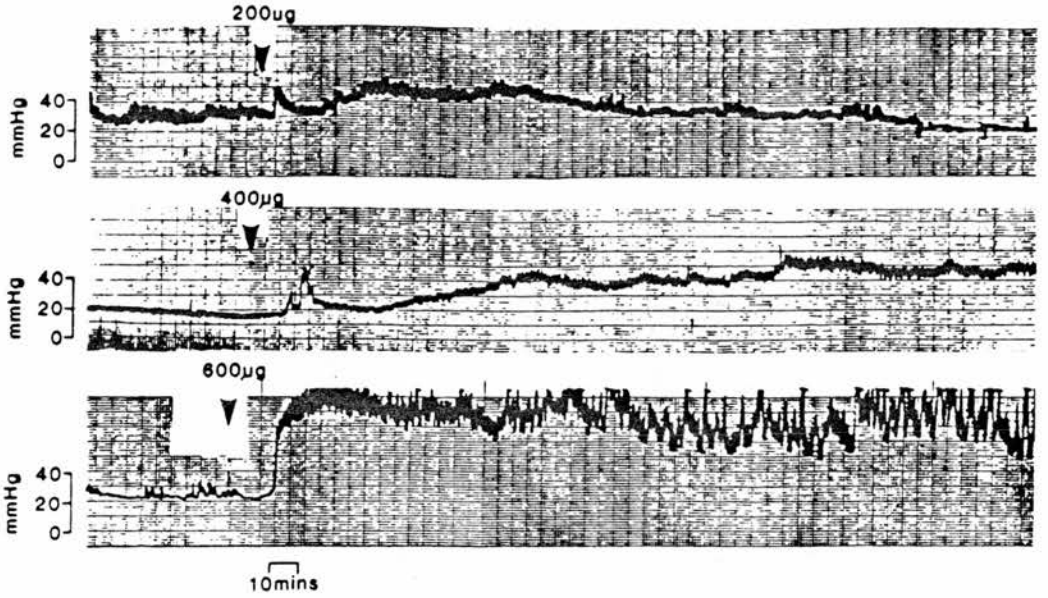
	Mean age in years (range)	Mean gestation in days (range)	Nulliparous women
Group 1	29 (19 - 38)	50 (42 - 56)	6 / 12
Group 2	26 (17 - 42)	49 (39 - 56)	26 / 40
Group 3	25 (18 - 35)	50 (39 - 56)	17 / 21

Characteristics of the women recruited to each group.

Group 1. (Misoprostol alone).

Following misoprostol, there was an increase in intrauterine pressure in ten out of twelve patients, but despite this increase in intrauterine tone, regular uterine activity did not develop (Figure 4.1). In the other two patients (both treated with 400 μg misoprostol), misoprostol failed to effect any change in intrauterine pressure. The change in intrauterine pressure following misoprostol administration was analysed both with respect to time and dose of misoprostol. Analysis of the effect of time after misoprostol administration on mean intrauterine pressure (all three doses of misoprostol) showed a greater mean intrauterine pressure at 30, 60 and 120 minutes after misoprostol administration, compared with the pretreatment value (Table 4.2). Comparison of different doses of misoprostol shows a greater intrauterine pressure in women given 600 μg misoprostol, compared with women given 400 μg or 200 μg misoprostol (all time points compared simultaneously). (Figure 4.2.)

Figure 4.1



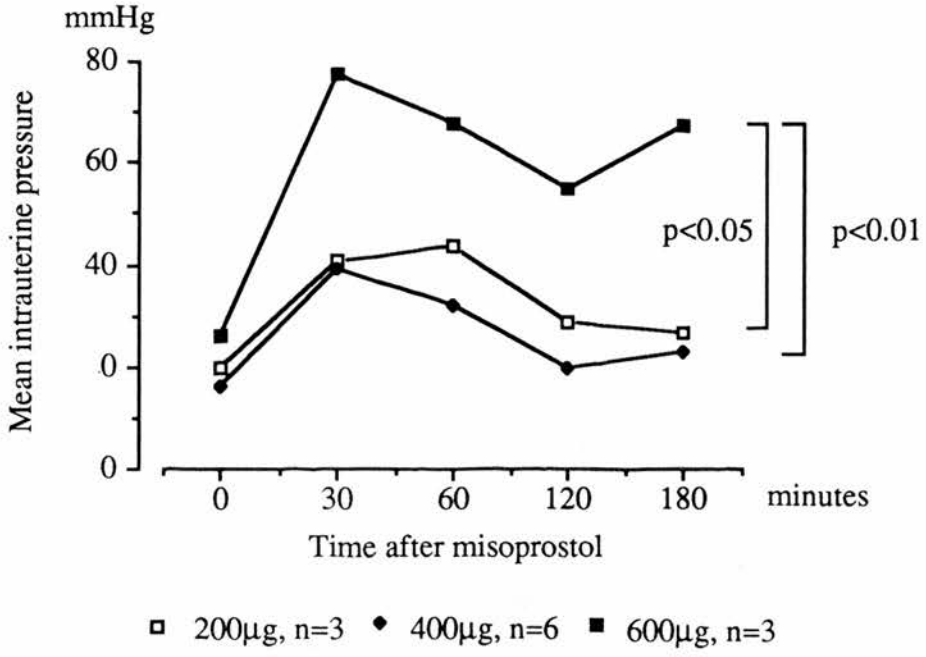
Effect of misoprostol alone on intrauterine pressure.

Table 4.2

Time after PG administration	0	30	60	120	180
200µg misoprostol	20.0 (12.0 - 33.4)	40.7 (30.0 - 55.2)	43.5 (28.4 - 66.5)	28.8 (16.6 - 50.0)	26.9 (21.5 - 33.4)
400µg misoprostol	16.0 (10.7 - 23.9)	39.3 (26.9 - 57.3)	32.1 (21.2 - 48.5)	20.0 (11.0 - 36.6)	22.8 (8.2 - 62.8)
600µg misoprostol	25.9 (22.3 - 29.9)	77.3 (74.8 - 80.0)	67.5 (53.7 - 84.5)	55.0 (40.3 - 75.2)	67.5 (48.3 - 94.0)
All three doses	19.1 (14.9 - 24.5)	47.0** (36.6-60.3)	41.5** (30.6 -56.2)	28.2* (18.9 -42.2)	33.9 (19.7 - 58.5)

Intrauterine pressure in mmHg (geometric mean and 95% C.I) at different time points after each of three different doses of misoprostol, and mean intrauterine pressure for all three doses combined. (*: $p<0.05$ compared with time 0; **: $p<0.01$ compared with time 0)

Figure 4.2



Mean intrauterine pressure following three different doses of misoprostol.

P values show overall significance of the differences in pressure.
(Data also shown in Table 4.2)

No patient given misoprostol alone developed prostaglandin related side effects such as nausea, vomiting or diarrhoea. One patient (who had received 400 µg misoprostol) developed mild abdominal pain which was relieved by paracetamol. Three other patients reported abdominal pain on direct questioning but did not require analgesia.

Group 2: (Clinical efficacy of misoprostol alone).

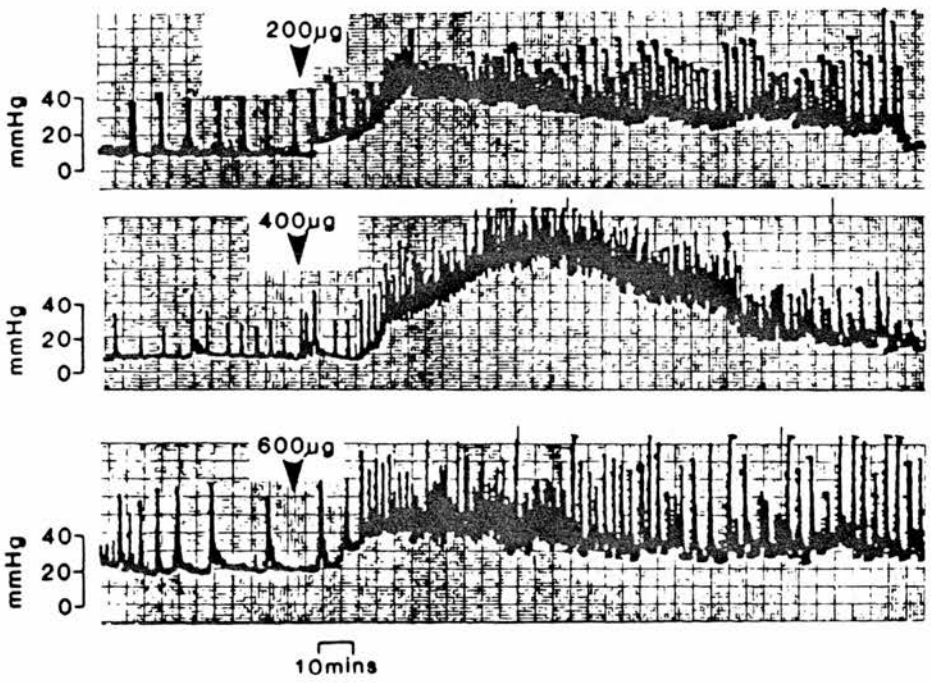
Twenty one of the forty patients had some bleeding in the seven days following the administration of misoprostol, although only two (42 and 44 days amenorrhoea) aborted completely, 6 and 72 hours respectively after prostaglandin intake. The remainder required surgical evacuation seven days later due to ongoing pregnancy (thirty two women), or incomplete or missed abortion (six women). Side effects were few with only one woman having either diarrhoea or vomiting. Ten women felt abdominal discomfort in the four hours following misoprostol, of whom three described the pain as severe.

Group 3. (Misoprostol pretreated with mifepristone).

In the group treated with mifepristone, there was the expected increase in uterine contractility compared to those in Group 1 (Figure 4.3), although a quantitative

analysis was not made. There was a further increase in uterine activity (in Montevideo units) following prostaglandin (Table 4.3). The significance of the increase during different time epochs (all three doses of misoprostol combined) is shown in Figure 4.4.

Figure 4.3



Intrauterine pressure tracing after three different doses of misoprostol in women pretreated with 200 mg mifepristone.

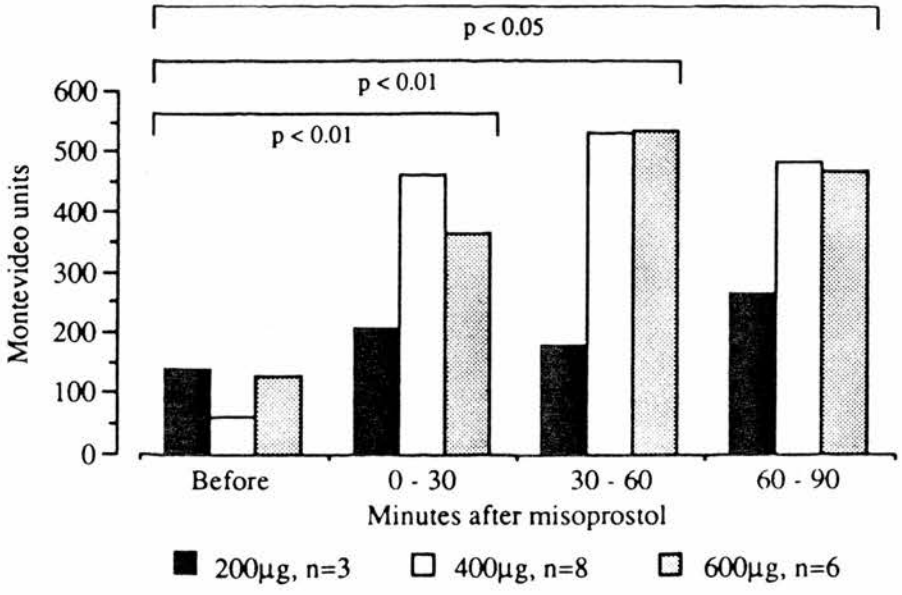
Table 4.3

Time after PG administration	Before	0 - 30 minutes	30 - 60 minutes	60 - 90 minutes
200µg misoprostol	143 (125 - 164)	208 (65 - 670)	181 (21 - 1563)	266 (20 - 3539)
400µg misoprostol	64 (30 - 137)	463 (330 - 650)	532 (348 - 815)	488 (310 - 766)
600µg misoprostol	130 (82 - 207)	363 (200 - 659)	536 (387 - 741)	469 (335 - 656)

Uterine activity in Montevideo units (geometric mean and 95% CI) in women pretreated with 200 mg mifepristone, during various time epochs before and after misoprostol administration.

There were no significant differences in uterine activity between the three doses of misoprostol. In four women uterine recordings were not possible due to technical reasons. In three of the remaining seventeen women, the recording was terminated prematurely before 90 minutes due to passage of the fetus (two of the women in the 600 μg group and one in the 200 μg group). In eighteen of the twenty one women, complete abortion occurred following treatment with misoprostol and mifepristone. Of the remaining three women who required surgical evacuation, two (one given 200 μg and one given 400 μg misoprostol in combination with 200 mg mifepristone) were found to have an ongoing pregnancy at a follow up visit fourteen days later. The third woman (given 400 μg misoprostol in combination with 200 mg mifepristone) passed a gestational sac and placental tissue following misoprostol and appeared to have an empty uterus one week later. However, she was subsequently admitted to another hospital with continuing vaginal bleeding. An ultrasound scan showed the presence of some material in the uterus and uterine evacuation was therefore performed. Unfortunately the material obtained was not sent for histological evaluation. Side effects of the combination therapy were not troublesome. Three patients vomited following misoprostol but one had vomited earlier that day and reported an improvement in symptoms after taking misoprostol. None of the women had diarrhoea, nine women (two following 200 μg misoprostol, six following 400 μg misoprostol, and one following 600 μg misoprostol) required analgesia in the form of paracetamol or dihydrocodeine and three (two following 400 μg misoprostol and one following 600 μg misoprostol) required opiate analgesia.

Figure 4.4.



Uterine activity following mifepristone and three different doses of misoprostol.

Discussion.

The effects of misoprostol on uterine tone as shown above are qualitatively similar to those seen with other prostaglandins. When the natural prostaglandins are given orally early in the second trimester, a high incidence of side effects occurs at doses sufficient to effect a change in uterine tone (Karim, 1971). In this study, misoprostol resulted in a significant increase in uterine tone without marked side effects. The low incidence of side effects with the use of misoprostol is in keeping with previously published reports. In one study only 8.5% of patients receiving 400 μg misoprostol per day for the treatment of peptic ulcer complained of diarrhoea over a four week period (Sontag et al, 1985). Misoprostol is known to have some uterine effects. In a study of pregnant women of 9-12 weeks gestation, 800 μg of misoprostol induced vaginal bleeding in 45% of women, and partial or complete abortion in 11% of women within 12 hours of tablet administration (Rabe et al, 1987). In the UK the Committee on Safety of Medicines has urged caution regarding the use of misoprostol in pre and post menopausal women, and advise against its use in women who are pregnant, or who are planning a pregnancy, because of the risk of provoking uterine activity (Committee on Safety of Medicines, 1989).

Although misoprostol induced an increase in uterine tone when given alone, only eight out of forty women bled or aborted, indicating that it would have little use as an abortifacient at this dose. Six women had either a missed or incomplete abortion and required surgical evacuation as well as the thirty-two women with ongoing pregnancies. Fetal malformations have been reported in babies born to women who have taken misoprostol in early pregnancy in an unsuccessful attempt to induce abortion (Fonseca et al, 1991). There is insufficient data to determine whether these abnormalites are due directly to misoprostol, but clearly misoprostol alone is ineffective as an abortifacient. The increased effectiveness in women who had been pretreated with mifepristone reflects the increased sensitivity to prostaglandins induced by antigestagens (Bygdeman and Swahn, 1985).

The observed increase in uterine activity following misoprostol in women pretreated with mifepristone (RU 486) are qualitatively similar to the effects of other prostaglandin analogues, whether administered by the intramuscular (Bygdeman and Swahn, 1985) or oral route (Swahn et al, 1990). Clinical effectiveness of the mifepristone and misoprostol therapy cannot be fully evaluated from this small study, but the fact that complete abortion occurred in eighteen out of twenty-one patients is encouraging. The success rate in this study was lower than that

reported in a larger study of 100 women of ≤49 days amenorrhoea where a complete abortion rate of 95% was achieved using 600 mg mifepristone followed 48 hrs later with 400 μg misoprostol (Aubeny and Baulieu, 1991).

Another orally active prostaglandin, arbaprostil, a PGE_2 analogue (15 (R)15 methyl PGE_2) which is used to treat peptic ulcers has been shown to have little or no effect on uterine activity or baseline uterine tone when a single dose (similar to that recommended for treatment) was given to pregnant women in the first trimester (Euler et al, 1989). At larger doses (1200 μg and over) vaginal spotting was induced in some women, but the incidence of side effects in association with this dose of prostaglandin were high.

In summary, oral misoprostol increased uterine tone in women in early pregnancy, but only resulted in bleeding and abortion in 20% of women. In contrast, in women pretreated with 200 mg mifepristone there was a striking increase in contractility and eighteen out of twenty-one women aborted completely. The side effects associated with the administration of misoprostol were minimal. Opiate analgesics were required by only 14% of women following mifepristone and misoprostol, compared with 21% of women following mifepristone and gemeprost (Chapter 2) at the same gestation. It is not clear whether the apparent lower incidence of side effects following mifepristone and misoprostol simply relates to a lower total dose of prostaglandin, and whether the same effect could be achieved by using a lower dose of gemeprost in combination with mifepristone. Further work is required to determine the clinical efficacy of mifepristone and misoprostol in women of up to 56 days amenorrhoea, and to compare the use of misoprostol with a bio-equivalent dose of other prostaglandins.

Chapter 5:

The effects of mifepristone in vivo on decidual prostaglandin production

Introduction

The data presented in Chapters 2 and 4 demonstrate that mifepristone is an effective abortifacient when used in combination with an exogenous prostaglandin. It also stimulates uterine activity in early pregnancy (Bygdeman and Swahn, 1985), but the mechanism of action of mifepristone in inducing these effects is uncertain. An increase in decidual prostaglandin production could explain both effects. There is good evidence that progesterone inhibits decidual prostaglandin synthesis, both in vitro and in vivo (see Chapter 1). In vitro studies have shown an increase in decidual prostaglandin production following the addition of mifepristone to decidual glandular cells in culture (Smith and Kelly, 1987). However, in vivo studies, where an increase in prostaglandin metabolite levels in the peripheral circulation have been used as an index of decidual prostaglandin production, have failed to show a demonstrable increase in decidual prostaglandin production 24-48 hours after 600 mg mifepristone in vivo both in the first and early second trimester (Hill et al, 1990a; Hill et al, 1990b). This Chapter describes results of a study to determine whether treatment with mifepristone in vivo results in an increase in decidual prostaglandin by obtaining decidua from women pretreated in vivo with mifepristone, and examining the ability of the tissue to generate prostaglandins in culture in vitro.

Methods

Patients

Forty-five women with a normal intrauterine pregnancy of less than 56 days amenorrhoea undergoing legal termination of pregnancy were recruited into the study. All women had grounds for termination of pregnancy under the 1967 Abortion Act. The study was described in detail to the patients and written informed consent obtained prior to enrolment. The protocol was approved by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board. On recruitment, full clinical history was taken and physical examination performed. A scan was performed to confirm an intrauterine pregnancy of $\leq$ 56 days gestation. Mifepristone $\pm$ indomethacin was given, and surgical termination performed under general anaesthesia as described below. Women were detained in hospital for four hours post operatively. Thereafter they were

allowed home with contraceptive advice, and anti D if appropriate. All women were seen for routine follow up one week after abortion.

Treatment

Women were allocated into one of eight groups: a control group received no treatment prior to surgical termination of pregnancy (n=5), three groups were given 600 mg (3 tablets) of mifepristone 24 hours (n=5), 36 hours (n=4) or 48 hours (n=3) prior to termination of pregnancy. A second cohort of women were also included in the randomisation but in addition received indomethacin in the form of a rectal suppository 100 mg every 12 hours from the time of administration of mifepristone. One group of women were given indomethacin alone, 100 mg PR every 12 hours from 48 hours before surgery, the last dose being given 12 hours before pregnancy termination (n=5). The remaining three groups of women were given mifepristone and indomethacin, with the last dose of indomethacin given 12 hours prior to surgical termination so that a total number of 2, 3, or 4 doses of indomethacin were given to women treated with mifepristone 24 hours (n=5), 36 hours (n=4), or 48 hours (n=3) prior to surgical termination respectively.

Operation

At operation under general anaesthesia, the vulva and vagina were cleansed with Betadine (povidone-iodine 10%), and draped to provide a sterile operative area.The cervix was dilated using Hegar dilators to a diameter of 8mm. Decidua was obtained by gentle curettage from the side wall of the uterus, and placed in liquid nitrogen (for measurement of endogenous levels) or ice cold transport media (as described below) for removal to the laboratory and subsequent tissue culture. Pregnancy termination was completed using suction curettage.

Materials

Mifepristone {RU486 (17α hydroxy 11β - [4 dimethyl amino phenyl] 17β -[1-propynyl] estra 4, 9 dien-3-one)} tablets (200 mg each) were a gift from Miss Angela Davey, Roussel Laboratories Ltd, Uxbridge U.K. RPMI 1640 medium (Gibco Ltd, Paisley, Scotland, UK) was supplemented with 200 mg/ml gentamicin and fungizone 2 μg/ml (both from Sigma Chemical Co., USA) and used as "transport medium". Tissues were cultured in "complete medium": RPMI 1640 supplemented with 2 μg/ml fungizone and 50 mg/ml gentamicin with 5%(v/v) calf serum (Gibco Ltd, Paisley, Scotland, UK) which had previously been stripped with charcoal (1 mg/ml). Progesterone (Sigma Ltd, Poole, Dorset, UK) 50 ng/ml or 500 ng/ml was added to

complete media in some of the wells. Mifepristone (175 ng/ml) (Roussel Laboratories Ltd, Uxbridge U.K) and progesterone (50 ng/ml) was added to complete media in a fourth set of wells

Extraction of endogenous prostaglandins

A portion of decidua was stored in liquid nitrogen until analysis. When ready to be assayed the tissues were removed from the liquid nitrogen. A piece of decidua weighing approximately 60 mg was homogenised in 5 ml ice cold ethanol at full speed (Polytron) for 5 seconds to extract the prostaglandins. After homogenisation, a 1 ml aliquot of the homogenate was spun at 12000 g for 2 minutes. An equal volume of methyl oximating solution (0.12M methoxyamine hydrochloride and 1.0M sodium acetate, pH 5.6-5.8) was added to 0.5 ml of the supernatant. The sample was then stored until ready to be assayed in the manner described below.

Culture of decidua

On arrival at the laboratory, the decidua was washed twice in complete medium. The tissue was then divided into small pieces of around $1mm^3$. Several pieces were then placed into each of 12 wells of a 24 well culture plate (Nunc, Denmark) with 1 of complete medium, medium supplemented with progesterone to a final concentration of 50 ng/ml or 500 ng/ml, or medium supplemented with progesterone and mifepristone to a final concentration of 50 ng/ml and 175 ng/ml respectively. Three replicates of each culture treatment were used. The culture plates were sealed with micropore tape and placed in an incubator at 37°C in 95% air and 5% CO_2. Media was changed daily and the spent media stored at room temperature after the immediate addition of methyl oximating solution in a 1:1 ratio. At the end of three days of culture, protein content of each of the wells was measured using the protein estimation technique of Lowry (Lowry et al, 1951).

Assay procedures

1. Prostaglandin radioimmunoassay

The prostaglandins were first converted to their methyl oxime derivative using the methyl oximating solution described above. Conversion is >95% when samples are treated at 20°C overnight. Radioimmunoassay using antisera to the methyloxime derivatives was performed. Cross reactivities of the antisera were as follows: $PGF_{2\alpha}$ antisera with $PGF_{1\alpha}$, 7.2%; $PGF_{3\alpha}$, 2.9%; $PGF_{2\beta}$, 3.5%; PGE_2, 1.1%; 6-oxo $PGF_{1\alpha}$,1.05%; 13,14-dihydro $PGF_{2\alpha}$, 1.0%, all other prostaglandins <0.2%; PGFM antisera (raised against the 13,14- dihyro-15-keto $PGF_{2\alpha}$ derivative) with 15-keto

$PGF_{2\alpha}$, 4.0%; 13,14-dihydro-15-keto $PGF_{2\alpha}$, 2.0%; 6,15-dioxo-13,14 dihydro$PGF_{1\alpha}$, 0.35%; PGD_2, 13,14-dihydro-15-keto PGE_2, 0.12%; 0.08%; $PGF_{1\alpha}$, 0.07%; PGE_2, 0.04%; all other prostaglandins <0.01%; PGE_2 antisera with PGE_1, 53%; PGE_3, 31%; 20 methyl PGE_2, 31%; 19-hydroxy PGE_1, 17.8%; 19-hydroxy PGE_2, 3.7%; 20-hydroxy PGE_2, 3.7%; 8 iso PGE_2, 2.9%; 15-keto PGE_2, 0.25%; all other prostaglandins < 0.2%. Cross reactivity was determined by the amount of prostaglandin that caused 50% of inhibition of binding of the appropriate labelled prostaglandin to the antibody (Kelly et al, 1986a; Kelly et al 1986b; Kelly et al, unpublished data). The sensitivity of the assay (the amount distinguishable from zero with a 95% confidence limit) was 2 pg. Intra-assay and interassay coefficients of variation were 11% and 14% for $PGF_{2\alpha}$, 12% and 13% for PGE_2, and 7.3% and 13.3% for PGFM. Prostaglandin results were expressed in ng as a ratio of the protein content of the tissue, expressed in milligrammes or grammes.

2. Protein quantification

Protein content of the tissue was measured by the method of Lowry et al (1951). This involves reaction of the protein with copper in alkali, and then the reduction of the phosphomolybdic-phosphotungstic reagent by the copper treated protein. The optical density of the sample is then read in a spectrophotometer at a wavelength of 640 nm. The colour change is dependent on the amount of protein present and can be compared with a standard curve of bovine serum albumin of known concentration. A disadvantage of the Lowry assay is that the amount of colour varies slightly with different proteins, however, in this situation, where mixed tissue protein is being measured, and an absolute value of protein content is not required, this variation is not a serious drawback.

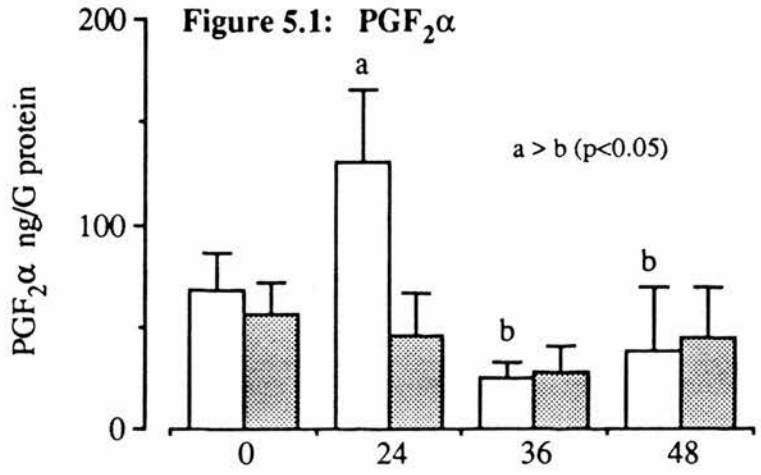
Statistics

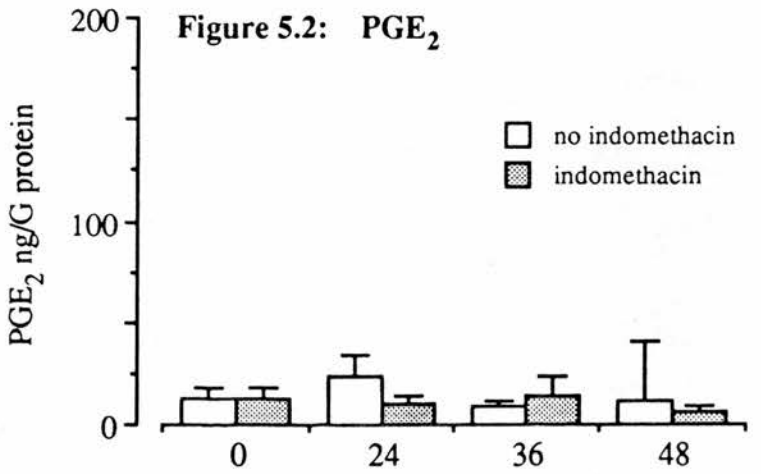
Statistically significant differences were found using analysis of variance using Newman Keuls procedure. Within subject variables were analysed using analysis of variance using the t-test. The data was log transformed before analysis to normalise the distribution. Since the 95% CI are wide, the graphs show geometric mean and 67% CI.

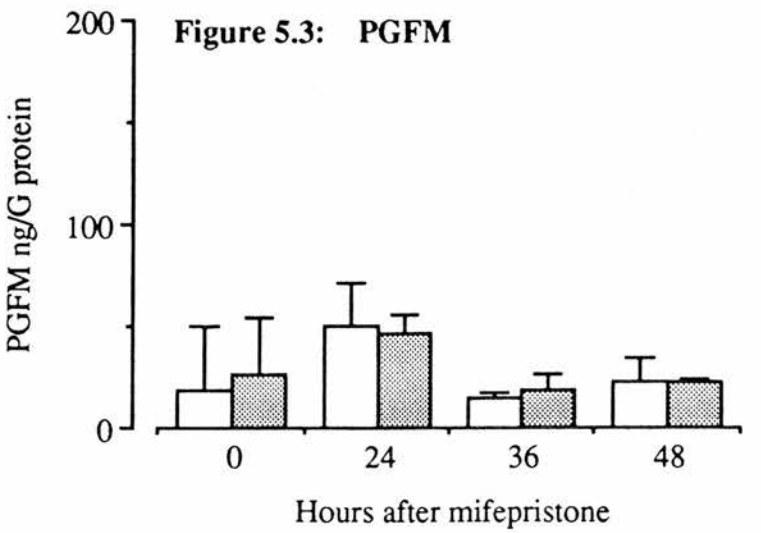
Results

Endogenous levels

The "endogenous concentrations" of PGF2α in the decidua were greater in tissue harvested from women 24 hours after treatment with mifepristone in vivo than 36 or 48 hours after mifepristone in vivo ($p<0.05$) (Figure 5.1). There was no significant effect of mifepristone in vivo on decidual concentrations of PGE_2 (Figure 5.2) or PGFM (Figure 5.3). Decidual prostaglandin concentrations in women pretreated with mifepristone and indomethacin for 24 hours were apparently lower than those in women pretreated with mifepristone alone, but the difference did not reach statistical significance.







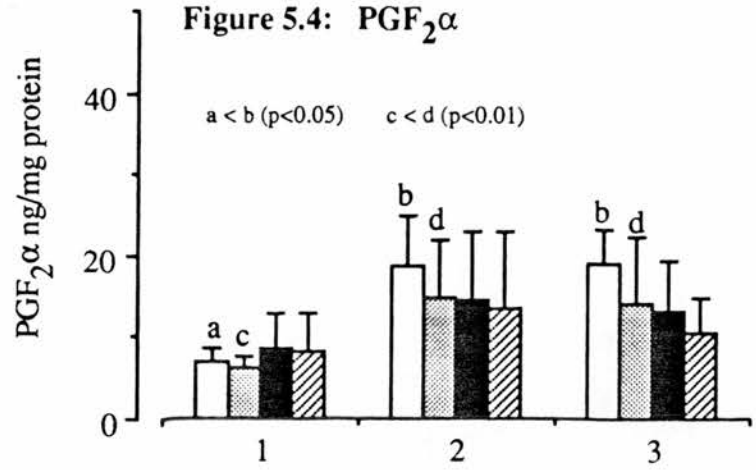
Endogenous decidual prostaglandin concentrations (geometric mean and 67% CI).

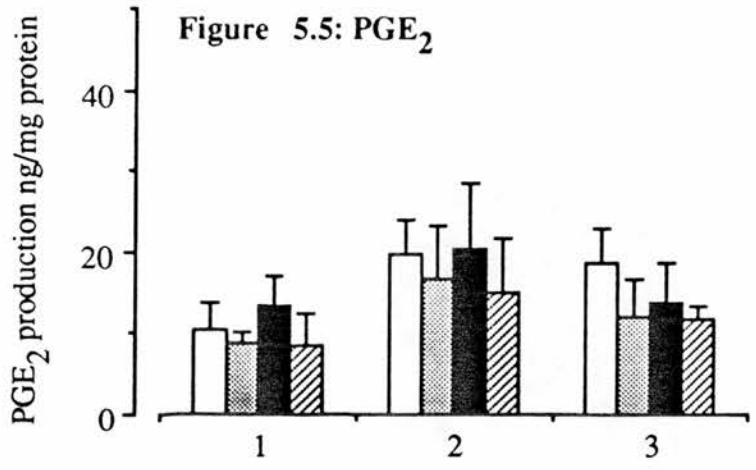
1. Effect of duration of culture on prostaglandin production

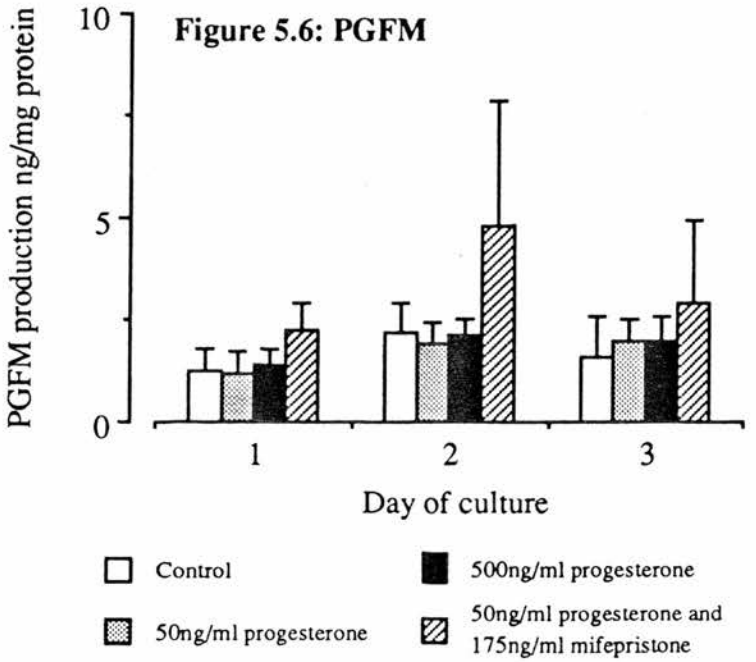
To determine the effect of duration of culture on prostaglandin production, prostaglandin production from control tissues (ie in the absence of in vivo treatment) on each day of culture was compared. (Figures 5.4; 5.5; 5.6). PGF2α production from decidua harvested from women in the control group was greater on the second and third day of culture than the first day, when cultured in complete medium only ($p<0.05$) or in 50 ng/ml of progesterone ($p<0.01$) (Figure 5.4). This increase in PGF2α production with duration of culture was not seen in decidua cultured either in a larger dose of progesterone (500 ng/ml) or in the presence of progesterone (50 ng/ml) and mifepristone (175 ng/ml) combined. There was no significant change in decidual PGE_2 or PGFM production with increasing duration of culture from the control group whatever the culture media used (Figures 5.5 and 5.6).

2. Effect of progesterone and mifepristone in vitro on prostaglandin production

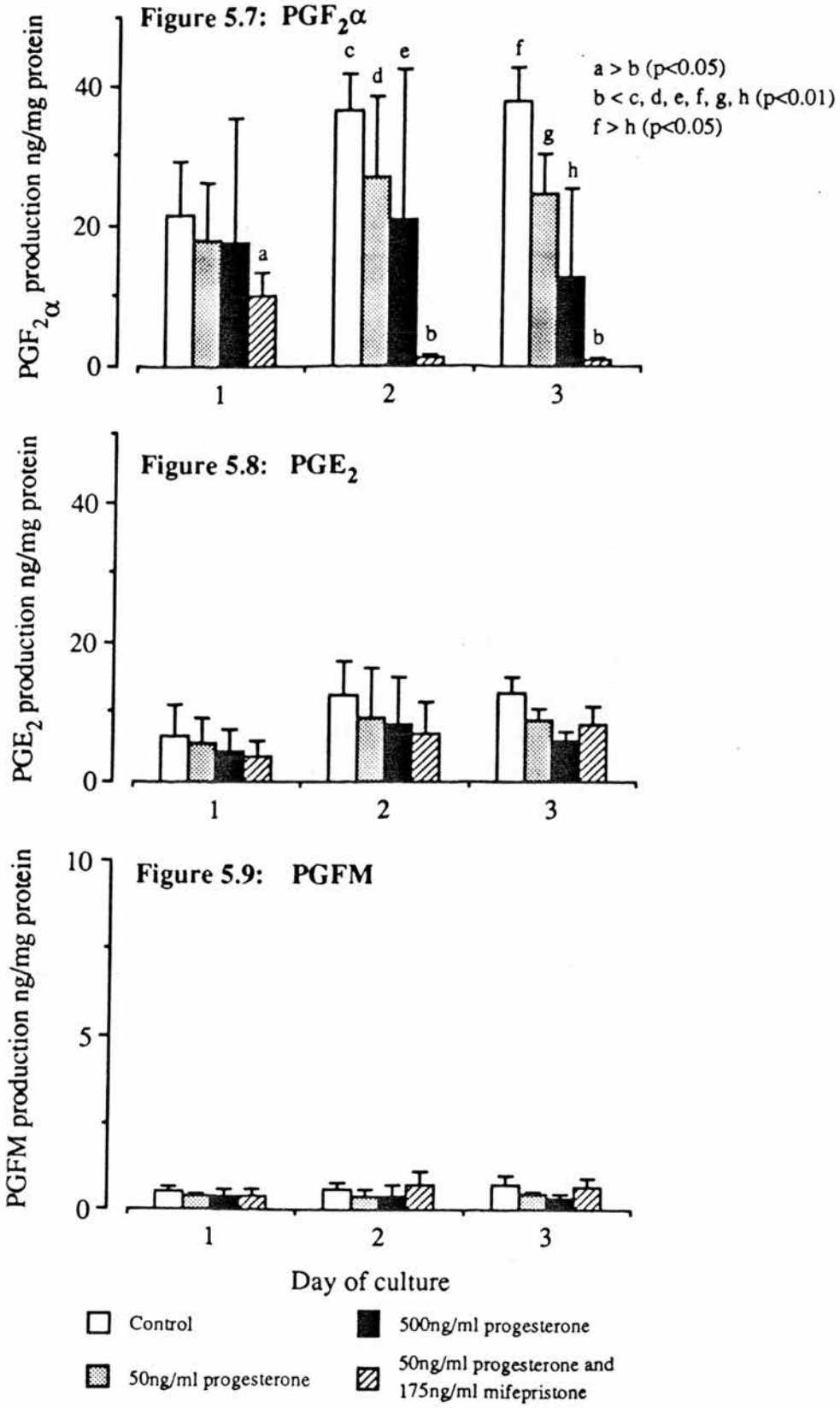
To determine the effect of progesterone and mifepristone in vitro, prostaglandin production from decidua harvested from women in the control group was compared under different culture conditions. Similarly, prostaglandin production from decidua harvested from women pretreated 24 hours earlier with mifepristone was compared under different culture conditions. The production of prostaglandins from decidua from women in the control group was low and unaffected by the addition of progesterone or progesterone and mifepristone to the medium (Figures 5.4; 5.5; and 5.6). However, in women pretreated with mifepristone 24 hours previously, the addition of progesterone (500 ng/ml) to the culture medium resulted in a significant reduction in $PGF_{2\alpha}$ production by day 3 of culture ($p<0.05$) (Figure 5.7). A lower dose of progesterone (50 ng/ml) had a smaller inhibitory effect on $PGF_{2\alpha}$ production, suggesting a dose related suppression of $PGF_{2\alpha}$ production by progesterone, although statistical significance could not be established for this. The addition of mifepristone (175 ng/ml) and progesterone (50 ng/ml) together to the culture media had a significantly greater inhibitory effect on $PGF_{2\alpha}$ production than 500 ng/ml progesterone alone, such that prostaglandin production from decidua cultured with mifepristone (175 ng/ml) and progesterone (50 ng/ml) was lower than prostaglandin production from decidua cultured either with 500 ng/ml progesterone, or in control media only, on day 2 and day 3 of culture ($p<0.01$). Different culture media had no significant effects on PGE_2 or PGFM production from decidua either from women in the control group, or the from women treated with mifepristone 24 hours previously (Figures 5.7; 5.8; 5.9).







Prostaglandin production in culture from decidua harvested from control patients. (geometric mean and 67% CI).



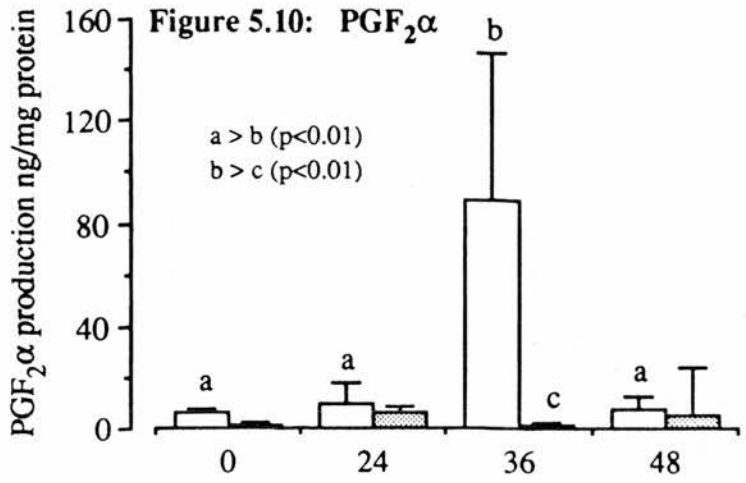
Prostaglandin production in culture from decidua harvested from women treated 24 hrs previously with mifepristone. (geometric mean and 67% CI).

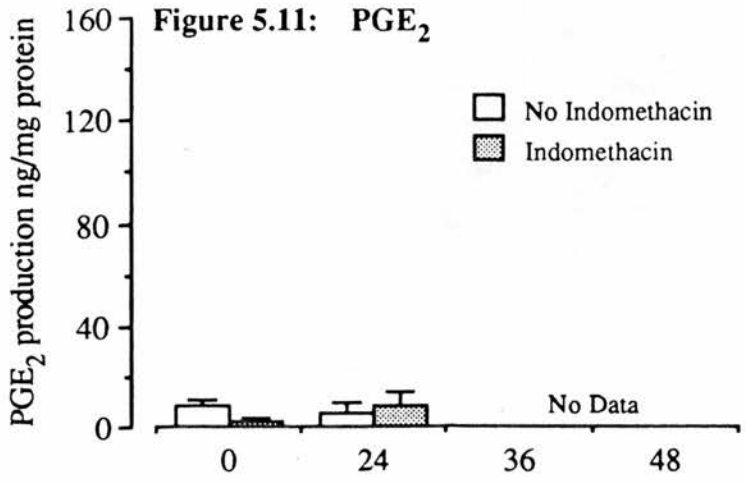
3. Effect of mifepristone (with or without indomethacin) in vivo.

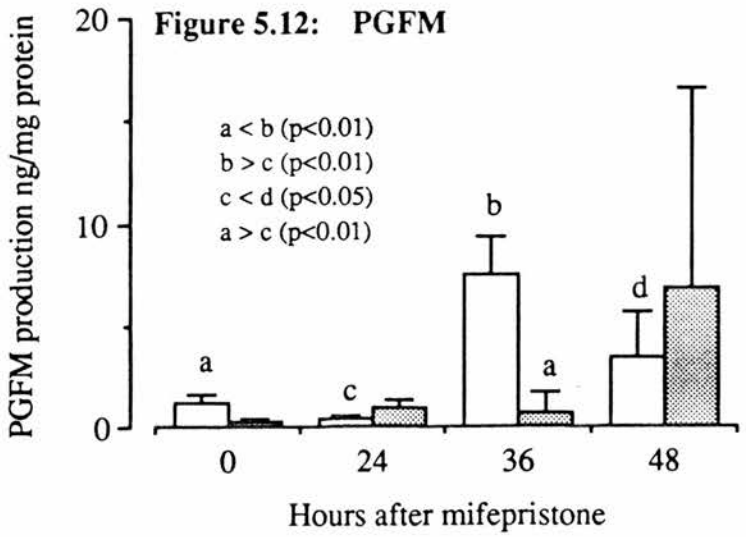
To assess the effects of pretreatment with mifepristone in vivo on the output of prostaglandins in vitro, decidual explants were cultured for 24 hours in the presence of progesterone (50 ng/ml) which simulated the concentration seen in plasma in pregnancy. The production of $PGF_{2\alpha}$ was significantly higher in women pretreated with mifepristone 36 hours previously, but had declined to control levels in the group pretreated with mifepristone for 48 hours (Figure 5.10). PGE_2 production in vivo was unaffected by pretreatment with mifepristone in vivo for 24 hours (Figure 5.11).

PGFM production was significantly decreased by 24 hours after mifepristone in vivo ($p<0.01$), but significantly increased by 36 hours after mifepristone in vivo ($p <0.0.5$) (Figure 5.12). The ratio of PGFM to $PGF_{2\alpha}$ shows a biphasic response with a decrease in PGFM production 24 and 36 hours after mifepristone in vivo, and an increase in PGFM production 48 hours after mifepristone in vivo.

Co-administration of indomethacin during mifepristone pretreatment inhibited the increase in $PGF_{2\alpha}$ ($p<0.01$) and PGFM ($p<0.05$) production seen following pretreatment with mifepristone alone for 36 hours (Figures 5.10 and 5.12). There was no significant effect of indomethacin on PGE_2 production.







Prostaglandin production in culture (supplemented by 50 ng/ml progesterone) from decidua harvested following mifepristone in vivo (geometic mean and 67% CI).

Discussion

The measured concentrations of prostaglandin (as "endogenous levels" in the first part of this study) do not correspond to the levels of prostaglandin in the tissue in vivo; rather they reflect the ability of the decidua to generate prostaglandins in response to the trauma of collection (Gréen, 1979). Maximal $PGF_{2\alpha}$ concentrations were seen 24 hours after mifepristone in vivo, in contrast to maximal production of $PGF_{2\alpha}$ in culture which was seen 36 hours after mifepristone in vivo. The reasons for this difference are unclear, but may relate to a difference between the amount of intracellular prostaglandin which can be extracted and measured after homogenisation of the tissue, and the ability of the tissue to secrete prostaglandin from intact cells into the surrounding culture medium.

The low decidual prostaglandin production in culture from women in the control group is in keeping with previous reports on decidual prostaglandin production in pregnancy (Maathuis and Kelly, 1978; Abel et al, 1980). The increase in prostaglandin production with duration of culture (from decidua from women in the control group) when cultured in the absence of steroid hormones may reflect a decreasing effect of endogenous progesterone. This hypothesis is supported by the lack of a change in prostaglandin production over duration of culture either when progesterone is added to the culture medium, or in women pretreated with mifepristone 24 hours previously, where the effects of endogenous progesterone are already antagonised by mifepristone in vivo. The addition of progesterone to the culture medium inhibited decidual prostaglandin production by the third day of culture only in women pretreated with mifepristone in vivo, and not in the control group. It may be that prostaglandin production is already maximally suppressed by the effect of endogenous progesterone in the control group. The inhibitory effects of progesterone in vitro on prostaglandin production from decidua in culture is in keeping with previous data on the effects of progesterone on endometrial prostaglandin production (Abel and Baird, 1980; Kelly and Smith, 1987).

In vitro treatment with mifepristone and progesterone inhibited prostaglandin production in women pretreated with mifepristone in vivo. These results are surprising since mifepristone, as a progesterone antagonist, might be expected to stimulate prostaglandin production in vitro.Stimulatory effects of mifepristone in vitro on decidual prostaglandin production have previously been demonstrated from decidual glandular cells (Smith and Kelly, 1987) and from endometrial stromal cells (Kelly et al, 1986a) in culture. In the former study, the increase in prostaglandin

production was seen both in the presence and absence of arachidonic acid, suggesting an effect of mifepristone on the cyclo-oxygenase enzyme. In contrast, incubation of fragments of secretory endometrium with mifepristone alone resulted in a significant decrease in $PGF_{2\alpha}$ production by the third day of culture, compared with controls, suggesting that mifepristone can act as a progesterone agonist in vitro (Kelly and Smith, 1987b). However, an equimolar concentration of progesterone was a greater inhibitor of prostaglandin production, and when equimolar concentrations of mifepristone and progesterone were added together to the culture medium, mifepristone partially antagonized the inhibitory effects of progesterone on $PGF_{2\alpha}$ production. There were no significant effects of mifepristone in vitro, either alone or in combination with progesterone on decidual prostaglandin production in the same system (Kelly and Smith, 1987b). In the study described here, the addition of mifepristone and progesterone in vitro to decidua harvested from untreated women had no significant effect, confirming the data from Kelly and Smith. When decidua harvested from women pretreated 24 hours earlier with mifepristone was cultured, the greater inhibitory effect of mifepristone and progesterone together, compared with a similar concentration of progesterone alone, may reflect action of mifepristone as a (partial) progesterone agonist in vitro.

Decidua from women pretreated in vivo with mifepristone for 36 hours released more $PGF_{2\alpha}$ in culture than decidua from women in the control group. Other groups have used peripheral levels of prostaglandin metabolites as an index of uterine prostaglandin production, and have failed to show an increase 24-48 hours after mifepristone in vivo, either in the first (Hill et al, 1990a) or the second trimester (Hill et al, 1990b). Similarly, no change in peripheral PGFM levels has been seen following the progesterone synthetase inhibitor epostane (Webster et al, 1985a). However, the data presented here suggest that mifepristone inhibits prostaglandin metabolism in addition to increasing prostaglandin synthesis, resulting in a decreased production of PGFM after pretreatment with mifepristone for 24 hours in vivo. It is unlikely, therefore, that peripheral serum levels of PGFM reflect in vivo decidual prostaglandin production following mifepristone.

Although pretreatment with mifepristone for 36 hours resulted in increased $PGF_{2\alpha}$ production, this was not seen 48 hours after mifepristone treatment in vivo. Since clinically, bleeding and abortion may occur at this time even prior to the action of exogenous prostaglandins, the lack of decidual prostaglandin production at this time is surprising. There are several possible explanations. Firstly, although progesterone

inhibits prostaglandin production, there is evidence that progesterone is required for the accumulation of arachidonic acid, the precursor of prostaglandin production.When endometrial stromal cells are incubated with arachidonic acid, a subsequently increased ability to generate prostaglandins is seen only when the cells are coincubated with progesterone (Kelly et al, 1986a). In vitro, the synthetic capacity of persistent proliferative endometrium is lower than that of normal secretory endometrium until arachidonic acid is added to the incubation media, suggesting that persistent proliferative endometrium and normal secretory endometrium have the same synthetic capacity, but that the low endogenous concentrations of $PGF_{2\alpha}$ in the former arise from lack of an endogenous precursor (Smith et al, 1982). In women pretreated with mifepristone for 48 hours, the stores of arachidonic acid may be depleted through conversion into prostaglandins, and lack of replacement in the antiprogestogenic environment. Further prostaglandin production may be limited 48 hours after mifepristone in vivo through lack of the precursor arachidonic acid. An alternative explanation may be that the decidua is partially necrotic by this stage, at least when viewed at the ultrastructural level (Schindler et al, 1985), so that the amount of functional tissue capable of prostaglandin production is reduced.

PGFM production in women pretreated with mifepristone in vivo for 24 hours was reduced, despite a non significant increase in the precursor $PGF_{2\alpha}$. The ratio of PGFM to $PGF_{2\alpha}$ which represents the ability of the tissues to catabolise $PGF_{2\alpha}$ was reduced 24 and 36 hours after mifepristone in vivo, compared to control tissues. These data suggest an inhibitory effect of mifepristone on prostaglandin metabolism. In vitro data suggests that progesterone stimulates prostaglandin metabolism (Kelly et al, 1986a), and mifepristone has been shown to inhibit prostaglandin metabolism in glandular decidual cells (Smith and Kelly, 1987). The net effect of mifepristone in vivo in stimulating prostaglandin production and inhibiting prostaglandin metabolism is to increase local decidual concentrations of prostaglandins. These effects on prostaglandin production and metabolism are in accord with the theory that pathophysiological processes which stimulate prostaglandin production inhibit metabolism, and vice versa (Hoult and Moore, 1979). In the clinical situation, when exogenous prostaglandins are given in combination with mifepristone, a decrease in prostaglandin metabolism would be particularly important and may explain the increased sensitivity to exogenous prostaglandins, but not oxytocin, seen following mifepristone.

It has been assumed that mifepristone stimulates prostaglandin production via its progesterone antagonistic effects. However, mifepristone is also a glucocorticoid antagonist (Philibert et al, 1981). The glucocorticoids are known to stimulate uterine prostaglandin production. Preincubation of dispersed endometrial cells in culture with cortisol ($5x10^{-7}$ - $5x10^{-5}$M) resulted in a dose dependent decrease in the ability of the tissues to generate PGF in response to stimulation by histamine (Skinner et al, 1984). It is likely, however, that it is the antiprogestogenic effect of mifepristone which influences prostaglandin production and not its antiglucocorticoid effect. Progesterone shows inhibitory action on PGF production by proliferative endometrium in tissue culture at concentrations where cortisol has no effect (Kelly and Smith, 1987a) and under in vitro systems where mifepristone stimulates prostaglandin synthesis, the antiprogestin ZK 98734 (which has very little antiglucocorticoid activity) had similar effects (Smith and Kelly, 1987; Kelly and Smith, 1987b).

Indomethacin is one of the group of non steroidal anti-inflammatory drugs and acts via inhibition of the cyclo-oxygenase enzyme to inhibit prostaglandin production (Lands and Hanel, 1983). The observed effects of indomethacin in inhibiting the mifepristone induced increase in prostaglandin production are in accordance with this. This will be further considered in the next Chapter when the effects of mifepristone with or without indomethacin on uterine activity are studied.

Summary

The observed effects of progesterone in vitro (and by inference in vivo) in inhibiting decidual prostaglandin production is in accordance with the known effects of progesterone. The inhibitory effect of mifepristone and progesterone on decidual prostaglandin production in vitro is more difficult to explain, but may reflect partial agonist activity of mifepristone, which has been demonstrated by other workers (Gravanis et al, 1985). In vivo, mifepristone clearly stimulates decidual prostaglandin production, but this increase can be inhibited by indomethacin.

Chapter 6:

Effects of mifepristone in vivo (with or without indomethacin) on uterine activity.

Introduction

Mifepristone stimulates uterine activity in pregnant women in the first and second trimester (Bygdeman and Swahn, 1985; Hill et al, 1990b) and sensitises the uterus to the action of exogenous prostaglandins (Bygdeman and Swahn, 1985). Progesterone withdrawal (Csapo et al, 1973b), and the use of inhibitors of progesterone synthesis (Webster et al, 1985a; Webster et al, 1985b) also have the same effect. The mechanism of action of this increase in uterine activity is as yet unclear. The inhibitory effects of progesterone on uterine activity act through various mechanisms (as reviewed in Chapter 1). The antiprogesterone mifepristone is known to stimulate gap junction formation in rat myometrium (Garfield and Baulieu, 1987), and to stimulate decidual prostaglandin in the human (Chapter 5), both of which effects will contribute to an increase uterine activity. In this Chapter, the results of a study to determine whether mifepristone also simulated uterine activity when decidual prostaglandin production was inhibited is described. Uterine activity was measured 24-48 hours after mifepristone administration, both when mifepristone was given alone and in conjunction with indomethacin. Uterine activity in the two groups was then compared. The contribution of the increase in decidual prostaglandin production to the increase in uterine activity seen following mifepristone could therefore be assessed.

Methods

Patients

Patients recruited for the protocol described in Chapter 5 were included in the study. The protocol was approved by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board, and written informed consent obtained from each patient, prior to enrolment in the study. Patients were randomised to treatment with mifepristone (± indomethacin) in one of eight groups as described in Chapter 5: a control group (n=8), three groups treated with 600 mg mifepristone 24 hours (n=8), 36 hours (n=6) or 48 hours (n=6) prior to surgical termination of pregnancy. Five women were treated with indomethacin alone (100 mg PR every 12 hours) from 48 hours prior to surgical termination of pregnancy, the last dose being given 12 hours prior to surgery. A further three groups of women were given mifepristone (600 mg) and indomethacin (100 mg PR every 12 hours from the time of mifepristone administration, the last dose

being given 12 hours preoperatively) either 24 (n=5), 36 (n=6) or 48 (n=5) hours prior to surgical termination respectively. Following measurement of uterine activity, surgical termination of pregnancy was performed as described in Chapter 5. After a four hour recovery period, women were allowed home if well, with anti D if necessary, and seen for follow up one week later.

Intrauterine pressure recordings

Intrauterine pressure was recorded for one hour immediately prior to surgical abortion. The equipment used, the method of catheter insertion and the method of calibration were as described in Chapter 4. Half way through the monitoring period, an intravenous injection of 10 μg of PGE_2 (Prostin E_2, Upjohn) was administered, to determine the sensitivity of the uterus to exogenous prostaglandins.

Calculation of uterine activity

Uterine activity was calculated in Montevideo units. The reasons for this, and the system used for identifying contractions are described in Chapter 4.

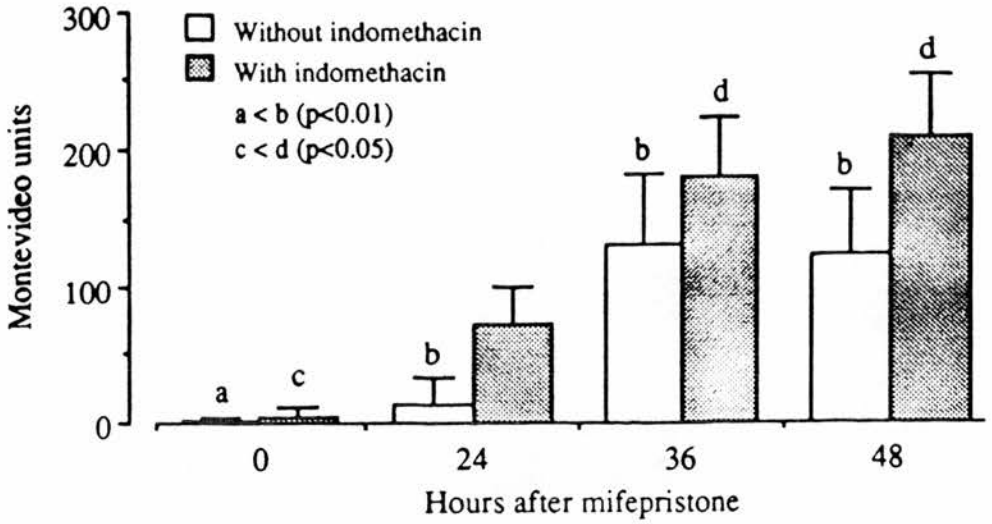
Statistics

Significant differences in uterine activity between the groups were found using analysis of variance using Newman Keuls procedure. The data was log transformed before analysis to normalise the distribution. A paired t test was used to determine the significance in the increase in uterine activity following prostaglandin E_2 injection. Graphs show geometric mean and 67% CI for clarity.

Results

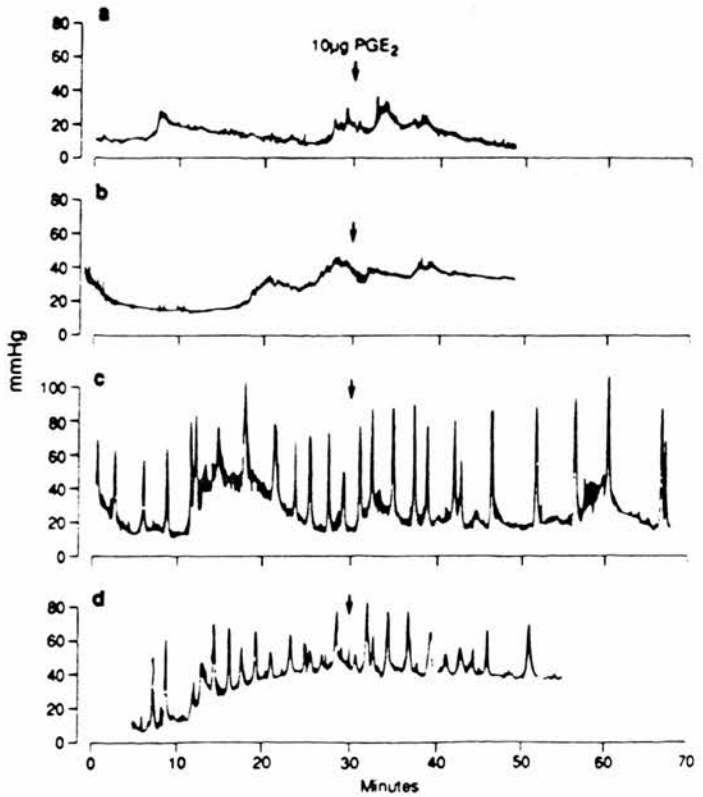
Uterine activity following mifepristone, with or without indomethacin, prior to treatment with exogenous prostaglandins is shown in Figure 6.1 (expressed in Montevideo units) and Figure 6.2 (four actual traces chosen at random). Technical problems with the recording apparatus precluded uterine activity measurement in five patients, so that results are based on tracings from 7 patients in the control group, and 6, 6, and 5 patients pretreated with mifepristone for periods of 24, 36, and 48 hours respectively. Tracings were obtained from 5 women in each of the four groups treated with indomethacin ± mifepristone.

Figure 6.1



Uterine activity following mifepristone in vivo (geometric mean and 67% CI).

Figure 6.2



Uterine activity in women of ≤ 56 days gestation. (a) Control. (b) Treated with indomethacin 100 mg bd for 48 hrs. (c). 36 hrs after mifepristone 600 mg. (d) 36 hrs after mifepristone 600 mg and indomethacin 100 mg bd.

Uterine activity was significantly greater in the three groups pretreated with mifepristone than in the control group ($p<0.01$). An increase in uterine activity was seen in four out of six women 24 hours after mifepristone, and in all women 36 and 48 hours after mifepristone. Within the group of women pretreated with mifepristone, duration of pretreatment had no significant effect on uterine activity. There was no difference in uterine activity when indomethacin and mifepristone were given, compared to uterine activity following mifepristone alone, for each treatment duration.

There was no change in uterine activity measured in Montevideo Units following 10 μg of PGE_2, either in the control group, or group pretreated with mifepristone alone.

Discussion

The mechanism of uterine contractility and its regulation, with particular reference to the role of progesterone, has been reviewed in Chapter 1. Both the antiprogestin mifepristone, and the progesterone synthetase inhibitor epostane stimulate uterine activity. In a study to determine the effect of epostane on uterine activity, twenty women of 8-11 weeks gestation, were randomly allocated to receive epostane 100 mg tid for three days, or to receive placebo tablets (Webster et al, 1985a). After treatment a Foley catheter was inserted in to the uterus, the balloon inflated with 2 ml of fluid and pressure changes recorded through a fluid column in the catheter. Uterine activity was greater in the epostane treated group compared with the placebo treated group. There was a significant inverse correlation between uterine activity 60 minutes after catheter insertion, and post treatment serum progesterone levels. In another study epostane resulted in a dose dependent increase in sensitivity to exogenous prostaglandin given in the form of a prostaglandin E_2 pessary, with a partial increase in uterine sensitivity following 100 mg epostane t.i.d for three days, and a consistent increase in uterine sensitivity to prostaglandin E_2 following epostane 400 mg daily for 3 days (Webster et al, 1985b). In the second trimester, treatment with epostane resulted in increased uterine activity compared to control women, and increased uterine sensitivity to exogenous prostaglandins but not to exogenous oxytocin (Selinger, 1988). Pretreatment with mifepristone also increases uterine activity in the first (Bygdeman and Swahn, 1985; Swahn and Bygdeman, 1988) and second trimester (Hill et al, 1990b). An increase in uterine sensitivity to exogenous prostaglandins, but not oxytocin can be seen following the use of mifepristone in the first trimester (Swahn and Bygdeman, 1988). When mifepristone 25 mg b.d is given, an increase in uterine activity can be seen in some women 24 hours after and in

all women 36 hours after mifepristone therapy (Swahn and Bygdeman, 1988). In non pregnant women in the luteal phase of the menstrual cycle, mifepristone causes an increase in uterine activity measured in Montevideo Units, but an increase in uterine sensitivity to exogenous prostaglandins has not been demonstrated (Gemzell et al, 1990). The progressive increase in uterine activity following mifepristone described in this Chapter is in accordance therefore with previous work on the antiprogesterones and progesterone synthetase inhibitors. Interestingly, there was an increase in uterine activity in four out of six women 24 hours after mifepristone, but in all women 36-48 hours after mifepristone, and again this is in agreement with previous data (Swahn and Bygdeman, 1988).

The prostaglandin synthetase inhibitor indomethacin has been shown to inhibit uterine activity in conditions associated with an increase in prostaglandin synthesis. In women with dysmennorhoea, indomethacin (100 mg rectally) inhibited uterine activity within one hour of administration (Lundstrom et al, 1979). Indomethacin has also been shown to prolong gestation in women in suspected preterm labour, compared to placebo (Zuckerman et al, 1984). Both of these conditions are associated with increased prostaglandin synthesis (Willman et al, 1976; Weitz et al, 1986). Therefore, in situations where an increase in uterine activity is caused by an increase in prostaglandin production, indomethacin appears to inhibit uterine activity, presumably by inhibiting endogenous prostaglandin production.

The failure of indomethacin to inhibit the mifepristone induced increase in uterine activity in this study was surprising. In Chapter 5, indomethacin was shown to inhibit the mifepristone induced increase in decidual prostaglandin production. Since indomethacin did not simultaneously inhibit uterine activity this suggests that factors other than an increase in decidual prostaglandin production are responsible for the increase in uterine activity seen following mifepristone. There are several possible explanations for this:

Firstly, mifepristone not only stimulates production of prostaglandins which increase uterine tone, but has also been shown (in vitro) to antagonise the progesterone induced inhibition of the uterine relaxant PGI_2, in rat myometrium (Jeremy and Dandona, 1986). When indomethacin is given with mifepristone, the production of both these prostaglandins may be inhibited, such that the balance between myometrial stimulation and relaxation is maintained. Secondly, indomethacin also inhibits prostaglandin metabolism (Hansen, 1976) so that the reduced amount of

PG synthesised is active for longer. Since indomethacin was not added to the culture media, data shown in Chapter 5 may not reflect PG concentration in decidua in vivo. Thirdly, some commentators (Kierse, personal communication) have suggested that uterine contractility is a function of myometrial, rather than decidual prostaglandin production, and that the effects of mifepristone, with or without indomethacin, may not be the same in the myometrium as in the decidua. By extrapolation from labour at term, the main source of the increase in prostaglandin levels in both amniotic fluid (Karim and Devlin, 1967) and peripheral plasma (Gréen et al, 1974) co-incident with an increase in uterine contractility is unclear, with the decidua (Karim and Devlin, 1967), the fetal membranes (McDonald et al, 1974) and the myometrium (Willman and Collins, 1976) all having been proposed. However, since indomethacin in the regime described here has been shown to inhibit uterine activity in conditions associated with increased prostaglandin production, one would expect indomethacin to inhibit uterine activity stimulated by any drug acting via increased prostaglandin synthesis, wherever the source of the increased prostaglandin production in the uterus.

The most convincing explanation for the lack of inhibition of the mifepristone induced increase in uterine activity by indomethacin is that the increase in uterine activity seen following mifepristone is not due to antagonism of the inhibitory effects of progesterone on prostaglandin production, but is mediated via antagonism of one of the other suppressive effects of progesterone on uterine activity (Chapter 1). Mifepristone appears to stimulate gap junction formation in rat myometrium (Garfield and Baulieu, 1987) and it is possible that the stimulatory effects of mifepristone on uterine activity act via this mechanism, rather than via increased prostaglandin production. Further studies are required to investigate the contribution of other mechanisms to the mifepristone induced increase in uterine activity.

The lack of a further increase in uterine activity following exogenous prostaglandin suggests that the dose of prostaglandin used was below the threshold dose. In mid pregnancy, the threshold dose of a single injection of PGE which stimulates a uterine contraction is 20 μg (Lundstrom, 1986). A dose of 10 μg of PGE_2 was chosen for the study described here, in the hope that the untreated uterus would not respond, but that following sensitisation with mifepristone (where an increase in myometrial sensitivity to intramuscular sulprostone of four fold can be seen) (Bygdeman and Swahn,1985), an increase in uterine tone might be observed. In addition, we were keen to avoid any cardiovascular side effects which can be observed following moderate-large doses of

intravenous prostaglandins (Karim et al, 1971). Since in early pregnancy the uterus is even more refractory to the effects of exogenous prostaglandins, it appears in retrospect that the dose of PGE_2 used in this study to test uterine response was too small. Further studies using larger doses of PGE_2 would be required to determine whether co-administration of indomethacin inhibits the increased sensitivity of the uterus to exogenous oxytocic agents.

Chapter 7:

The effects of mifepristone on the cervix in early pregnancy

Introduction

The data presented in Chapter 6 show that mifepristone stimulates uterine activity, an action which contributes to its abortifacient effect. The effect of mifepristone on the cervix has also been studied. The largest published study involved 230 primigravidae of 10-12 weeks amenorrhoea, given 0-100 mg mifepristone in a double blind randomised fashion 24 and 12 hours prior to surgical termination of pregnancy (World Health Organization Task Force, 1990). Cervical dilation observed at operation was significantly greater in women pretreated with mifepristone (mean 6.5 mm) compared to those treated with placebo (mean 5.4 mm). Resistance to further dilation was assessed subjectively at operation. Significantly greater ease of cervical dilation was observed in mifepristone treated women, an effect which was dose dependent. Lower cervical resistance in mifepristone treated patients was also recorded objectively using a strain gauge in a study of 80 primigravidae of 7-13 weeks gestation. The sum of the peak force required to insert dilators from 4-10 mm was 84.3N in women given placebo tablets, and 46.0N in women pretreated 30 hours earlier with 600 mg mifepristone (Cohn and Stewart, 1991). The duration of mifepristone pretreatment also appears to be important. In a study of 55 primigravidae at 7-10 weeks gestation, the resistance of the cervix to further dilation was significantly less in a group of women given 100 mg mifepristone 36 and 48 hours prior to termination of pregnancy, compared to women treated with mifepristone 36 and 24 hours prior to surgery (Rådestad et al, 1990). A ripening effect of mifepristone on the cervix has also been observed in other studies where parous and nulliparous women were studied together, or when non pregnant women were studied (Rådestad et al, 1988; Durlot et al, 1988; Gupta and Johnson, 1990).

Therapeutically, the ripening effect of mifepristone on the cervix can be exploited to avoid cervical trauma during surgical termination of pregnancy, but it is also likely to aid the abortion process during medical termination of pregnancy. In this Chapter, the results of a study to determine the effects of mifepristone on the cervix in early pregnancy, both in terms of the force required to dilate the cervix further, on collagen, glycosaminoglycan and neutrophil content, and on the ability of the cervix to generate prostaglandins in culture are reported.

Methods

Thirty-five women of between ten and twelve weeks gestation undergoing legal termination of pregnancy were recruited into the study, and randomised to receive either a placebo tablet or 200 mg oral mifepristone 36 hours before surgical termination of pregnancy. At operation, the free passability of the cervix and the force required to dilate the cervix was measured, and a cervical biopsy taken. The ability of the cervix to generate prostaglandins in tissue culture, and the glycosaminoglycan and neutrophil elastase content of the cervix were subsequently assessed. The protocol was approved by the local Reproductive Medicine Ethical Subcommittee, and was part of a multicentre trial supported by the WHO.

Protocol

Recruitment

Patients were recruited from those scheduled for suction termination of pregnancy, as described in Chapter 2. Prior to recruitment, a clinical history, and full physical examination were performed. Details of the study were given to the patient, and written consent obtained. Patients were informed of their right to withdraw from the study should they wish.

Mifepristone administration

On admission to the study, details of the patients menstrual cycle, the date of the last menstrual period, and previous obstetric and medical history were recorded on a data sheet. Details of the general physical examination, height and weight were recorded. A pelvic ultrasound scan was performed and mean gestational sac diameter noted. Blood was taken for typing of the patient's blood group and haemoglobin estimation. Exclusion criteria for the study were:

- age less than sixteen;
- recent irregular menstrual cycles;
- ectopic pregnancy;
- amenorrhoea of < 70 or > 84 days;
- chronic disease affecting the respiratory, gastrointestinal, endocrine, genitourinary, neurological or cardiovascular system;
- contraindications to mifepristone;
- regular use of prescription drugs or the oral contraceptive pill in any of the three months prior to presentation.

Patients were given a single tablet containing either 200 mg mifepristone or placebo. The study was performed in a double blind randomised fashion, patients being assigned in order of recruitment to a coded treatment pack made up by the WHO in Geneva. Women were allowed home immediately after taking the tablets, asked to note any bleeding and to avoid the use of non steroidal anti-inflammatory drugs. Surgical termination of pregnancy was scheduled for thirty-six hours after tablet administration.

Termination of pregnancy

On readmission to hospital for termination of pregnancy, a short symptom questionnaire was completed, and routine clinical observations performed. Termination of pregnancy was performed in theatre under general anaesthesia, with the patient in the lithotomy position. After washing the perineum with 10% povidone-iodine, the operative area was draped with sterile towels, and a bimanual pelvic examination performed. A Sim's speculum was inserted into the vagina, and the cervix grasped with two vollsellae. Traction was exerted on the volsellae, and a trucut biopsy taken of the anterior lip of the cervix, in the sagittal plane, at 75^{o} to the cervical canal and approximately 1 cm from the external cervical os. The cervical biopsy was divided into two, one piece placed in "complete" culture media (see Chapter 5) and the other in formaldehyde. The free passability of the cervix (the maximum size of cervical dilator which could be passed through the cervix without encountering resistance as judged clinically) was recorded. The cervix was dilated using a set of cylindrical dilators of progressively greater diameters, starting with a dilator of 3 mm and increasing by increments of 1 mm . The dilators were attached to a strain gauge and the peak force required to insert each dilator through the internal os recorded. The cervix was dilated to 12 mm (or 10 mm if dilation was proving excessively difficult), and routine suction termination of pregnancy performed. Blood loss was estimated after sieving the products of conception and removing fetal and placental tissue. The duration of the operation, from the start of cervical dilation to completion of abortion was recorded.

The patient was reviewed six hours post operatively, and a further blood sample taken for haemoglobin estimation, prior to discharge. A follow up appointment was scheduled for eight weeks post operatively, and barrier methods of contraception advised until the next menstrual period.

Follow up

At the follow up visit, details of any post operative complications, the duration of bleeding and the interval to the next menses recorded. Pelvic examination was performed, and the patient discharged from further follow up if well.

Cervical biochemistry

A portion of the cervical biopsy was fixed in buffered 4% formaldehyde solution, dehydrated and embedded in paraffin, to enable 5 micron sections to be cut longitudinally through each specimen. The sections were then deparaffinised with xylene, and rehydrated through graded ethanol. The following stains were used:

1. Sirius Red (0.1%) (BDH, UK) in saturated aqueous picric acid for one hour. The sections were then washed for 2 minutes in 0.01M hydrochloric acid, dehydrated, washed with xylene and coverslipped with Eukitt (O.Kindler Gmbh and Co). This stain is specific for polymerised collagen. The dye molecules attach to the collagen fibrils in such a way that their long axes are parallel, substantially increasing the birefringencey of the collagen. Thus collagen concentrations in different biopsies can be compared by measuring the percentage transmission of polarised light through each specimen (Junqueira et al, 1979).

2. Alcian Blue (0.05%) in 0.025M acetate buffer (pH 5.8) with either 0.06M, 0.5M or 1.0M magnesium chloride for twelve hours. The sections were then rinsed in absolute alcohol and washed with xylene, before coverslipping with Eukitt. This method has previously been used to stain for the major classes of glycosaminoglycans in the cervix (Scott and Dorling, 1965). The differential concentrations of magnesium chloride provide electrolyte cations which compete with dye cations for binding sites on tissue polyanions; with the different glycosaminoglycans responding to different electrolyte concentrations: 0.06M, 0.5M and 0.1M magnesium chloride stain selectively for hyaluronic acid, chondroitin/dermatan sulphate and keratan sulphate respectively. The intensity of staining at each magnesium chloride concentration can be used to compare glycosaminoglycan concentrations in each biopsy, by measuring percentage light transmission.

3. Immunohistochemical staining was used to measure neutrophil elastase content. Tissue sections were incubated with 3% hydrogen peroxide in methanol for 30 minutes to inhibit endogenase peroxidase activity, rinsed in distilled water and incubated for 30 minutes in normal rabbit serum (Dakopatts X902; Dako Ltd, UK) diluted 1: 5 with Tris buffered saline (TBS). They were then incubated for 30 minutes with sheep polyclonal antibody to human neutrophil elastase (ICN Biochemicals Ltd, UK), washed in TBS for 2 x 5 minutes, incubated for 30 minutes with 1:2500 rabbit anti-sheep immunoglobulin/biotinylated (Vector Ltd, UK), washed in TBS 2 x 5 minutes, incubated with avidin-biotin complex / horseradish peroxidase (Dakopatts K355; Dako Ltd, UK), washed in TBS 2 x 5 minutes and incubated for 5 minutes with peroxidase substrate (3, 3-diaminobenzidine tetrahydrochloride) solution. Sections were dehydrated, cleared in xylene and coverslipped with Eukitt. The number of neutrophils in two random fields of the connective tissue of each biopsy specimen were then counted excluding those within blood vessels.

Quantitation of material

1. Collagen

Slides were screened under an image analyser (Olympus Cue 2 Analyser System with 386 computer and densitometry software 2.2, Olympus UK), under x40 magnification. Two polarising filters were used, one below the condenser and the other above the objective lens. A plain area of glass (containing no specimen) was used as the reference optical density, with the polarising filters set in parallel (100% light transmission). The polarising filters were then reset at 90^{o} to each other, so that no light was transmitted. The specimen was then moved under the lens, and the optical density (% light transmission) of 5 separate areas of around 3500 microns square were analysed so that the median optical density could be calculated. Since the birefringency was greater with increasing collagen concentrations, the percentage light transmission was also greater.

2. Glycosaminoglycan content

Slides were screened as above, but without polarising filters. The instrument was again calibrated using a plain area of glass to give 100% light transmission. Since staining is proportional to glycosaminoglycan content, the greater the glycosaminoglycan content, the lower the optical density.

3. Neutrophil elastase

The number of neutrophils in two random high powered fields of the connective tissue were counted (magnification x 40). Cells in blood vessels were not included in the quantification. Mean neutrophil count was recorded.

Cervical prostaglandin production

Cervical biopsies for culture were transported to the laboratory in "complete media" as defined in Chapter 5. The biopsies were washed in complete media, cut into pieces weighing approximately 0.25 mg, and divided between two culture wells. Complete media (1 ml) was added to each well, and the plates incubated overnight (as described in Chapter 5). At the end of 24 hours of culture, spent media was removed. Methyl oximating solution (0.5ml) was added to 0.5ml of spent media, and the sample stored until analysis.

Assay procedures

Prostaglandin concentration of the spent media was measured by radioimmunoassay. Details of the PGE_2 and $PGF_{2\alpha}$ assay were as described in Chapter 5. Prostaglandin E metabolites were measured by radioimmunoassay to the methyl oxime derivative of 13, 14 dihydro-15-keto PGE_2. Again, samples were converted to the methyl oxime derivative using the methyl oximating solution described in Chapter 5. Conversion is >95% when samples are treated at 20°C overnight. Cross reactivities to the antisera to 13, 14 dihydro-15-keto PGE_2 were: 15 keto PGE_2, 11.7%; 15-keto $PGF_{2\alpha}$, 0.94%; 13, 14 dihydro-15-keto $PGF_{2\alpha}$, 0.19%; 13, 14 dihydro $PGF_{2\alpha}$, 0.11%; PGE_2, 0.05%; PGE_3, 0.03%; PGE_1, 0.02%; all other prostaglandins <0.02%. The protein content of the cervical biopsy was measured by the method of Lowry (see Chapter 5).

Statistics

Normally distributed data: age, gestation, weight, height, haemoglobin concentration, and optical density in the two groups were compared using analysis of variance. Age, sac diameter, and prostaglandin levels were log transformed (to generate a normal distribution) and then compared using analysis of variance. Cycle length, duration of menstruation, cervical dilation, duration of operation, blood loss, change in haemoglobin concentration, duration of bleeding, interval to next menses and cervical resistance in the two groups were compared using Mann Whitney U. The chi squared test was used to explore the difference in the proportion of women bleeding after tablet administration, and cervical neutrophil concentrations in the two groups.

Results

Clinical study

On breaking the code at the end of the study, eighteen women were found to have been treated with the placebo, and seventeen women with 200 mg mifepristone. There were no significant differences between the groups on recruitment (Table 7.1).

Table 7.1.

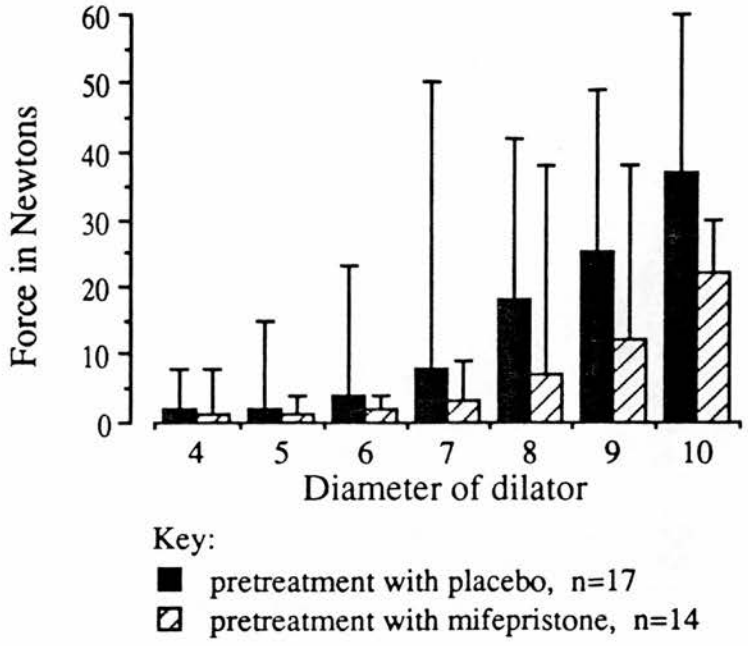
	Placebo n=18	Mifepristone n=17
Age (years)	21.8 (20.0 - 23.9)	20.4 (18.9 - 22.0)
Gestation (days)	74.3 (70.8 - 77.9)	73.0 (70.7 - 75.3)
Weight (kg)	62.8 (59.5 - 66.2)	58.8 (55.8 - 61.7)
Height (cm)	165.0 (162 - 168)	163.0 (161 - 165)
Diameter of gestational sac (mm)	47.0 (40.6 - 54.6)	46.2 (42.3 - 50.6)
Haemoglobin (g/dl)	13.2 (12.8 - 13.5)	13.0 (12.6 - 13.4)
Cycle length (days)	28 (28 - 35)	28 (28 - 35)
Duration of menses (days)	5 (4 - 7)	5 (3 - 7)

Patient characteristics on recruitment, mean and 95% CI, except cycle length and duration of menses.

Although some women of less than 70 days were found to have been recruited on reviewing the data, since they were evenly distributed between the groups, it was decided to include them in the analysis. Only two women (both in the mifepristone treated group) reported vaginal bleeding in the 36 hours after tablet ingestion (ns). Median (range) cervical dilation at operation in the mifepristone treated group was 5 mm (2-8), and 6mm (2-9) in the placebo group (ns). Median (range) total force required to dilate the cervix from 4-10 mm was 47N (9-120N) in the mifepristone treated group, compared with 93N (34-247N in the placebo treated group ($p<0.0005$) (Figure 7.1). Blood loss, and operating time were similar in the two groups, median and range in the placebo group of 220 ml (10-600) and 12 minutes (7-25) respectively, compared with 200 ml (100-350) and 10 minutes (7-20) in the mifepristone treated group. Fall in haemoglobin concentration was not significantly different between the groups: 0.6 g/dl in the placebo treated group compared to 0.4g/dl in the group treated with mifepristone. Median (range) duration of vaginal bleeding and interval from

abortion to the next menses was 7 days (2-15) and 37 days (25-52) in the placebo group, compared to 7.5 (2-16) and 37 (24-54) in the mifepristone treated group.

Figure 7.1



Force required to insert dilators of various sizes in women treated with placebo or mifepristone: median and upper limit of range.

Biochemical study.

Mean (95%CI) optical density in each of the groups is shown in Table 7.2. There was no significant difference in the optical density of stained cervical biopsies between the group treated with mifepristone and the group treated with placebo. Neutrophil elastase count was very low in both groups (ns between the groups).

Table 7.2

	Placebo	Mifepristone
AB 1.0	95.3% (94.2 - 96.4%) n = 8	95.9% (94.7 - 97.1%) n = 8
AB 0.6	82.4% (80.0 - 84.9%) n = 4	93.2% (88.9 - 97.5%) n = 7
AB 0.06	87.4% (84.4 - 90.4%) n = 8	72.4 % (53.0 - 91.8%) n = 6
PSR	0.54% (0.19 - 0.89%) n = 8	0.39% (0.07 - 0.70%) n = 7
NPL	0.5 (0 - 1) n = 9	0.857 (0 - 3) n = 7

Optical density and NPL count. Mean and 95% CI (ns. between the groups).

Prostaglandin production

Prostaglandin concentrations in the spent media were expressed as a ratio of protein content in the cervical biopsy. There was no significant difference in mean PGE_2, $PGF_{2\alpha}$ or PGEM concentrations between the group pretreated with mifepristone and the group pretreated with placebo (Table 7.3).

Table 7.3

	Placebo	Mifepristone
PGE2 ng/mg protein	1.00 (0.39 - 2.58) n = 6	0.87 (0.542 - 1.39) n = 6
PGF2α ng/mg protein	1.80 (0.46 - 7.14) n = 5	1.42 (0.51 - 3.93) n = 6
PGEM ng/mg protein	1.41 (0.77 - 2.57) n = 6	1.46 (0.097 - 2.19) n = 6

Mean (95% CI) prostaglandin concentration in media incubated with cervical biopsies (taken from women pretreated with mifepristone 200 mg or placebo) for 24 hours. (ns between the groups).

Conclusion

The data described here confirm the cervical softening effect of mifepristone. The total force required for cervical dilation in women pretreated 36 hours previously with 200 mg mifepristone was 50% that required for cervical dilation in women pretreated with placebo. These findings are very similar to those in a study comparing pretreatment with 600 mg mifepristone or placebo 30 hours prior to cervical dilation, where the force required for cervical dilation in women treated with mifepristone was 55% that found in women pretreated with placebo (Cohn and Stewart, 1991). A randomised, quantitative comparison of the cervical ripening effect of different doses of mifepristone has yet to be performed, although data on the ease of cervical dilation as assessed subjectively in a multicentre trial has shown a dose related effect of mifepristone on the cervix (World Health Organization Task Force, 1990). In contrast to other studies (Durlot et al, 1988; Gupta and Johnson, 1990; World Health Organization Task Force, 1990; Lefebvre et al, 1990; Cohn and Stewart, 1991) we failed to demonstrate any significant difference in cervical dilation between the mifepristone treated group, and the placebo treated group at the start of surgical termination of pregnancy. The reasons for this are not clear, but may merely be a consequence of the relatively small sample size employed. The lack of an effect of mifepristone pretreatment on operating time and blood loss during operation is in agreement with other studies (World Health Organization Task Force, 1990; Cohn and Stewart, 1991), although a recent study comparing the effect of 200 mg mifepristone or 1 mg gemeprost with placebo for preoperative cervical ripening in women of 63-91 days amenorrhoea showed lower blood loss in both the active treatment groups, compared with the placebo group (Henshaw and Templeton, 1991).

The data presented here fail to demonstrate differences in cervical collagen, hyaluronic acid, chondroitin/dermatan sulphate or keratan sulphate concentrations between the group of women pretreated with placebo, and those pretreated with mifepristone. This implies that the ripening effects of mifepristone on the cervix are mediated by effects other than a change in collagen or glysocaminoglycan concentrations. There is little data available on the effects of the antiprogesterones on cervical collagen or GAG concentrations in the human. Guinea-pig cervix has been studied in late pregnancy using an electron microscope to determine the morphological effects of the antiprogesterone onapristone (ZK 98 299) (Hegele-Hartung et al, 1989). Following subcutaneous administration of 1 mg onapristone on day 59 of pregnancy, greater collagen degradation, proliferation of fibroblasts, oedema and

polymorphonuclear leucocyte infiltration was observed in cervical biopsies harvested 16-24 hours after treatment, compared to cervical biopsies from control animals at the same gestation. The changes observed in the mifepristone treated animals were similar to those seen during spontaneous cervical ripening in control animals when biopsies were harvested on day 65 of pregnancy (spontaneous delivery in this species normally occurring on day 67 of pregnancy). Using electrophoresis and susceptibility to degradation by specific mucopolysaccharidases to determine concentrations of the different glycosaminoacids in the cervix of the rat (an animal in whom spontaneous delivery normally occurs from day 22- 23), greater hyaluronic acid concentrations were observed in biopsies harvested at 22 days gestation when animals were pretreated 24 hours earlier with 10 mg oral mifepristone, compared with cervical biopsies from animals pretreated 24 hours earlier with vehicle only (Cabrol et al, 1991). There were no differences in total glycosaminoglycan, heparan sulphate, dermatan sulphate or chondroitin sulphate concentrations between the mifepristone and placebo treated group. Electron microscopic analysis of human cervical tissue harvested shortly after pretreatment with mifepristone in the first trimester shows dissolution of collagen fibres, accumulation of mast cells and outgrowth of blood capillaries compared with cervical tissue harvested from a group of women treated with a placebo However, the same author has failed to demonstrate any change in cervical tissue morphology in individual patients, after treatment with mifepristone, using cervical biopsies harvested from the same patients prior to treatment as a control (Rådestad, 1991). The lack of firm quantitative or qualitative evidence of an effect of mifepristone on cervical collagen or glycosaminoacid concentration contrasts with data on the effects of the PGE_1 analogue gemeprost on cervical biochemistry. Lower collagen content was observed in biopsies from primigravidae in the first trimester treated with gemeprost, compared with biopsies from a control group treated with placebo three hours prior to termination of pregnancy, using quantitative techniques identical to those described in this Chapter (Greer et al - submitted data, 1991). A modest influx of neutrophils was also observed in the group pretreated with gemeprost (compared to placebo), but there was no change in glycoasaminoglycan concentrations. This implies, therefore, that not only does mifepristone induce cervical ripening via mechanisms other than a change in collagen or glycosaminoacid concentrations, but that the mechanism of action of mifepristone in inducing cervical ripening may differ from that of the prostaglandins. The lack of any effect of mifepristone pretreatment on the ability of the cervix to generate prostaglandins in tissue culture as described in this Chapter is not, therefore, surprising. Cervical biopsies taken from pregnant women have previously been shown to produce

prostaglandins in vitro using a superfusion technique with a trend to an increase in prostaglandin production during active dilation (Ellwood et al, 1980). An increase in the ability of the cervix to convert arachidonic acid to PGE_2 and $PGF_{2\alpha}$ has also been demonstrated, with an increase in conversion with increasing duration of pregnancy. However no correlation has been observed between cervical softening as assessed by the Bishop score, and the ability of the cervix to convert [^{14}C]arachidonic acid to any one of the prostaglandins (Christensen et al, 1985). Since prostaglandins ripen the cervix (Calder et al, 1977) and as mifepristone antagonises the inhibitory effect of progesterone on prostaglandin production from both the decidua (Chapter 5) and the myometrium (Jeremy and Dandona, 1986), it might be supposed that the ripening effect of mifepristone on the human cervix is due to increased prostaglandin production. The data described here fail to support this hypothesis, and are in agreement with data showing a (non-significant) trend for a decrease in the ability of the cervix to convert arachidonic acid to $PGF_{2\alpha}$ and PGE_2 following pretreatment with mifepristone in vivo (100 mg 48 and 36 hours, or 36 and 24 hours prior to biopsy) (Rådestadt et al, 1990). Data on the effects of pretreatment with mifepristone (10 mg 24 hours prior to harvesting tissues) in the guinea-pig show no change in the prostaglandin synthetic or metabolic activity of homogenised cervical tissue (Kelly and Bukman, 1990), despite significant effects on amnion, chorion and myometrium/decidua. Further support to the data shown here that increased endogenous cervical prostaglandin production does not play a role in the mechanism of cervical ripening induced by antiprogesterone is evinced by guinea-pig studies in which the prostaglandin synthetase inhibitor indomethacin was given in conjunction with the antiprogesterone ZK 98 299, but failed to inhibit cervical ripening (Chwalisz et al, 1987).

If mifepristone ripens the cervix neither by an increase in cervical prostaglandin production, nor via a change in cervical collagen or glycosaminoglycan content, it may have other direct effects on the cervix, possibly by antagonism of the effects of progesterone. Alternatively, the mechanism of cervical ripening following mifepristone may be passive, with the active effect being the increase in myometrial contractility following mifepristone. This is less likely, however, since in none of the human studies on the effect of mifepristone as a cervical ripening agent in the first trimester is uterine activity or abdominal pain a significant feature. In an animal study, neither myometrial activity nor premature labour was noted in guinea-pigs in whom significant cervical ripening was induced by mifepristone (Chwalisz et al, 1987). There is limited data on the effects of progesterone on the human cervix. In vitro

treatment with progesterone inhibits the accumulation of hydroxyproline containing peptides (used as an index of collagen breakdown) in media of cervical biopsies in culture from non-pregnant women (Wallis and Hillier, 1981). There is greater but conflicting evidence on the effect of mifepristone on the cervix in animals. In the pregnant sheep at term progesterone fails to inhibit cervical softening (Stys et al, 1978). In the guinea-pig, progesterone blocks the oestradiol 17β stimulated increase in collagen (type 1) cleavage products in the cultured cervix of the non pregnant guinea-pig (Rajabi et al, 1991). In studies in the rabbit, both oestradiol 17β and progesterone decreased the level of matrix metalloproteinases such as procollagenase and prostromelysin, and increased the level of tissue inhibitor of metalloproteinases in the culture media of rabbit uterine cervical fibroblasts; the overall effect being an inhibition of cervical collagen breakdown by progesterone and oestradiol 17β (Sato et al, 1991). Although these studies indicate that progesterone tends to inhibit cervical ripening, an action in accord with the effect of mifepristone as a cervical ripening agent, none of these studies indicates any mechanism of action of progesterone on the cervix other than via inhibition of collagen breakdown. If the action of mifepristone in the human cervix is to antagonise this effect of progesterone, one would expect a change in optical density as assessed by the picrosirius red method in this Chapter. Indeed, since significant cervical ripening was observed in the cohort of women treated with mifepristone from whom cervical biopsies were analysed, compared to women treated with placebo from whom cervical biopsies were also analysed, differences in the dose and duration of pretreatment with mifepristone cannot be credited with the lack of an effect of mifepristone on cervical collagen or glycosaminoacid concentrations seen here. The remaining possibilities are either that the changes in collagen or glycosaminoacid concentration are too subtle to be detected in a small group of patients by the methods described here; that mifepristone antagonises some as yet unknown effect of progesterone on the cervix, or that mifepristone has a direct effect, the mechanism of action of which is again entirely unclear. Further work is required to elucidate which of these hypotheses is correct.

Chapter 8.

Conclusions.

The data presented here confirm that mifepristone, in combination with a prostaglandin, is a safe and effective agent for menstrual induction. Data in Chapter 2 shows that the currently recommended dose of mifepristone (600 mg) could be reduced, with no reduction in efficacy of treatment. The full multicentre data is required to determine whether any small differences in efficacy exist between the regimes. Where mifepristone is unavailable or contra-indicated, gemeprost alone can be used for menstrual induction, but the efficacy of a six hourly regime (to a maximum of 3 mg) described here is less than that of 200-600 mg mifepristone followed 48 hours later by 1 mg gemeprost, and the side effects in terms of analgesic requirements are greater. Other prostaglandins can also be used in combination with mifepristone. Data in Chapter 4 show a dose-responsive effect of the orally active prostaglandin analogue, misoprostol, on uterine activity and indicate that the combination of mifepristone and misoprostol could be used for menstrual induction. The importance of mifepristone pretreatment is indicated by the fact that misoprostol alone (400 μg) is insufficient to induce abortion. Whether the mifepristone-misoprostol combination has advantages over mifepristone-gemeprost in terms of reduced side effects, has yet to be shown in a randomised study.

The mechanism of action of mifepristone in inducing abortion is still unclear. Whilst the data in Chapter 5 shows that mifepristone in vivo stimulates the ability of the decidua to generate prostaglandin when subsequently cultured in vitro, inhibition of this increase in prostaglandin synthetic capacity by co-administration of indomethacin fails to inhibit the mifepristone stimulated increase in uterine activity (Chapter 6). An increase in uterine prostaglandin production is not, therefore, an obligatory part of the mechanism of action of mifepristone in stimulating uterine activity and it may be that other mechanisms such as an increase in gap junction formation are more important. The data in Chapter 7 demonstrates the ripening effect of mifepristone on the cervix, but the mechanism of action of this effect is still unclear, since a change in cervical prostaglandin synthetic capacity, collagen or glycosaminoglycan production does not seem to be involved.

In summary, therefore, the ideal dose of mifepristone and the type and dose of prostaglandin for menstrual induction is yet to be determined. The mechanism of action of mifepristone in inducing menstruation is still unclear, and further work is required to elucidate this. It is estimated that 64,000 women per year in the United Kingdom will undergo legal termination of pregnancy at less than nine weeks gestation. The fact that the mechanism of action of mifepristone in inducing menstruation is still unclear should not prevent the combination of mifepristone and a prostaglandin being used to treat these women. All the available evidence indicates that this method would be more acceptable than the currently used technique of vacuum aspiration.

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Appendix.

Published Papers.

The following papers have been published based on the text of this thesis : -

Original Articles.

1. Norman JE, Kelly RW, Baird DT (1991). Uterine activity and decidual prostaglandin production in women in early pregnancy in response to mifepristone with or without indomethacin in vivo. Human Reproduction **6:** 740 - 744.

2. Norman JE, Wu WX, Kelly RW, Glasier AF, McNeilly AS, Baird DT (1991). Effects of mifepristone in vivo on decidual prostaglandin synthesis and metabolism. Contraception **44:** 89 - 98.

3. Norman JE, Thong KJ, Baird DT (1991). Uterine contractility and induction of abortion in early pregnancy by misoprostol and mifepristone. Lancet **338:** 1233 - 1236.

4. Norman JE, Thong KJ, Rodger MW, Baird DT (In Press).
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Review Articles.

1. Norman J (1990). The antiprogestin RU 486 (mifepristone). The Medical Letter - USA **32:** 112 - 113.

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Uterine activity and decidual prostaglandin production in women in early pregnancy in response to mifepristone with or without indomethacin *in vivo*

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Mifepristone stimulates uterine activity in pregnant women in the first trimester. In-vitro studies have shown that the addition of mifepristone to culture medium increases the ability of decidual cells to generate prostaglandins. In this study, pregnant women of < 56 days gestation were treated with mifepristone (600 mg) 24, 36 or 48 h prior to surgical termination of pregnancy. Another cohort of women were similarly treated with mifepristone, but also received indomethacin (100 mg b.d.). Control groups, given indomethacin alone (100 mg b.d. for 48 h) or no treatment, were also included. Uterine activity was measured immediately prior to surgical termination of pregnancy and the ability of decidua obtained at operation to generate prostaglandins in culture was measured. Uterine activity was increased in all groups pretreated with mifepristone, an increase which was not prevented by co-administration of indomethacin. In the groups pretreated with mifepristone 36 h previously, the production of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by decidua *in vitro* was increased. In the group in whom indomethacin was given in addition to mifepristone 36 h prior to the study, there was a marked reduction in the ability of decidua to generate $PGF_{2\alpha}$. In spite of this suppression, the increase in uterine activity was similar to the group which had not received indomethacin. These results suggest that factors other than an increase in decidual prostaglandin production are responsible for the increase in uterine activity seen following mifepristone administration *in vivo*.

Key words: mifepristone/prostaglandins/decidua/abortion/uterine contractility

Introduction

Mifepristone (RU486) has now undergone extensive clinical trials for the induction of abortion in early pregnancy and is found to be very effective in combination with an exogenous prostaglandin for the termination of early pregnancy (Van Look and Bygdeman, 1989). The mechanism of action of mifepristone in inducing an increase in uterine activity, increasing sensitivity to exogenous prostaglandin and in terminating early pregnancy is, however, unknown. Mifepristone increases prostaglandin synthesis when added to cultures of endometrial or decidual cells *in vitro* (Kelly *et al.*, 1986a; Smith and Kelly, 1987).

We have attempted to investigate whether the increase in uterine activity seen after mifepristone treatment (Bygdeman and Swahn, 1985) is due to an increase in synthesis of prostaglandins by the uterine decidua. Mifepristone was administered to women in early pregnancy prior to surgical termination of pregnancy, and prostaglandin synthesis was inhibited in a proportion of these patients with the use of indomethacin. Uterine activity was measured for an hour prior to surgery and then correlated with prostaglandin production from decidua from the same patients in tissue culture.

Materials and methods

Subjects

Women requesting termination of pregnancy with a normal intrauterine pregnancy of < 56 days amenorrhoea were recruited into the study. All women satisfied the conditions of the 1967 Abortion Act, and written informed consent was obtained from each patient. The protocol had been passed by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board.

Women were allocated into a control group (n = 8) (who received no treatment prior to surgical termination), or were given 600 mg (three tablets) of mifepristone either 24 (n = 8), 36 (n = 6) or 48 (n = 6) h prior to termination of pregnancy. A further group of women were similarly treated with mifepristone but were also given indomethacin in the form of a rectal suppository 100 mg every 12 h from the time of administration of mifepristone. The last dose of indomethacin was given 12 h prior to surgical termination so that a total number of 2, 3, or 4 doses of indomethacin were given to women treated with mifepristone 24 (n = 5), 36 (n = 6), or 48 (n = 5) h prior to surgical termination, respectively. Five women were not treated with mifepristone but were given indomethacin 100 mg rectally every 12 h prior to pregnancy termination.

The allocation of women into the above groups was randomized. Intrauterine pressure recordings were made for 1 h immediately prior to surgical termination, and uterine decidua collected at termination of pregnancy for culture.

Intrauterine pressure recordings

Equipment

A flexible catheter tipped with a pressure transduce (Gaeltec Ltd, Dunvegan, Isle of Skye, Scotland) was used (pressure range 0–300 mmHg). This was connected to an amplifier and thence to a pen recorder.

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Transducer insertion

Under sterile conditions, the cervix was visualized with the aid of a vaginal speculum, the catheter was grasped with a pair of sponge forceps and passed through the cervical canal into the uterus. When resistance to further insertion was felt at a depth of around 8 – 10 cm, the catheter was withdrawn 1 – 2 cm so that it would be lying free in the uterine cavity. The other end of the catheter was then connected via an amplifier to a pen recorder. A vaginal tampon was inserted to hold the catheter in place, and the catheter taped to the patient's leg.

Calibration

Prior to each insertion the transducer and amplifier were calibrated as follows. The system was zeroed at atmospheric pressure, i.e. when the transducer was free in air, a pressure of 0 was recorded on both the amplification system and the pen recorder. The transducer was then placed at the bottom of a column of water at a depth of 43 cm (equivalent to a pressure of 31.6 mmHg). The gain on the amplifier was adjusted so that this pressure resulted in a deflection of 31.6 mm on the pen recorder. When high intrauterine pressures were experienced, the sensitivity of the system was halved by halving the sensitivity of the pen recording system so that the records were kept on one page. The catheter was immersed in 2% glutaraldehyde solution ('Cidex'; Surgicos Ltd, Livingston, Scotland) for sterilization prior to insertion into the patient. The paper was allowed to run at a speed of 5 mm/min. Following a 'settling down' period of 10 min, a 60 min recording of intrauterine pressure was obtained.

Calculation of uterine activity

Intrauterine recordings were subsequently analysed and expressed in Montivideo units. Montivideo units (as defined by Caldeyro-Barcia *et al.*, 1957) are the mean amplitude of contractions (in mmHg) multiplied by the frequency in 10 min. We were unable to find reports of an objective scoring system for discriminating between single contractions, the noise of the recording system and an increase in baseline intrauterine pressure when calculating Montivideo units. We therefore devised the following system to reduce observer bias:

(i) Any increase in uterine activity lasting >1 min was considered to be a change in baseline tone rather than a single contraction. These changes in baseline tone were not included in any calculation of Montivideo units.

(ii) To distinguish a contraction from electrical 'noise' in the system, we considered an increase in tone as a contraction only if the amplitude of the increase was more than twice that of the variability around the baseline, and if the increase in uterine tone lasted < 1 min.

(iii) For an episode of increased uterine pressure where the pressure dipped towards the baseline but did not reach it before rising again to a further peak, we considered this as two separate contractions only if the difference between the pressure trough and the baseline was <20% of the difference between the smaller peak and the baseline. Otherwise this was considered as one contraction (provided it lasted < 1 min) with the greatest peak of intrauterine pressure taken as the amplitude of contraction.

In practice all significant increases in intrauterine pressure which appeared to be contractions were included by this system. It is possible that contractions of low amplitude would not hav been included under this system, but the contribution of thes contractions to the total score of Montivideo units would not hav been great. Certainly, the score in Montivideo units correlate with a naked eye impression of uterine activity.

Termination of pregnancy and tissue collection

The intrauterine pressure transducer was removed immediatel prior to operation. Surgical termination of pregnancy wa performed under general anaesthesia. Under sterile condition with the patient in the lithotomy position the cervix was dilate to 8 mm, and decidua obtained by gentle curettage of the sid wall of the uterus. This was placed immediately into ice-col transport medium (see below). Uterine evacuation was complete using suction in the normal manner. Anti D was given to rhesu negative patients. Patients rested for 4 h after the surgica procedure and were then allowed to return home if accompanied A follow-up appointment was scheduled for the following week

Tissue culture

Materials

RU486 (17α-hydroxy 11β-[4 dimethyl amino phenyl 17β-[1-propynyl] estra 4, 9 dien-3-one) tablets (200 mg each were a gift from Roussle Laboratories Ltd, Uxbridge UK RPM 1640 medium (Gibco Ltd, Paisley, Scotland, UK) wa supplemented with 200 μg/ml gentamycin and 2 μg/ml fungison both from Sigma Chemical Co. Ltd (Poole, Dorset, England and used as 'transport medium'. Tissues were cultured in 'complete medium': RPMI 1640 supplemented with 2 μg/m fungisone and 50 μg/ml gentamycin with 5% (v/v) fetal calf serum (Gibco Ltd, Paisley, Scotland, UK) which had previously been stripped with 1 mg charcoal/ml.

Methods

On arrival at the laboratory, the decidua was washed twice in complete medium. The tissue was then divided into small pieces of around 1 mm^3. Several pieces were then placed into each of 3 wells of a well culture plate (Nunclon, Nunc, Denmark) with 1 ml of complete medium. The culture plates were sealed with micropore tape and placed in a plastic box in an incubator at 37°C in 95% air and 5% CO_2. Media was changed daily and the spent media stored at room temperature after the immediate addition of methyl oxamating solution (0.12 M methoxyamine hydrochloride and 1.0 M sodium acetate, pH 5.6 – 5.8) in a 1:1 ratio. The decidua was cultured for 3 days, and protein content of the wells measured at the end of the culture period.

Decidua was cultured from women pretreated with mifepristone for 24 (n = 5), 36 (n = 4) and 48 (n = 3) h and from women in the control group (n = 5). In women treated similarly but with the addition of indomethacin, the numbers in each group were 5, 5, 4 and 3 respectively.

Assay procedures

Radioimmunoassay of the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) content of the media has already been reported (Kelly *et al.*, 1986b). Protein content of the culture wells was measured using the protein estimation technique of Lowry *et al.*, (1951). $PGF_{2\alpha}$ results are expressed as mean daily prostaglandin per mg protein.

Statistics

Statistically significant differences were found by analysis of variance using the Newman–Keuls procedure. The data were log transformed before analysis to normalize the distribution.

Results

Mean duration of amenorrhoea at time of operation was 48 days; at time of treatment with mifepristone ± indomethacin (or at time of operation in the control group) mean serum oestradiol was 1930 pmol/l, mean serum progesterone 28.3 ng/ml and mean serum HCG concentration 44905 IU/l. There was no significant difference between the treatment groups with respect to the above variables.

Uterine activity

Effects on uterine contractility of pretreatment with mifepristone with or without indomethacin are shown in Figure 1 (expressed in Montivideo units) and Figure 2 (two actual traces chosen at random). Technical problems with the recording apparatus meant that uterine activity was not recorded in a total of six patients, so that results are based on tracings from seven patients in the control group, and 6,6, and 5 patients pretreated with mifepristone for periods of 24, 36 and 48 h respectively. Tracings were obtained from five women in each of the four groups treated with indomethacin. In some women, vaginal bleeding became apparent prior to the end of the recording of uterine activity. Since the catheter was not expelled, these recordings were assumed to be an accurate reflection of uterine activity and therefore included in the calculations.

The uterine activity was significantly greater in the three groups pretreated with mifepristone than in the control group ($P < 0.01$). An increase in uterine activity was seen in four out of six women 24 h after mifepristone and in all women 36 and 48 h after mifepristone. There was no significant difference in uterine contractility within the three groups of women pretreated with mifepristone. Addition of indomethacin had no effect on uterine activity either in the control group of women who did not receive mifepristone, or in the groups treated with mifepristone. The apparent overall increase in uterine activity in groups treated with indomethacin compared to groups not treated with indomethacin was not statistically significant.

Prostaglandin production

$PGF_{2\alpha}$ production in cultures from decidua from women in each of the treatment groups is shown in Figure 3. It was not possible to measure $PGF_{2\alpha}$ in the culture media from two patients due to limited amounts of sample: one from the group pretreated with mifepristone alone 36 h previously and one from the group pretreated with mifepristone and indomethacin for 36 h.

The production of $PGF_{2\alpha}$ by decidua collected from women pretreated 36 h earlier with mifepristone was greater than in the control group ($P < 0.01$). Indomethacin had no effect on $PGF_{2\alpha}$ production from decidua from women not pretreated with mifepristone but inhibited the mifepristone-stimulated increase in $PGF_{2\alpha}$ production ($P < 0.01$ in tissue from women pretreated 36 h previously with mifepristone).

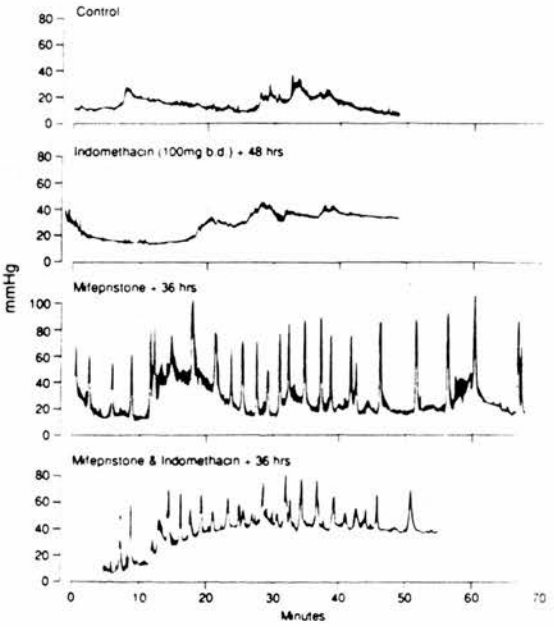

Fig. 2. Recordings of uterine activity in pregnant women of < 56 days gestation.

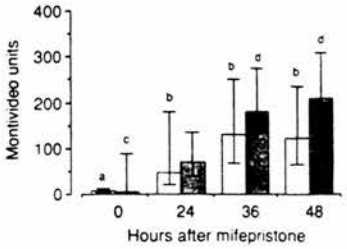

Fig. 1. Uterine contractility following administration of mifepristone *in vivo* (Montevideo units); mean and 95% confidence limits. □, Not pretreated with indomethacin; ■, pretreated with indomethacin. a < b ($P < 0.01$); c < d ($P < 0.01$).

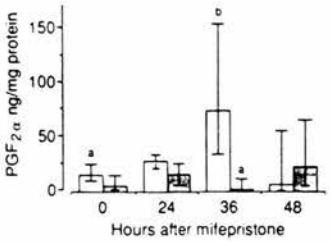

Fig. 3. Daily $PGF_{2\alpha}$ production by decidua following mifepristone *in vivo*; mean and 95% confidence limits. □, Not pretreated with indomethacin; ■, pretreated with indomethacin. a < b ($P < 0.01$).

Discussion

This study demonstrates that a single dose of mifepristone (600 mg) resulted in an increase in uterine activity. This confirms the findings of Swahn and Bygdeman (1988) who demonstrated an increase in uterine activity as early as 24 h after commencement of treatment of 25 mg mifepristone b.d. In both this study, and that of Swahn and Bygdeman, uterine activity developed in some (but not all) patients by 24 h after the start of treatment; it occurred in all subjects 36 or 48 h after the start of treatment. It appears from these studies that duration of treatment with mifepristone may be more important than total dose of mifepristone for stimulation of uterine activity.

$PGF_{2\alpha}$ has a well described stimulatory effect on uterine activity in both pregnant and non-pregnant women (for review see Lundstrom, 1986). Production of $PGF_{2\alpha}$ by decidual explants in culture reached a maximum 36 h after pretreatment with mifepristone. This coincides with the presence of uterine activity in all subjects, and with the peak of uterine activity measured in Montevideo units. $PGF_{2\alpha}$ metabolism is decreased in women pretreated 24 h previously with mifepristone compared to metabolism in a control group (Norman *et al.*, 1991). An increase in decidual $PGF_{2\alpha}$ production would appear to be at least partially responsible for the increase in uterine activity seen at 36 h following treatment with mifepristone. However, uterine activity was still high 48 h after pretreatment with mifepristone, whereas $PGF_{2\alpha}$ production from decidua harvested 48 h after pretreatment with mifepristone was not significantly increased (compared to control). Some factor other than increased decidual $PGF_{2\alpha}$ production must therefore contribute to the increase in uterine activity seen 48 h after pretreatment with mifepristone. This theory correlates with the observed effects of indomethacin in this study.

Indomethacin is a non-steroidal anti-inflammatory drug which inhibits prostaglandin synthesis via an inhibitory effect on the cycloxygenase enzyme (Lands and Hanel, 1983). Indomethacin (100 mg rectally) inhibits uterine activity in women with dysmenorrhoea (Lundstrom *et al.*, 1979) and arrests premature labour (Zuckerman *et al.*, 1984). Both dysmenorrhoea (Willman *et al.*, 1976) and premature labour (Weitz *et al.*, 1986) are associated with increased prostaglandin synthesis. Therefore, in situations where an increase in uterine activity is caused by an increase in prostaglandin production, indomethacin appears to inhibit uterine activity.

In this study, indomethacin had no effect on the mifepristone-induced increase in uterine activity despite a significant inhibitory effect on the mifepristone-induced ability of decidua to generate $PGF_{2\alpha}$ in tissue culture. There are several possible explanations for this finding.

The inhibitory effects of progesterone on uterine activity are mediated via hyperpolarization of resting membrane potential, increased junctional resistance and decreased availability of intracellular calcium, in addition to inhibition of prostaglandin production (for review see Garfield *et al.*, 1988). The effect of mifepristone on uterine activity may be via antagonism of any one of these effects. Indeed, mifepristone has been shown to have a stimulatory effect on gap junction formation in rats (Garfield and Baulieu, 1987). An increase in the number of gap junctions may be the major mechanism of action of mifepristone on uterine activity, with increased prostaglandin production playing only a minor role.

Secondly, myometrial activity is stimulated by $PGF_{2\alpha}$ and PGE_2 but inhibited by PGI_2 (at least in the non-pregnant state) (see Lundstrom, 1986 for review). In cultured rat myometrial explants, mifepristone has been shown to increase PGI_2 production (Jeremy and Dandona, 1986). The immediate metabolite of PGI_2 is the largest conversion product of radio-labelled arachidonic acid in homogenates of human myometrium (Christensen, 1984). In the myometrium, therefore, indomethacin may inhibit the mifepristone-induced production of this inhibitory prostaglandin, in addition to its effects on decidual $PGF_{2\alpha}$ production and thus have no net effect on uterine activity. Although for obvious reasons we were only able to measure decidual and not myometrial prostaglandin production, there is evidence that changes in decidual rather than myometrial prostaglandin production are largely responsible for the initiation of uterine contractility during labour at term (Casey and MacDonald, 1988). It is likely that uterine activity throughout pregnancy is similarly dependent on decidual and not myometrial prostaglandin production.

Thirdly, prostaglandin receptors concentrations were not measured. Indomethacin inhibits prostaglandin production but may also alter prostaglandin receptor concentration so that the remaining $PGF_{2\alpha}$ has a proportionally greater effect.

In summary, mifepristone has a stimulatory effect on uterine activity in early pregnancy. This increase in uterine activity is associated with an increase in decidual $PGF_{2\alpha}$ production in culture 36 h after pretreatment with mifepristone *in vivo*. Indomethacin is unable to inhibit the mifepristone-induced in uterine activity, despite having an inhibitory effect on the ability of the tissue to generate $PGF_{2\alpha}$. This suggests that mechanisms other than an increase in decidual prostaglandin production contribute to the abortifacient effect of mifepristone. An increase in myometrial gap junctions in response to mifepristone may be important.

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EFFECTS OF MIFEPRISTONE IN VIVO ON DECIDUAL PROSTAGLANDIN SYNTHESIS AND METABOLISM

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ABSTRACT

Mifepristone is an effective abortifacient in combination with an exogenous prostaglandin but its mechanism of action is unknown. Mifepristone stimulates prostaglandin production from decidua in tissue culture. To determine whether this effect also operates in vivo, we treated women with mifepristone 24, 36 and 48 hours prior to surgical termination. Decidua was removed at operation and the ability of the tissue to generate prostaglandin in culture subsequently assessed. Pretreatment with mifepristone 36 hours prior to termination of pregnancy resulted in an increased production of $PGF_{2\alpha}$ in tissue culture ($p<0.01$). A significant decrease in PGFM production was seen 24 hours after pretreatment with mifepristone in vivo ($p < 0.01$). These results suggest that the increased uterine activity observed after administration of mifepristone may be due to stimulation of endogenous prostaglandin production and inhibition of prostaglandin metabolism.

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INTRODUCTION

The antiprogestin mifepristone (RU486) is an effective abortifacient in early pregnancy with a success rate in inducing complete abortion of around 62% when used alone up to 56 days amenorrhoea, and around 95% when used in conjunction with an exogenous prostaglandin at the same gestation[1]. The mechanism of action of mifepristone in inducing bleeding and abortion is however unknown. There is an increase in the production of prostaglandins following the addition of mifepristone to decidual cells in vitro[2]. The following studies were undertaken to investigate the mechanism of action of mifepristone in inducing abortion, and in particular to determine whether treatment with mifepristone in vivo results in an increase in endometrial prostaglandin production.

In this study we have measured the decidual concentrations of PGE_2, $PGF_{2\alpha}$, and metabolites of $PGF_{2\alpha}$ (PGFM), and the production of these prostaglandins in culture, following pretreatment with mifepristone in vivo. The effects of the duration of tissue culture on prostaglandin production, and the effects of in vitro treatment with progesterone were also investigated.

MATERIALS AND METHODS

Materials

Mifepristone {RU 486 (17α hydroxy 11β -[4 dimethyl amino phenyl] 17β- [1-propynyl] estra 4, 9 dien-3-one)} tablets (200 mg each) were a gift from Miss Angela Davey, Roussel Laboratories Ltd, Uxbridge, UK. RPMI 1640 medium (Gibco Ltd, Paisley, Scotland UK) was supplemented with 200 μg/ml gentamycin and fungizone 2 μg/ml (both from Sigma Chemical Co., USA) and used as "transport medium". Tissues were cultured in "complete medium": RPMI 1640 supplemented with 2 μg/ml fungizone and 50 μg/ml gentamycin with 5% (v/v) calf serum (Gibco Ltd, Paisley, Scotland UK) which had previously been stripped with charcoal (1 mg/ml). Progesterone (Sigma Ltd, Poole, Dorset, UK) in ethanol, 2g progesterone / litre ethanol, was added to complete media in some of the wells, to a final concentration of 50 ng/ml or 500 ng/ml progesterone. The final concentration of ethanol in these wells was 1 part in 400,000 and 1 part in 40,000, respectively.

Patients

Twenty-four women requesting termination of pregnancy with a normal intrauterine pregnancy of less than 56 days gestation were recruited into the study. All women satisfied the conditions of the 1967 Abortion Act, and written informed consent was obtained from each patient. The protocol was approved by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board. Women were randomised into one of four groups. Group 1 (the control group, n=8) received no treatment prior to surgical termination, the remaining women were given 600 mg (3 tablets) of mifepristone 24 hours (group 2, n=7), 36 hours (group 3, n=4) or 48 hours (group 4, n=5) prior to

termination of pregnancy. At operation under general anaesthesia, decidua was obtained by gentle curettage and placed in liquid nitrogen (for measurement of endogenous levels) or ice cold transport media as described above for removal to the laboratory and subsequent tissue culture. Pregnancy termination was completed using suction curettage.

Endogenous levels

A portion of decidua was stored in liquid nitrogen until analysis. When ready to be assayed, the tissues were removed from the liquid nitrogen. A piece of decidua weighing about 60 mg was homogenised in 5 ml ice cold ethanol at full speed (Polytron) for 5 secs to extract the prostaglandins. After homogenisation, a 1 ml aliquot of the homogenate was spun at 12000 g for 2 minutes. To 0.5 ml of the supernatant, an equal volume of methyl oximating solution (0.12M methoxyamine hydrochloride and 1.0M sodium acetate, pH 5.6-5.8) was added. The sample was then stored until ready to be assayed in the manner described below.

Tissue culture

Decidua from five patients in each of groups 1 and 2 and four patients in each of groups 3 and 4 was set up in tissue culture. On arrival at the laboratory, the decidua was washed twice in complete medium. The tissue was then divided into small pieces of around 1 mm^3. Several pieces were then placed into each of 9 wells of a 24-well culture plate (Nunc, Denmark) with 1 ml of complete medium, or medium supplemented with progesterone to a final concentration of 50ng/ml or 500 ng/ml. Three replicates of each culture treatment were used. The culture plates were sealed with micropore tape and placed in an incubator at 37°C in 95% air and 5% CO_2. Media was changed daily and the spent media stored at room temperature after the immediate addition of methyl oximating solution in a 1:1 ratio. At the end of three days of culture, protein content of each of the wells was measured using the protein estimation technique of Lowry[3]. $PGF_{2\alpha}$ levels were measured on only the third day of culture in one patient in group 3. PGE_2 levels were measured in groups 1 and 2 only. PGFM levels were measured in only four patients in group 2, but on all cultured samples in the other groups.

Radioimmunoassay

Radioimmunoassay was used to determine the PGE_2, $PGF_{2\alpha}$, and PGFM content of the media. The method used has already been reported[4,5,6]. Prostaglandin results are expressed as a ratio of protein content of the decidua. PGE_2 levels were only measured in media from control subjects and patients pretreated with mifepristone 24 hrs previously since repeat measurements for $PGF_{2\alpha}$ and PGFM used all the media.

Statistics

Statistically significant differences were found using analysis of variance using Newman-Keuls procedure. Within subject variables were analysed using analysis of variance using the t-test. The data were log transformed before analysis to normalise the distribution.

RESULTS

Endogenous levels

Decidual concentrations of $PGF_{2\alpha}$, PGE_2 and PGFM varied greatly with each group (Fig 1). $PGF_{2\alpha}$ concentration was greater in group 2 than in group 3 or 4 ($p<0.05$). Decidual concentrations of PGE_2 and PGFM mirrored $PGF_{2\alpha}$, but no significant differences were observed between the groups.

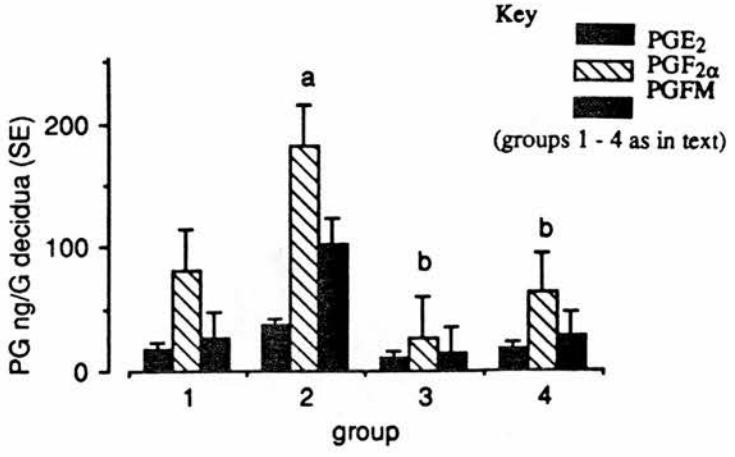

Fig. 1: Decidual concentration of prostaglandin following mifepristone in vivo.
a > b ($p<0.05$)

In vitro culture data

In order to investigate the effect of tissue in culture and of steroid hormones, decidua from group 1 (control untreated) was cultured for up to 3 days in vitro. $PGF_{2\alpha}$, PGE_2 and PGFM production from decidua harvested from women in the control group is shown in Fig. 2. The production of prostaglandins from decidua of women in the control group was low and unaffected by the addition of progesterone to the medium. Decidual $PGF_{2\alpha}$ production (Fig. 2a) was greater on the second and third day in culture than on the first day, when cultured in the absence of progesterone (complete media only) ($p<0.05$) or in the presence of a small dose of progesterone (50 ng/ml) ($p<0.01$). $PGF_{2\alpha}$ production did not change significantly when tissue was cultured in the presence of a higher dose of progesterone (500 ng/ml). Progesterone had no effect on production of PGE_2 or PGFM in culture, and there was no significant change in PGE_2 or PGFM

production over the three-day culture period whether or not progesterone was added to the culture medium (Fig. 2b and 2c).

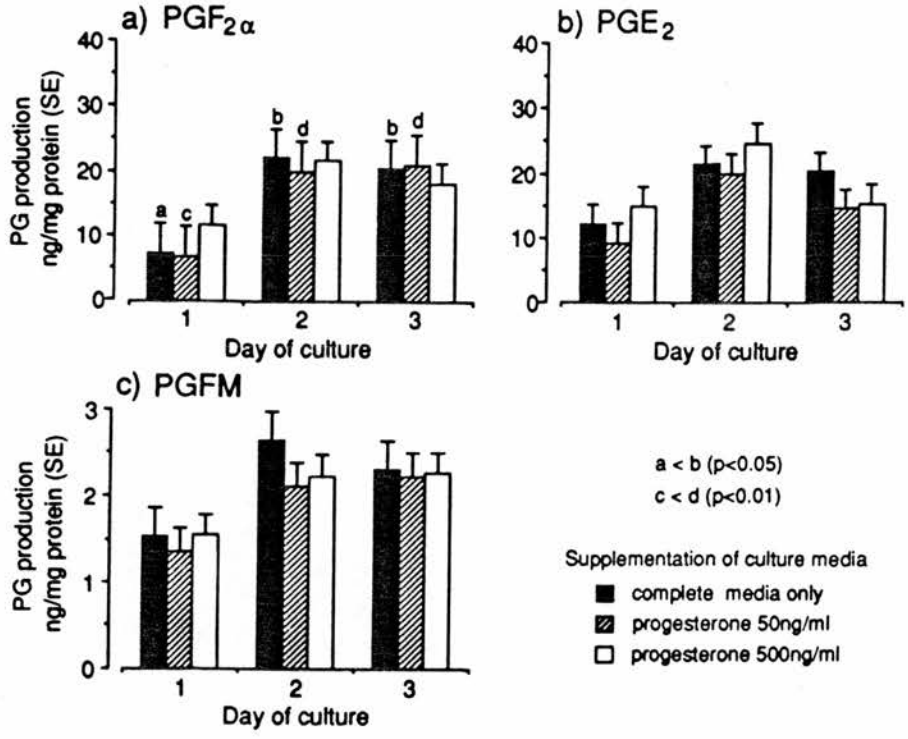

Figure 2: Decidual prostaglandin production from control tissue (group 1) in culture.

The production of prostaglandins by control decidua was extremely low and unlikely to be suppressed by progesterone. Accordingly, the effect of progesterone was tested on decidua removed from women who had been pretreated 24 hours previously with mifepristone (group 2). It was hypothesised that the mifepristone-induced increase in prostaglandin synthesis might be reversed by the addition of progesterone in vitro. In women pretreated with mifepristone 24 hours previously (group 2), the addition of progesterone (500 ng/ml) significantly reduced $PGF_{2\alpha}$ production by day 3 of culture ($p<0.05$) (Fig. 3a). A lower dose of progesterone (50 ng/ml) had a smaller inhibitory effect on $PGF_{2\alpha}$ production, suggesting a dose-related suppression of $PGF_{2\alpha}$ production by progesterone, although we were not able to establish statistical significance for this. Progesterone had a similar (non-significant) effect on PGE_2 production (Fig. 3b). There was no significant change in production of $PGF_{2\alpha}$, PGE_2 or PGFM from decidua throughout the three-day culture period, either when cultured in control media only, or when cultured in the presence of progesterone.

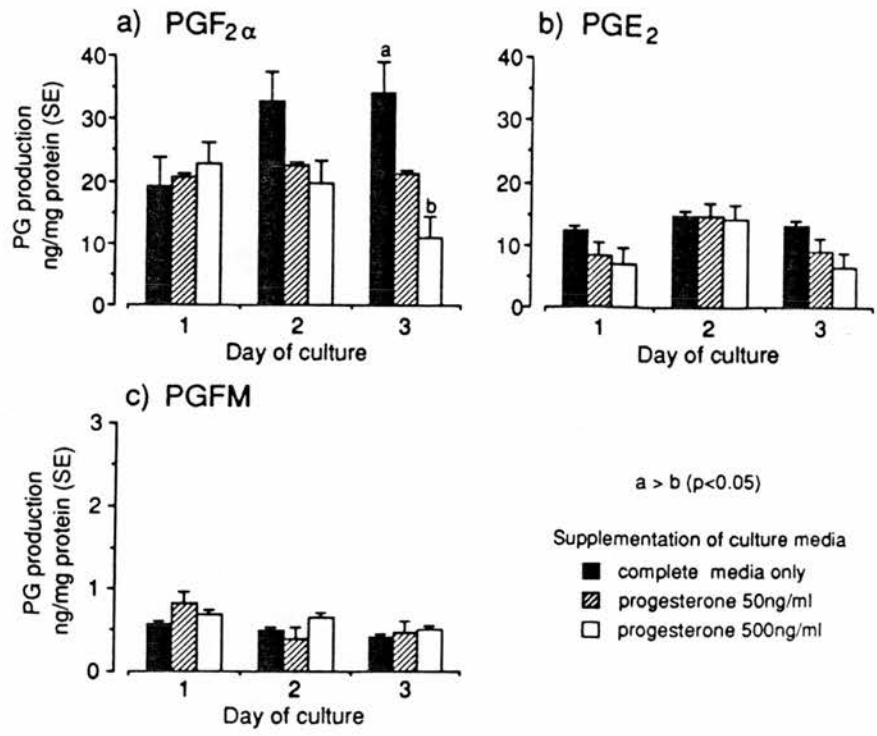

Figure 3 : Decidual prostaglandin production in culture 24 hours after mifepristone in vivo (group 2).

Effect of mifepristone in vivo

To assess the effects of pretreatment with mifepristone in vivo on the output of prostaglandins in vitro, decidual explants were cultured for 24 hours in the presence of progesterone (50 ng/ml) which simulated the concentration seen in the plasma in pregnancy. The production of $PGF_{2\alpha}$ was significantly higher in group 3 ($p<0.01$), but had declined to control levels in group 4 (Fig.4a). The production of PGFM was significantly reduced in group 2 compared with group 1 ($p<0.01$) (Fig. 4b). However in group 3, PGFM production was significantly increased in comparison with group 1 ($p<0.01$). The ratio of PGFM to $PGF_{2\alpha}$ shows a biphasic response, with a decrease in groups 2 and 3 and an increase in group 4 (data not shown).

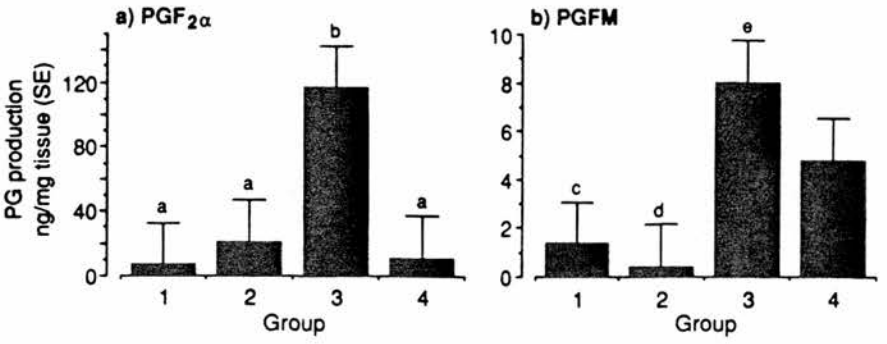

Figure 4: Prostaglandin production in culture following mifepristone in vivo.
a < b ($p<0.01$)
c > d ($p<0.01$)
c < e ($p<0.01$)

DISCUSSION

This study demonstrates that decidua removed from women pretreated 36 hours previously with mifepristone releases more $PGF_{2\alpha}$ than control tissue when cultured in vitro for 24 hours. There was a (non-significant) trend for an increase in the production of $PGF_{2\alpha}$ by decidua 24 hours after treatment with mifepristone in vivo. The concentration of $PGF_{2\alpha}$ in the decidua was also increased 24 hours after pretreatment with mifepristone in vivo, compared with concentrations 36 and 48 hours after pretreatment. It is unlikely that concentration as measured in vitro reflects the endogenous levels of prostaglandin in the tissue; rather it reflects the ability of the tissue to generate prostaglandins in response to the trauma of collection[7]. Thus the production of prostaglandins from explants of tissue in vitro may be a better reflection of the relative production of prostaglandins in vivo than the measured concentration of prostaglandin in a sample of decidua.

The output of prostaglandin E_2 and $F_{2\alpha}$ by explants of decidua from women in group 1 was low. Decidual "concentrations" of $PGF_{2\alpha}$ and PGE_2 are lower in pregnancy[8] probably due to an inhibitory effect of progesterone on prostaglandin production[9]. Progesterone inhibits prostaglandin production even in the presence of excess arachidonic acid[10]. The increased release of $PGF_{2\alpha}$ in tissue recovered from women pretreated with mifepristone in vivo may reflect partial antagonism of the suppressive effect of progesterone. This correlates with previous reports where mifepristone in vitro has been shown to stimulate decidual $PGF_{2\alpha}$ and PGE_2 production from progesterone-treated explants in a similar culture system [11].

The increase in decidual prostaglandin production from day 1 to day 2 and 3 of tissue culture in group 1 suggests a decreasing effect of endogenous progesterone as culture progresses. This hypothesis is supported by the lack of a change in prostaglandin

production when progesterone (500 ng/ml) is added to the culture medium, and by the lack of a change in prostaglandin production with duration of culture in group 2, where the effects of endogenous progesterone are already partially antagonised by treatment with mifepristone in vivo.

In the present study, the addition of progesterone to the culture medium resulted in a suppression of the release of $PGF_{2\alpha}$ by the third day of culture in women pretreated with mifepristone in vivo (group 2) bringing production down to the levels seen in the first 24 hours for the control group. A suppressive effect of progesterone in culture was not seen in decidua from women in the control group. Prostaglandin production is low in the control group and further suppression may be difficult to detect. In group 2, prostaglandin production had been stimulated by mifepristone in vivo (Fig. 1 and 4a). This effect was partially reversed by the addition of progesterone in vitro and a suppressive effect of progesterone on prostaglandin production was seen.

Twenty-four hours of pretreatment with mifepristone in vivo (group 2) resulted in a significant reduction in PGFM production in culture despite an increase in its precursor, $PGF_{2\alpha}$. In both group 2 and group 3, the ratio of PGFM to $PGF_{2\alpha}$ (which represents the ability of the tissue to metabolise $PGF_{2\alpha}$) was reduced, compared with group 1. This implies mifepristone antagonizes the stimulatory effects of progesterone on prostaglandin metabolism[5]. Again this confirms data with mifepristone in vitro showing an inhibitory effect of mifepristone on prostaglandin metabolism[2].

During pregnancy the myometrium shows little spontaneous activity which is reflected by the absence of uterine contractions. By 24 hours after administration of mifepristone, there is an increase in spontaneous uterine activity[12], and a markedly enhanced sensitivity to exogenous prostaglandins. The present findings of increased generation of $PGF_{2\alpha}$ in vitro by decidua collected from women pretreated with mifepristone in vivo is compatible with the hypothesis that the increased uterine contractility is due to increased release of prostaglandins by the decidua in vivo. Moreover, the fall in the output of PGFM at 24 hr may reflect a relative decrease in the metabolism of $PGF_{2\alpha}$ which would make the effective local concentration of the hormone higher. However, the fall in the concentration of $PGF_{2\alpha}$ in the tissue collected from women treated 48 hours previously (group 4) is more difficult to explain because bleeding and abortion is often occurring at this time. Possible reasons for the decrease in $PGF_{2\alpha}$ and production include the development of decidual necrosis following mifepristone[13] so that only a portion of the tissue is functional by 48 hours after mifepristone. Alternatively, the decrease in prostaglandin production in group 4 may represent antagonism by mifepristone of the accumulation of cellular stores of prostaglandin precursor, since progesterone appears necessary to prime the cells with arachidonic acid[14]. It is likely that factors other than increased prostaglandin production, such as increased number of gap junctions between myometrial cells[15] or increased number of receptors for prostaglandins, are important in the increase in uterine activity seen 48 hours after mifepristone.

In summary, this study confirms that mifepristone stimulates decidual prostaglandin production and inhibits prostaglandin metabolism. These effects are in keeping with the action of mifepristone as a progesterone antagonist, and partially explain its abortifacient

activity. However, it appears that factors other than an increased production of prostaglandins may also be responsible, at least in the later stages of the abortion process, and further work is needed to clarify this.

Acknowledgements
We are grateful to Sisters Jo Wu, Heideh Hillier and Maria Dewar for the excellent nursing care and data recordings of the patients in this study, and to Sister Doreen Wills and her staff on ward 54, SMMP for their continuing help. Thanks are also due to Alison Reavely, Paul Mustard and Pam Holland for performing some of the prostaglandin assays. The secretarial assistance of Margaret Harper, and the assistance of Tom McFetters and Ted Pinner in the Graphics Department is gratefully acknowledged.

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Uterine contractility and induction of abortion in early pregnancy by misoprostol and mifepristone

J. E. NORMAN K. J. THONG D. T. BAIRD

A combination of the antiprogestagen mifepristone and an exogenous prostaglandin given by intramuscular injection or intravaginal pessary is a highly effective means of inducing abortion in early pregnancy. However, the search for a stable oral prostaglandin preparation has been largely unsuccessful.

The effect of misoprostol, an orally active prostaglandin used to treat peptic ulcer, on uterine contractility was investigated in 33 women in early pregnancy (under 56 days' amenorrhoea). After administration of misoprostol in doses ranging from 200 μg to 600 μg, there was a significant increase in uterine pressure. In a second group of women who were given 200–1000 μg misoprostol 48 h after the administration of 200 mg mifepristone, there was a significant increase in the amplitude and frequency of uterine contractions. Complete abortion took place in 18 of the 21 women who received misoprostol after mifepristone, but in only 2 of 40 women given misoprostol alone.

Our findings show that misoprostol increases uterine activity in early pregnancy and suggest that, in combination with mifepristone, it may be a highly effective method of inducing therapeutic abortion.

Lancet 1991; **338**: 1233–36.

Introduction

The antiprogestagen, mifepristone, will induce abortion in early pregnancy.[1] When mifepristone is combined with a synthetic prostaglandin given by vaginal pessary (gemeprost)[2] or an intramuscular injection (sulprostone)[3] 36 to 48 h later, the complete abortion rate is about 95%. However, neither route of administration of prostaglandin is ideal. Reports of cardiovascular compromise and even death from myocardial infarction[4] after intramuscular sulprostone are probably related to high peak blood concentrations of drug. Gemeprost, as part of a vaginal pessary, must be refrigerated before use, and absorption from the vagina is variable between individuals; gemeprost has also been reported to cause cardiovascular complications.[5]

A stable, orally active prostaglandin that could be used in combination with mifepristone has been sought. Oral PGE_2 has proved disappointing,[6] but Swahn and colleagues[7] have shown that the combination of mifepristone and 9-methylene PGE_2 results in complete abortion rates of around 95%. Although that study showed the feasibility of using an orally active prostaglandin, the 9-methylene PGE_2 was given in solution and would be impracticable for routine clinical use. An orally active prostaglandin in tablet form would be more acceptable to women and could be important in developing countries where access to medical facilities is limited.

Misoprostol is an orally active prostaglandin ([15-S]-15-methyl-PGE_2 methyl ester) marketed for the treatment of peptic ulcer. The manufacturers claim that the structure of misoprostol leads to fewer gastrointestinal side-effects than other prostaglandins.[8,9] The unauthorised use of misoprostol as an abortifacient is widespread in some countries, including Brazil.[10]

The aims of this study were to determine the effects of misoprostol alone on uterine tone and contractility in pregnant women at an early gestation (under 56 days). Misoprostol (200–1000 μg) was also given to women pretreated with mifepristone (200 mg) to determine whether enhanced sensitivity to misoprostol could be seen after mifepristone administration. The clinical effects of combination abortifacient therapy were also assessed.

Subjects and methods

Women with a normal intrauterine pregnancy of up to 56 days' gestation and who requested termination of pregnancy were invited to take part in the study. All women satisfied the conditions of the 1967 Abortion Act and gave written informed consent. The protocol was approved by the Reproductive Medicine Ethical Subcommittee of Lothian Health Board.

Part 1

The effects of different doses of misoprostol alone on uterine activity were studied in 12 women scheduled for suction termination of pregnancy. 3 women were given one 200 μg misoprostol tablet, 6 women received two misoprostol tablets (total dose 400 μg), and the remaining 3 women received three misoprostol tablets (total dose 600 μg). Intrauterine pressure was monitored for 30 min before misoprostol was given and for the next 3 h. At the end of the monitoring period, suction termination was completed in the usual way.

Part 2

40 women were given 400 μg misoprostol seven days before surgical termination of pregnancy. They were asked to record any symptoms or bleeding. Seven days later, immediately before

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CLINICAL DETAILS OF SUBJECT GROUPS

—	n	Mean age (range)	Mean gestation in days (range)	Nulliparous women
Part 1	12	29 (19–38)	50 (42–56)	6
Part 2	40	26 (17–42)	49 (39–56)	26
Part 3	21	25 (18–35)	50 (39–56)	17

termination, a clinical assessment, an ultrasound scan, and a measurement of serum human chorionic gonadotropin concentrations were completed to find out whether there was either an ongoing pregnancy or an incomplete or complete abortion. The pregnancy was then ended by suction termination.

Part 3

21 women were recruited to determine the effect of mifepristone and misoprostol on uterine contractility in early pregnancy (under 56 days' amenorrhoea) and to assess the clinical outcome of this therapy. Intrauterine pressure monitoring was started 48 h after oral treatment with 200 mg mifepristone. Baseline readings were taken over 30 min, and then a single dose of misoprostol was given orally. 4 women received 200 μg misoprostol (one tablet), 10 received 400 μg misoprostol (two tablets), and 7 received 600 μg misoprostol (three tablets). Intrauterine pressure monitoring was continued either for a further 3 h or until abortion occurred, whichever was the shorter time period.

3 h after misoprostol was given, subjects were examined to assess whether abortion had taken place. In 3 women who received 600 μg misoprostol, a further dose of 400 μg misoprostol was given after examination, because no products of conception had been passed vaginally. Surgical termination of pregnancy was not carried out in these women but a follow-up appointment ensured that abortion was complete. Complete abortion was defined as an empty uterus on ultrasound scan with falling concentrations of human chorionic gonadotropin. Women in whom abortion was not complete by the second follow-up were scheduled for surgical termination of pregnancy.

In parts 1, 2, and 3, a short symptom questionnaire was completed before and 3 h after misoprostol was given.

Intrauterine pressure recordings

A flexible catheter with a pressure transducer at its tip (Gaeltec, Dunvegan, Isle of Skye) was passed through the cervix to record intrauterine contractions and then connected to an amplifier and pen recorder. The transducer and amplifier were calibrated for each subject. Details of calibration, catheter insertion, and calculation of uterine activity in Montevideo units have been described previously.[11]

Uterine activity

The effects of exogenous prostaglandins on the uterus are qualitatively and quantitatively different in women pretreated with mifepristone compared with a control group.[3] In women not pretreated with mifepristone, a small dose of exogenous prostaglandin leads to an increase in uterine tone only. Discrete uterine contractions occur only with large doses of exogenous prostaglandin. In women pretreated with mifepristone, spontaneous uterine activity can be recorded. Exogenous prostaglandin increases the frequency and amplitude of this uterine activity. Uterine pressure tracings from women in part 1 were therefore analysed differently from those in part 3: intrauterine tone before and at various times after misoprostol was calculated for women in part 1, whereas recordings from women in part 3 were expressed in Montevideo units.[11]

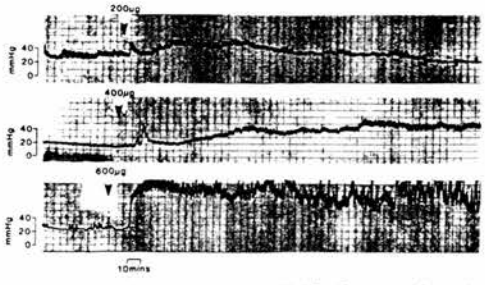

Fig 1—Intrauterine pressure (mm Hg) in 3 women in early pregnancy who received 200 μg, 400 μg, or 600 μg oral misoprostol.

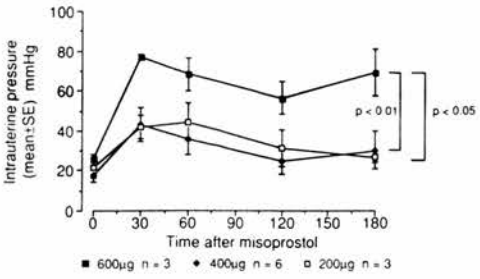

Fig 2—Mean (SE) intrauterine pressure (untransformed data) after three doses of misoprostol.

Statistics

The effects of both misoprostol dose and the time after misoprostol administration on intrauterine pressure and uterine activity were calculated by analysis of variance (Newman-Keuls procedure). Data were log transformed before analysis, although the values were given as means (and standard errors) of untransformed data for the sake of clarity.

Results

The clinical characteristics of women in each group are shown in the table.

Part 1

Before misoprostol was given, there was negligible uterine activity and the mean (SE) intrauterine pressure (all three doses of misoprostol combined) was 19·1 (2·6) mm Hg. In 10 of the 12 patients, there was an increase in intrauterine pressure after misoprostol but, despite this increase in intrauterine tone, regular uterine activity did not develop (fig 1). In the other 2 patients (both treated with 400 μg misoprostol), misoprostol caused no change in intrauterine pressure. Analysis of the effect of time after misoprostol administration on mean intrauterine pressure (all three doses of misoprostol combined) showed a greater intrauterine pressure after misoprostol administration: 49·7 (6·0) mm Hg at 30 min; 47·5 (6·0) mm Hg at 60 min; and 40·8 (8·4) mm Hg at 120 min ($p < 0·01$, $p < 0·01$, and $p < 0·05$, respectively, compared with time zero). Analysis of the effects of different dose regimens showed a greater intrauterine pressure in women given 600 μg misoprostol than in women given 400 μg or 200 μg misoprostol (all time points compared simultaneously; fig 2).

No patient given misoprostol alone complained of prostaglandin-related side-effects such as nausea, vomiting, or diarrhoea. 1 patient (who had received 400 μg misoprostol) had mild abdominal pain that was relieved by paracetamol. 3 patients reported abdominal pain that did not require analgesia.

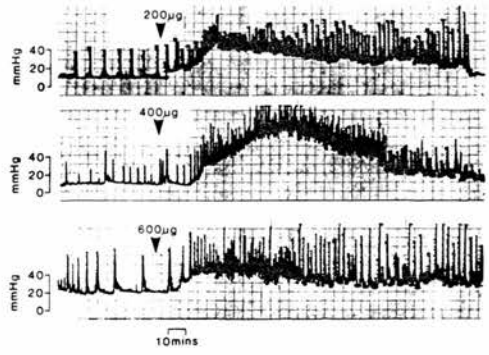

Fig 3—Intrauterine pressure (mm Hg) in 3 women in early pregnancy who received 200 µg, 400 µg, or 600 µg oral misoprostol 48 h after 200 mg mifepristone.

Part 2

21 of the 40 women studied had some bleeding in the seven days after administration of misoprostol, although only 2 (42 and 44 days' amenorrhoea) aborted completely, 6 h and 72 h, respectively, after prostaglandin intake. The remainder required surgical evacuation seven days later because of either ongoing pregnancy (32 women) or incomplete or missed abortion (6 women). Side-effects were few, with only 1 woman having either diarrhoea or vomiting. 10 women felt abdominal discomfort in the 4 h after misoprostol administration and 3 described the pain as severe.

Part 3

In the group treated with mifepristone, there was an expected apparent increase in uterine contractility compared with those in part 1 (fig 3). There was an increase in uterine activity (in Montevideo units) after prostaglandin administration; the significance of the increase during different time periods (all three doses of misoprostol combined) is shown in fig 4. There were no significant differences in uterine activity among the three doses of misoprostol. In 4 women, uterine recordings were not possible because of technical limitations. In 3 of the remaining 17 women, the recording was ended prematurely (before 90 min) because of passage of the fetus (2 women given 600 µg and 1 woman given 200 µg). In 18 of 21 women, complete abortion took place after treatment with misoprostol and mifepristone. Of the remaining 3 women who required surgical evacuation, 2 (1 given 200 µg and 1 given 400 µg misoprostol in combination with 200 mg mifepristone) were found to have an ongoing pregnancy at follow-up 14 days later. The third woman (given 400 µg misoprostol in combination with 200 mg mifepristone) passed a gestational sac and placental tissue after misoprostol and appeared to have an empty uterus one week later. However, she was subsequently admitted to another hospital with continuing vaginal bleeding. An ultrasound scan showed the presence of some material in the uterus and uterine evacuation was therefore completed. The material obtained was not sent for histological examination. Side-effects of combination therapy were minor. 3 patients vomited after misoprostol, but 1 had vomited earlier that day and reported an improvement in symptoms after taking the drug.

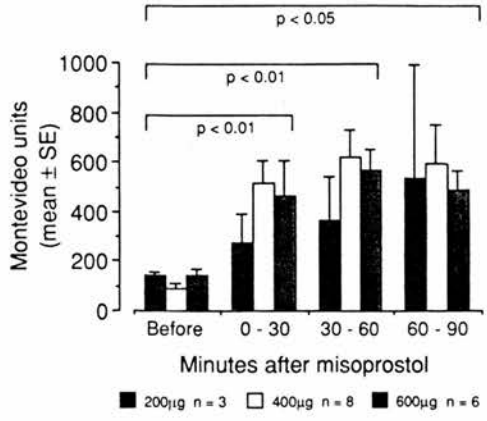

Fig 4—Mean uterine activity (untransformed data) before and at varying times after misoprostol at three doses after pretreatment with mifepristone.

None of the women had diarrhoea, although 9 women (2 after 200 µg misoprostol, 6 after 400 µg misoprostol, and 1 after 600 µg misoprostol) required analgesia (paracetamol or dihydrocodeine) and 3 (2 after 400 µg misoprostol and 1 after 600 µg misoprostol) required opioid analgesia.

Discussion

The effects of misoprostol on uterine tone are qualitatively similar to those seen with other prostaglandins. When prostaglandins are given orally, a high frequency of side-effects are reported at doses sufficient to cause a change in uterine tone.[12] In our study, misoprostol led to a significant increase in uterine tone without important adverse effects; this low frequency of side-effects after misoprostol is in keeping with previous reports.[13] Misoprostol is known to have some uterine effects. In a study of pregnant women of 9–12 weeks gestation, 800 µg misoprostol induced vaginal bleeding in 45% of women, and a partial or complete abortion in 11% of women, within 12 h of tablet administration.[14] In the UK, the Committee on Safety of Medicines has urged caution over the use of misoprostol in premenopausal and postmenopausal women and advises against its use in women who are pregnant or planning a pregnancy because of risk of vaginal bleeding.[15]

Although misoprostol led to an increase in uterine tone when given alone, pregnancy continued in 80%, indicating that it would have little use as an abortifacient at this dose. Congenital malformations have been reported in babies born to women who have taken misoprostol in early pregnancy in an unsuccessful attempt to induce abortion.[10] Whether these abnormalities can be directly attributed to misoprostol remains unclear. The increased efficacy of misoprostol in women who had been pretreated with mifepristone reflects the enhanced sensitivity to prostaglandins induced by antiprogestagens.[3]

The increased uterine activity after misoprostol administration in women pretreated with mifepristone is qualitatively similar to the effects of other prostaglandin analogues, whether given by the intramuscular[3] or oral[7] route. However, the only other available orally active 9-methylene PGE_2 is not available in a form that would enable large-scale commercial production. Natural PGE_2 given orally in doses small enough to be free of

gastrointestinal side-effects does not seem to induce uterine activity in early pregnancy, despite the increase in uterine sensitivity after pretreatment with mifepristone.[7]

The rate of complete abortion with mifepristone combined with exogenous prostaglandin ranges from 85%–100%.[16,17] The clinical value of mifepristone and misoprostol therapy cannot be fully evaluated from our small study, but the finding that complete abortion took place in 18 of 21 patients is encouraging. Furthermore, 95% of women in early pregnancy (under 49 days' amenorrhoea) aborted completely after administration of 600 mg mifepristone in combination with 600 μg misoprostol.[18]

Arbaprostil, an orally active PGE_2 analogue (15[R]15-methyl PGE_2) that is used to treat peptic ulcers, has been shown to have little or no effect on uterine activity or baseline uterine tone when a single dose (similar to that recommended for treatment) is given to pregnant women in the first trimester.[19] At larger doses (up to 1200 μg), vaginal spotting is induced in some women, but the frequency of side-effects in association with this dose of prostaglandin is high. If our encouraging clinical results are confirmed in a larger series, the combination of mifepristone and misoprostol may prove to be a safe, effective, and reasonably inexpensive means of medical termination of early pregnancy.

We thank Sister Maria Dewar and Sister Heideh Hillier for the excellent nursing care and data collection in this study, and Sister Doreen Wills and her staff on Ward 54, Simpson Memorial Maternity Pavilion for their continuing help. The secretarial assistance of Margaret Harper and the assistance of Mr T. McFetters and Mr E. Pinner in our Graphics Department is gratefully acknowledged.

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SHORT REPORTS

Safety and immunogenicity of *Pseudomonas aeruginosa* conjugate A vaccine in cystic fibrosis

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To assess the safety and immunogenicity of a *Pseudomonas aeruginosa* octavalent O-polysaccharide-toxin A conjugate vaccine, 22 patients (mean age 7 years) with cystic fibrosis who had no history of colonisation with *P aeruginosa* were immunised with the vaccine. Adverse reactions were mild and self-limiting. IgG antibody concentrations to all vaccine antigens were significantly raised after vaccination and remained so for 12 months. Immunisation produced opsonic and toxin A neutralising antibodies. A booster dose given at 12 months led to an anamnestic response. There was no significant change in clinical status after vaccination. Further work to assess efficacy in patients with cystic fibrosis can now be considered since our findings support the safety and immunogenicity of the vaccine.

Lancet 1991; **338**: 1236–37.

Bronchopulmonary infections due to *Pseudomonas aeruginosa* are a leading cause of morbidity and mortality in patients with cystic fibrosis (CF).[1] Once established in the lower respiratory tract, the organism is virtually impossible to eradicate. Repeated pulmonary exacerbations, which often are fatal, are common. The acquisition of antibiotic resistance by infecting strains also greatly complicates treatment.

We have recently described the safety and immunogenicity of a *P aeruginosa* octavalent O-polysaccharide (O-PS)-toxin A conjugate vaccine in man.[2] Strains of *P aeruginosa* that initiate colonisation are recognised by anti-lipopolysaccharide (LPS) antibodies, which eradicate the organism via opsonophagocytic killing; such antibodies could potentially prevent or impede

control patients also took 500 mg of calcium daily throughout one study and during the etidronate-free periods in the other (T Storm et al, N Engl J Med, 322:1265, May 3, 1990; NB Watts et al, N Engl J Med, 323:73, July 12, 1990).

ADVERSE EFFECTS — With 400 mg of etidronate per day in women with postmenopausal osteoporosis, no definite adverse effects were detected. After two or three years at this dosage, defects in bone mineralization have not been observed. In patients with Paget's disease, allergic skin rashes have been reported.

DOSAGE AND COST — The dosage of etidronate used in these studies for treatment of postmenopausal osteoporosis was 400 mg once daily two hours before or after a meal for 14 days, every 3 to 3½ months. Sixty 400-mg tablets cost the pharmacist $145.67, according to *Drug Topics MicREData*, Nov 1990.

CONCLUSION — Although not approved for such use by the US Food and Drug Administration, etidronate appears to be effective and well tolerated for treatment of postmenopausal osteoporosis, at least for two or three years. How it compares in effectiveness to other drugs for this indication and whether concurrent use with one or more of the other drugs would provide additional benefit remain to be determined.

MIFEPRISTONE (RU 486)

Recent articles in the press have suggested that mifepristone (RU 486 – Roussel Uclaf) can effectively and safely induce an abortion. The drug is currently available only in France and China, but the manufacturer has also applied for a license in the United Kingdom. Mifepristone has been used on an investigational basis in the USA as an abortifacient and for treatment of Cushing's disease and breast cancer, but no manufacturer has applied to the US Food and Drug Administration for approval to market the drug in this country.

MECHANISM OF ACTION — Mifepristone is a 19-norsteroid analog with a high affinity for progesterone receptors that acts as an antiprogestin (A Ulmann et al, Sci Am, 262:42, 1990). The drug also binds strongly to glucocorticoid receptors and, to a lesser extent, to androgen receptors, and stimulates synthesis of prostaglandins by cells of the early human decidua. Both the prostaglandins and withdrawal of progesterone stimulate uterine contractility. Treatment with mifepristone during early pregnancy leads to detachment and expulsion of the products of conception.

PHARMACOKINETICS — After oral administration, plasma concentrations of mifepristone generally reach a peak within one to two hours, with an elimination half-life of about 20 hours (ML Swahn et al, Contraception, 34:469, 1986). The drug is extensively metabolized and excreted mainly in bile. Serum concentrations of active metabolites, but not the parent drug, are generally dose-dependent (O Heikinheimo et al, Hum Reprod, 2:379, 1987).

EFFECTIVENESS — Clinical studies have shown that treatment with mifepristone alone can terminate early pregnancy. In one study, 400 to 800 mg in divided doses was 82% to 89% effective (B Couzinet et al, N Engl J Med, 315:1565, 1986). Another study found an 82% incidence of complete abortion after a single 600-mg dose (DA Grimes et al, Am J Obstet Gynecol, 162:910, 1990). A summary of studies found that the incidence of complete abortion fol-

lowing a single 600-mg dose was 84% with less than 42 days of amenorrhea, and 58% if the duration of amenorrhea was longer (A Ulmann, Contraception, 36 suppl:27, 1987).

The incidence of complete abortion increases if a small dose of a prostaglandin analog is added (withdrawal of progesterone increases the sensitivity of the myometrium to prostaglandins). A study in women with less than 56 days of amenorrhea found that mifepristone (150 mg daily for four days) led to complete abortion in 12 (60%) of 20 women, but given with an intravaginal suppository of the prostaglandin gemeprost (*Cervagème* – not available in the USA), complete abortion occurred in 18 (95%) of 19 women (IT Cameron et al, Contraception, 34:459, 1986). A Chinese clinical trial in 283 women found that the incidence of abortion was slightly more than 50% with mifepristone alone and more than 90% with mifepristone plus a prostaglandin (SR Zheng, Acta Obstet Gynecol Scand, suppl 149:19, 1989).

In an uncontrolled multicenter survey conducted in France, a single 600-mg dose of mifepristone followed 36 to 48 hours later by intramuscular sulprostone (*Nalador* – not available in the USA) or intravaginal gemeprost induced complete abortion in 96% of about 2000 women with up to 49 days of amenorrhea. Differences in effectiveness between different doses or routes of prostaglandins were small, but the mean time to expulsion of the products of conception ranged from 4.5 hours after the highest dose of sulprostone (0.5 mg) to 22.7 hours after intravaginal gemeprost (L Silvestre et al, N Engl J Med, 322:645, 1990). Another multicenter survey found that mifepristone given with intravaginal gemeprost was effective at inducing abortion in 95% of about 600 women with up to 63 days of amenorrhea (UK Multicentre Trial, Br J Obstet Gynaecol, 97:480, 1990). Mifepristone regimens have been reported to be ineffective in aborting ectopic pregnancies (JH Levin et al, Am J Obstet Gynecol, 163:543, August 1990).

BLEEDING — The bleeding pattern is similar with mifepristone alone or given with a prostaglandin; bleeding starts, in general, one to two days after treatment and lasts one to two weeks. In one study, patients started to bleed between 10 and 58 hours (median 35 hours) after being given the drugs, with a median duration of 13 days (range one to 44 days) (MW Rodger and DT Baird, Contraception, 40:439, 1989). The amount of blood loss tends to be more than during a normal menstrual period. About 1% of patients have required curettage, and a few have had transfusions.

ADVERSE EFFECTS — Except for bleeding, adverse effects with mifepristone plus a prostaglandin have generally been mild. Crampy abdominal pain occurs in most patients for a few hours after administration of the prostaglandin. Transient nausea, vomiting, diarrhea, and headache can also occur. Prostaglandins can cause adverse cardiovascular effects; among more than 20,000 patients, the manufacturer reports that one myocardial infarction and one ventricular arrhythmia occurred within three hours after injection of the prostaglandin, but no maternal deaths have been reported. Endometritis and salpingitis have occurred rarely. No fetal abnormalities have been observed, and three apparently healthy children were recently born to women who had taken mifepristone alone early in pregnancy (BH Lim et al, Lancet, 336:257, July 28, 1990).

CONCLUSION — A single oral dose of mifepristone followed by an intramuscular or intravaginal dose of a prostaglandin can terminate early intrauterine pregnancy in about 95% of women. The drugs commonly cause transient abdominal pain and one to two weeks of bleeding, which may sometimes require curettage.

New drugs

Antiprogesterones

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Inhibitors of progesterone synthesis, antibodies to circulating progesterone and progesterone receptor antagonists all act as antiprogesterones. Epostane, azastene and trilostane all inhibit 3β hydroxy steroid dehydrogenase, an essential enzyme in the pathway to synthesis of progesterone. Preliminary clinical trials using the antiprogestogenic activity of these drugs have shown some promise (van der Spuy et al, 1983; Birgerson et al, 1987). As yet only trilostane has a product licence, for its antiglucocorticoid properties. Mifepristone (RU486), ZK 98 299 and ZK 98 734 are all progesterone receptor antagonists. They offer a revolutionary approach to fertility control, but the very success of mifepristone has attracted adverse attention from the anti-abortion lobby. Mifepristone, which has undergone the most clinical trials, is at present only available in France and China. Antibodies to circulating progesterone, although effective at terminating pregnancy in mice, have not yet been developed clinically.

This article will concentrate on the progesterone receptor antagonists and in particular on mifepristone.

Progesterone receptor antagonists

Mifepristone is a progesterone receptor antagonist, i.e. it binds to the progesterone receptor to exert its effect. It binds more strongly to the progesterone receptor and to the glucocorticoid receptor than do progesterone and dexamethasone respectively, but binds only weakly to the androgen receptor (Philibert et al, 1981). Binding to the glucocorticoid receptor confers antiglucocorticoid properties.

Structure

Mifepristone is derived from the synthetic progestin norethindrone, with a dimethylaminophenyl side chain at the 11β position which confers antagonistic activity. In addition a propynyl side chain at the 17α position increases the affinity of the drug for the progesterone receptor (Teutsch, 1985) (*Fig. 1*). ZK 98 299 and ZK 98 734 (Schering) are structurally similar and differ from mifepristone in their in-vitro action only in their relative antiglucocorticoid and antiprogestogenic activities. However, these last two substances have not yet undergone extensive clinical trials in humans.

Mechanism of receptor interaction

Two mechanisms have been invoked to explain how mifepristone interacts with the progesterone receptor to produce an antagonistic effect. Mifepristone may stabilize a non-DNA-binding form of the receptor either by stabilizing the

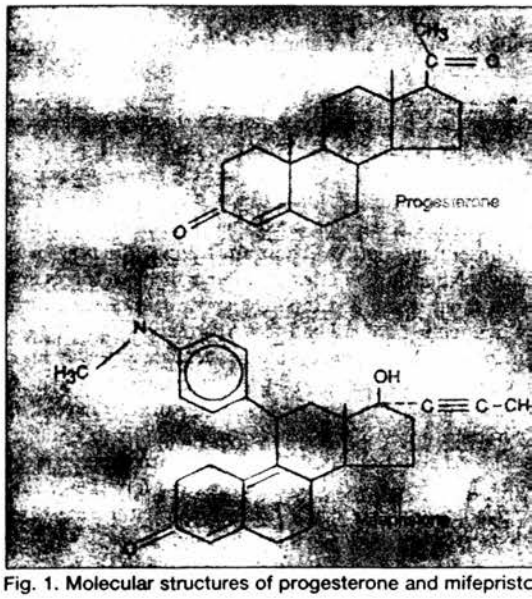

Fig. 1. Molecular structures of progesterone and mifepristo

progesterone receptor–heat shock protein complex, or allowing the heat shock protein to dissociate but prevent the necessary conformational change in the receptor for o mal binding to the hormone response elements of D (Baulieu, 1988). Transcription cannot then occur.

Pharmacology

Following oral administration the half-life of mifepriste in the human is around 20–24 h. Mifepristone circula bound to an alpha-1 acid glycoprotein which is saturated fairly low doses of the drug (Philibert et al, 1986). Unbou mifepristone is rapidly metabolized and excreted in the b (90%) or in the urine (10%). Metabolism of mifepristone p ceeds along two main pathways: mono- and then demethylation of the diaminophenyl side chain, and c dation of the propynyl side chain (Deraedt et al, 1985). Th metabolites also have antiprogestogenic and a glucocorticoid activity – around 30% that of the origi drug, which may well contribute to the overall biologi effect.

Effects of antiprogesterones

Luteal phase administration

Administration of mifepristone in the luteal phase of menstrual cycle has been shown to induce luteolysis and v inal bleeding (Schaison et al, 1985; Shoupe et al, 1987). V inal bleeding may occur independently of luteolysis and thought to be due to a direct effect of mifepristone

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the endometrium, possibly mediated via an increase in prostaglandin production by glandular cells (Kelly et al, 1986) or a decrease in endometrial aromatase activity (Tseng et al, 1986). In these women a second episode of vaginal bleeding may occur at the end of the luteal phase at the time of luteolysis. Luteolysis appears to be secondary to changes in gonadotrophin secretion, such that a decrease in amplitude (Schaison et al, 1985; Shoupe et al, 1987) and frequency (Critchley and Baird, 1986) of pulsatile gonadotrophin release is seen following mifepristone administration. These effects are similar to those following exogenous progesterone administration, and mifepristone may be acting as a progesterone agonist on the hypothalamic–pituitary axis in this instance. Factors influencing luteolysis include dosage of mifepristone and timing of administration; luteolysis is more likely to occur when mifepristone is given in the mid to late luteal phase, and when high doses are given (Nieman and Loriaux, 1988).

Follicular phase administration

Administration of mifepristone in the follicular phase delays ovulation and disrupts folliculogenesis (Liu et al, 1987). On ultrasound scanning, collapse of the dominant follicle is seen with a new wave of follicle formation starting when treatment is discontinued. Unlike the effects of mifepristone in the luteal phase, these effects do not seem to be mediated via a change in gonadotrophin secretion.

Effects on fertilization and embryogenesis

In the rat, administration of mifepristone results in accelerated ovum transport along the fallopian tube (Roblero et al, 1987). The receptive period available for implantation is delayed and widened (Sarantis et al, 1988). In the human, mifepristone does not appear to affect the final stages of oocyte development or to affect the in-vitro fertilization rate of these oocytes when given to women 35 h before egg collection (Messinis and Templeton, 1988).

Effects on early pregnancy

Progesterone is essential for the maintenance of early pregnancy. Removing the source of progesterone (Csapo et al, 1973), inhibiting progesterone synthesis using a 3β hydroxy steroid enzyme inhibitor such as epostane (Webster et al, 1985), and blocking the action of progesterone at the receptor level using mifepristone (Swahn and Bygdeman, 1988) all result in the development of uterine activity. In a proportion of cases, abortion occurs (*Fig. 2*). An increased sensitivity to exogenous prostaglandin has also been demonstrated following pretreatment with epostane or mifepristone.

The use of mifepristone or epostane in the second trimester also results in the development of spontaneous uterine activity and increased sensitivity to exogenous prostaglandin (Selinger, 1988; Hill et al, 1990a).

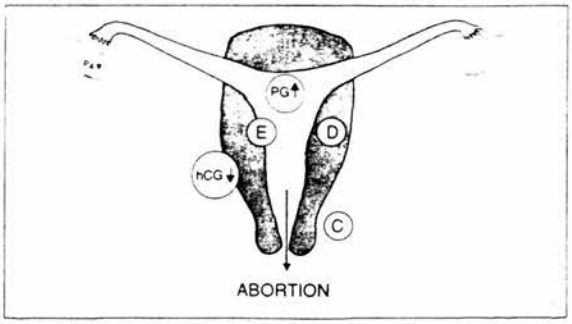

Fig. 2. Effects of mifepristone on the early pregnant uterus. C=cervical dilatation; D=decidual necrosis; PG ↑ =increased prostaglandin production from endometrium; P_4 ↓ =decreased progesterone production from corpus luteum; hCG ↓ = decreased placental production of human chorionic gonadotrophin; E=embryo.

Effects on the cervix

When given in the first trimester, mifepristone results in variable degree of cervical dilatation and ripening (Radestad et al, 1988; World Health Organisation, 1990). Morphologically, dissolution of the collagen fibres, accumulation of mast cells and ingrowth of blood capillaries can be seen (Van Look and Bygdeman, 1989). These effects were initially thought to be due to increased local prostaglandin E_2 release. In a recent study, however, homogenates of cervical biopsies from women pretreated with mifepristone had no greater ability to generate prostaglandin E_2, prostaglandin $F_{2\alpha}$, thromboxane B_2 or 6-keto prostaglandin $F_{1\alpha}$ from radiolabelled arachidonic acid than did homogenates of cervical biopsies from a control group of women (Radestad et al, 1990). This suggests that cervical effects may be mediated by factors other than an increase in prostaglandin production.

Effects on pregnancy at term

The use of mifepristone to induce labour has been investigated in monkeys. In one study, mifepristone appeared to provoke cervical ripening but this was insufficient to induce labour (Wolf et al, 1989). However, the combination of mifepristone and oxytocin was very effective, with 12 out of 14 fetuses being delivered within 48 h. Interestingly, monkeys delivered to the mifepristone-treated animals showed a transient increase in weight gain compared with control animals; this effect appeared to be secondary to accelerated colostrum production and immunoglobulin appearance in breast milk. Evidence from a previous study on the use of mifepristone to induce labour is less encouraging; mifepristone induced uterine activity in five macaques but cervical ripening did not occur, and delivery by caesarean section (for disproportionate labour) was necessary (Halushka et al, 1987).

Clinical use

The proven and potential uses of mifepristone are shown in *Tables 1* and *2* respectively.

Table 1. Proven uses of mifepristone

Early medical abortion
Cervical ripening agent
Postcoital contraceptive
Ovulation inhibitor
Induction of labour following fetal death
Treatment of Cushing's syndrome

Table 2. Further potential uses of mifepristone

Contraceptive agent
Induction of labour at term
Treatment of progesterone-dependent cancers
Manipulation of the implantation window in in-vitro fertilization

Abortifacient activity

It is as an abortifacient that mifepristone has been most widely investigated, and it is for this application that it is potentially most useful. Used alone in women at up to 56 days gestation, mifepristone induces abortion in around 62% (Van Look and Bygdeman, 1989). However, when used in combination with exogenous prostaglandin given 2–4 days after mifepristone therapy, the complete abortion rate can be improved to 95–100% (Rodger and Baird, 1987; Dubois et al, 1988; Silvestre et al, 1990). The minimum effective dose of mifepristone and the optimal dose and type of prostaglandin are unknown. Studies in Edinburgh on women of less than 56 days gestation have used 600 mg mifepristone followed 48 h later by 0.5–1 mg of vaginal gemeprost (a prostaglandin E_1 analogue) to achieve a complete abortion rate of 95% (Rodger and Baird, 1987). Vaginal bleeding lasted a median of 12 days and median blood loss (measured objectively) was 72 ml (range 15–398 ml). Ten per cent of women required opiate analgesia.

In the second trimester, priming with mifepristone for 24–36 h significantly reduced the induction–abortion interval when abortion was subsequently induced with prostaglandins (Urquhart and Templeton, 1987; Rodger and Baird, 1990). The dose of prostaglandin required and the necessity for opiate analgesia were also significantly reduced.

Mifepristone can also be used to ripen the cervix in women at an intermediate stage of gestation undergoing surgical termination of pregnancy.

Inhibition of ovulation

Mifepristone has been shown to inhibit ovulation (Luukkainen et al, 1988), but the minimum dose required is as yet unknown. Further studies in this area are currently being undertaken by the World Health Organisation. The use of mifepristone to inhibit ovulation would potentially be useful for contraception, but this has not yet been addressed directly. Unopposed oestrogenic stimulation of the endometrium might be a problem with long-term daily use.

Postcoital contraception

Three studies to date have examined the effectiveness of mifepristone as a contraceptive in the luteal phase (Ulmann, 1987; van Santen and Haspels, 1987; Lahteenmaki et al, 1988). A mean failure rate of 4% per treated cycle is comparable with that of other postcoital contraceptive methods (Van Look, 1987). In addition mifepristone does not seem to have the same high frequency of side effects (nausea and vomiting) as other hormonal regimens. A WHO-sponsored study to compare 600 mg mifepristone with the Yuzpe regimen is ongoing. In addition to its lower incidence of side effects, mifepristone may be effective for a longer period after intercourse than are the current hormonal methods.

Labour induction

The ability of mifepristone to induce labour at term has not yet been fully evaluated in the human. A recent French study has reported on the use of mifepristone to induce labour in humans and has shown some success (Frydman et al, 1991). Animal work suggests it has potential in this area. Mifepristone in combination with an oxytocic agent might induce labour in a more controllable manner than do the current available agents. Mifepristone was effective in inducing labour in some patients following fetal death in the third trimester (Cabrol et al, 1985; Padayachi et al, 1988).

If mifepristone is to be used to induce labour, it has to be shown to be harmless to the fetus. Mifepristone does cro the placenta and studies in the second trimester have show a significant increase in fetal aldosterone levels (but n change in fetal progesterone, oestradiol or cortisol levels) 4 after maternal administration of 600 mg mifepristone (Hi et al, 1990b). The significance of this is unclear.

Non-reproductive effects

The antiprogesterones, by virtue of their antiglucocorticoi activity, also have potential outwith the field of human repro duction. In a patient with Cushing's syndrome due to ectop secretion of adrenocorticotrophic hormone, treatment wit mifepristone resulted in a resolution of all symptoms an signs of the disease (Nieman et al, 1985). Mifepristone ha been shown to lower intraocular pressure in rabbits and ma be of benefit in the treatment of glaucoma (Phillips et a 1984). In women with breast cancer resistant to conventiona therapy, mifepristone may be useful as adjuvant treatmen (Romieu et al, 1987).

Availability

Mifepristone is at present only available in France and China Roussel have applied for a product licence for mifepriston in the UK, and it is to be hoped that this revolutionary dru will be available in the UK soon.

Conclusion

Mifepristone is the first of a number of antiprogesterone being developed. It is of proven use as a medical abortifacien (in combination with prostaglandins), as a cervical ripene in the late first trimester and as a postcoital contraceptiv agent. Further studies are needed to evaluate its use to induc labour and as a contraceptive. As with other antihormones the use of mifepristone provides a unique opportunity to in vestigate the effects of the natural hormone (progesteron in this case) in a variety of physiological situations.

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