From the Department of Woman and Child Health, Division of Obstetrics and Gynecology, Karolinska Institutet, Stockholm, Sweden

MISOPROSTOL – PHARMACOKINETICS AND EFFECTS ON UTERINE CONTRACTILITY AND CERVICAL RIPENING IN EARLY PREGNANCY

Annette Aronsson

Stockholm 2007
“Women are not dying because of diseases we cannot treat. They are dying because societies have yet to make the decision that their lives are worth saving.”

Professor M.F. Fathalla.
Former President of the International Federation of Gynaecology and Obstetrics,
Professor of Obstetrics and Gynaecology. Assiut Uni

To Emma
ABSTRACT

Misoprostol is an orally active synthetic PGE1 analogue which has become an important drug in obstetric and gynaecological practice because of its uterotonic and cervical priming actions. It is safe, cheap, widely available and stable at room temperature. Misoprostol has been found to be useful for medical abortion, cervical priming prior to surgical abortion, evacuation of the uterus in cases of incomplete abortion, missed abortion and intrauterine foetal death, induction of labour and treatment and prevention of post-partum haemorrhage. It was discovered in clinical studies that the misoprostol tablets licensed for oral use could be administered by other routes and that this could influence efficacy. A better understanding of the impact of the route of administration would thus help to further improve misoprostol regimens for several indications. The aims of this thesis were therefore to evaluate the effect of different administration routes and doses of conventional misoprostol and a new slow release (SR) formulation of misoprostol, focusing on its effects on uterine contractility and the pharmacokinetic profile of misoprostol free acid (MPA). In addition, the effect of misoprostol on inflammatory mediators in the cervix was investigated. Healthy women with a normal intrauterine pregnancy in the first trimester who requested vacuum aspiration for termination of pregnancy were recruited for the studies.

Uterine contractility was studied after treatment with oral (400 µg), vaginal (400 µg) or sublingual misoprostol (200 or 400 µg). Regardless of the dose and route of administration, the first effect of misoprostol to be observed was a rise in tonus which was more pronounced after oral or sublingual administration. Following sublingual and vaginal administration, regular uterine contractions developed, while oral treatment had only a minor effect on uterine contractions. The response to oral treatment with 400 and 800 µg SR misoprostol was also compared to the response to 400 µg conventional oral misoprostol. SR misoprostol was found to have less effect on uterine tonus, although it was more potent in terms of inducing uterine contractions. The pharmacokinetic profile of MPA following administration of SR misoprostol compared to conventional vaginal and sublingual misoprostol revealed lower peak plasma levels but a longer lasting elevation of these plasma levels.

The effect of 400 µg oral or vaginal misoprostol on inflammatory mediators in the cervical tissue was compared with an untreated control group. Immunohistochemical analysis was performed on cervical biopsies. Treatment with misoprostol was associated with higher levels of leukocytes (CD45), and monocytes (CD68) in the cervix compared to untreated controls. A greater staining of MMP 8 and MMP 9 was found following treatment, while TIMP 1 and 2 expression did not differ between the treatment and control groups.

Taken together, the results indicate that the peak MPA serum levels seem to correlate with uterine tonus, while the duration of elevated serum levels seems to correlate with the effect on uterine contractility. A certain threshold level of MPA is needed, but once this is reached; the duration of elevated MPA over this critical threshold seems to be the most important parameter in terms of inducing contractions. The findings relating to the pharmacokinetic properties and the effect on uterine contractility of different routes of administration will enable clinicians to design optimal regimens for various clinical applications.

Key words: misoprostol/ induced abortion/ pregnancy/sublingual administration/cervical ripening/slow-release/uterine contractility/ pharmacokinetics
LIST OF PUBLICATIONS

I. Effects of misoprostol on uterine contractility following different routes of administration.
   Annette Aronsson, Marc Bygdeman and Kristina Gemzell-Danielsson.

II. The effect of orally and vaginally administered misoprostol on inflammatory mediators and cervical ripening during early pregnancy.

III. Effects slow release misoprostol on uterine contractility in early pregnancy.

IV. Pharmacokinetic profiles up to 12 h after administration of vaginal, sublingual and slow-release oral misoprostol.
   Annette Aronsson, Christian Fiala, Olof Stephansson, Fredrik Granath, Bernhard Watzer, HW Schweer and Kristina Gemzell-Danielsson
CONTENTS

1 INTRODUCTION ......................................................................................1
  1.1 "The missing drug" in a global perspective.......................................1
  1.2 Anatomy and physiology of the uterus..............................................3
    1.2.1 Corpus uteri ...........................................................................3
    1.2.2 Uterine contractility...............................................................3
    1.2.3 Cervix uteri ...........................................................................4
    1.2.4 Extracellular matrix...............................................................5
    1.2.5 Cervical ripening ...................................................................6
  1.3 Prostaglandins....................................................................................7
    1.3.1 History ...................................................................................7
  1.3.2 Synthesis and metabolism .....................................................8
  1.4 Misoprostol........................................................................................9
    1.4.1 Source....................................................................................9
    1.4.2 Pharmacology of misoprostol ................................................9
      1.4.2.1 Structure and chemistry of misoprostol ..................9
      1.4.2.2 Pharmacokinetics ..................................................10
    1.4.3 Different routes of administration of misoprostol ............11
      1.4.3.1 Oral route ................................................................11
      1.4.3.2 Vaginal route.........................................................12
      1.4.3.3 Sublingual route ..................................................13
      1.4.3.4 Buccal route ..........................................................14
      1.4.3.5 Rectal route ...........................................................14
    1.4.4 Effects of misoprostol on the uterus and the cervix ............14
      1.4.4.1 Uterus....................................................................15
      1.4.4.2 Cervix....................................................................15
    1.4.5 Side-effects and incidence of foetal malformations ............16
  1.5 Induced abortion..............................................................................17
    1.5.1 Surgical abortion ................................................................17
      1.5.1.1 Priming agents ......................................................17
      1.5.1.2 Intracervical tents..................................................18
      1.5.1.3 Gemeprost.............................................................18
      1.5.1.4 Misoprostol ...........................................................18
      1.5.1.5 Mifepristone…………………………………….18
    1.5.2 Medical abortion..................................................................19
      1.5.2.1 Mifepristone..........................................................19
      1.5.2.2 Misoprostol-alone regimens for medical abortion20
  2 AIMS OF THE STUDY ...........................................................................21
  3 MATERIAL AND METHODES..............................................................22
    3.1 Patients........................................................................................ 23
    3.2 Study design................................................................................ 24
    3.3 Day of surgery............................................................................. 24
      3.3.1 Pain relief ........................................................................ 24
      3.3.2 Misoprostol...................................................................... 25
    3.4 Intrauterine pressure recording (papers I and II)...................... 25
    3.5 Tissue collection (paper II).......................................................... 26
      3.5.1 Immunohistochemistry .................................................... 26
      3.5.2 Pharmacokinetics and analysis of misoprostol acid (paper IV).... 27
    3.7 Statistics...................................................................................... 27
  4 RESULTS............................................................................................. 29
4.1 Uterine contractility (papers I and III) ........................................ 29
  4.1.1 Tonus .............................................................................. 29
  4.1.2 Contractions ................................................................. 29
4.2 The effect of misoprostol on inflammatory mediators and
cervical ripening during early pregnancy (paper II) .................... 30
  4.2.1 CD45 (leukocyte common antigen) ................................. 30
  4.2.2 CD68 (monocyte/macrophage cell marker) ..................... 30
  4.2.3 MMPs and TIMPs ........................................................... 30
4.3 Pharmacokinetic profiles up to 12h after administration using
different routes and formulations of misoprostol (paper IV) ...... 31
5 DISCUSSION .............................................................................. 33
  5.1 Pharmacokinetics ............................................................... 33
    5.1.1 Oral and vaginal administration ................................... 33
    5.1.2 Sublingual administration ............................................ 34
    5.1.3 SR misoprostol ............................................................ 35
    5.1.4 Pharmacokinetics in human breast milk ......................... 37
  5.2 Uterine contractility .............................................................. 37
  5.3 Effects of misoprostol on cervical ripening ............................ 40
  5.4 Side-effects and acceptability ............................................. 42
  5.5 Methodological considerations .......................................... 43
6 CONCLUSIONS ........................................................................... 45
7 ACKNOWLEDGEMENTS .............................................................. 47
8 REFERENCES .............................................................................. 50
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>Anova</td>
<td>Analysis of variance</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>Cmax</td>
<td>Peak serum levels of MPA</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>HIV/AIDS</td>
<td>Human immunodeficiency virus/Acquired immunodeficiency syndrome</td>
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<tr>
<td>LC/MS/MS</td>
<td>Liquid chromatography/ tandem mass spectrometry</td>
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<td>IgG</td>
<td>Immunoglobulin G</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
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<tr>
<td>mmHg</td>
<td>Millimeter mercury</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<tr>
<td>MPA</td>
<td>Misoprostol acid</td>
</tr>
<tr>
<td>MU</td>
<td>Montevideo units</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
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<tr>
<td>NSAID</td>
<td>Non steroidal anti-inflammatory drugs</td>
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<tr>
<td>PG</td>
<td>Prostaglandin</td>
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<tr>
<td>PGDH</td>
<td>15-hydroxyprostaglandin dehydrogenase</td>
</tr>
<tr>
<td>PLA/C</td>
<td>Phospholipase A2/C</td>
</tr>
<tr>
<td>SR</td>
<td>Slow release</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitor of MMP</td>
</tr>
<tr>
<td>Tmax</td>
<td>Time to peak serum level of MPA</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1 INTRODUCTION

1.1 “THE MISSING DRUG” IN A GLOBAL PERSPECTIVE

Misoprostol has become an important drug in both obstetric and gynaecological practice because of its uterotonic and cervical priming actions which has a number of advantages – it is safe, cheap, widely available and stable at room temperature when compared to other prostaglandin analogues. It has been extensively studied in reproductive health and shown to be useful for medical abortion, cervical ripening before surgical abortion, and evacuation of the uterus for various medical reasons, including incomplete abortion, missed abortion, intrauterine foetal death and severe pre-eclampsia, induction of labour and treatment and prevention of post-partum haemorrhage (www.misoprostol.org).

Regardless of culture, religion or political persuasion, women have always had recourse to abortion and will probably continue to do so. Induced abortion is used as a method of fertility regulation worldwide, and it is estimated that about 46 million pregnancies are terminated annually (Odlind, 1997, Alan Guttmacher Institute, 1999). Sadly, the complications of induced abortions remain significant causes of maternal mortality and morbidity around the world, but this applies particularly to countries that do not provide access to safe abortion services. When women do have access to safe abortion and safe contraceptive methods, the total abortion rate declines (Bongaarts and Westoff, 2000). There is however nowhere in the world where the rate is zero, and there are a number of reasons for this (WHO 2003). These reasons are complex, although the main ones include lack of both access and adequate information. In addition, no contraceptive method is ever 100% effective. Even if all women and men used contraceptives perfectly and consistently, nearly six millions unwanted pregnancies would occur every year.

At the global level, more than 8 million women suffer from poor reproductive health and serious pregnancy-related illness and disability. Every year, more than 500,000 women die from complications of pregnancy and childbirth. In developing countries, one woman in 16 may die of pregnancy-related complications, compared to one in 2800 in developed countries (WHO 2004). Even if most of the deaths occur in Asia, the risk of dying is highest in Africa (www.developmentgoals.org/Maternal_Health.htm).
Most of these deaths could be avoided if preventive measures were taken and adequate care was available. For every woman who dies, many more suffer from extremely serious conditions that may affect them for the rest of their lives. And every minute, every day, a woman dies somewhere in the world as a result of complications arising during pregnancy and childbirth. Tragically, the majority of these deaths are avoidable. They could, as stated above, be prevented if women had access to family planning information and services, care for abortion-related complications and, where abortion is not prohibited by law, safe abortion care.

The medical causes of maternal deaths are similar throughout the world. The single most common cause – accounting for a quarter of all maternal deaths – is severe bleeding, generally occurring post-partum. Sepsis, which is often a consequence of poor hygiene during delivery or of untreated sexually transmitted diseases, accounts for 15% of maternal deaths.

Complications after unsafe abortions are responsible for a substantial proportion, 13%, of maternal deaths. In some parts of the world, 50% of all maternal deaths are associated with unsafe abortions. In addition, hypertensive disorders of pregnancy, particularly eclampsia, are the cause of approximately 12% of all maternal deaths, while prolonged or obstructed labour accounts for about 8%. This is often caused by cephalopelvic disproportion or by abnormal lie. Disproportion is more common where malnutrition is endemic, and it is worse where girls marry young and become pregnant before they are fully grown.

Altogether, around 20% of maternal deaths are the result of pre-existing conditions that are exacerbated by pregnancy or its management. One of the most significant of these indirect causes of death is anaemia which, as well as causing death through cardiovascular arrest, is thought also to underlie a substantial proportion of indirect deaths. Other important indirect causes of death include malaria, hepatitis, heart diseases, and HIV/AIDS, which is increasing in some settings (www.developmentgoals.org/Maternal_Health.htm).

Despite the obvious potential that misoprostol has for reducing maternal mortality and morbidity, the patent holder has never applied for licences for any indication in the fields of obstetrics and gynaecology. The drug is only licensed for the treatment of
gastric ulcers. The only other approved indication in Sweden, using the mifegyne label (Exelgyn, Paris, France), is for medical abortion up to 49 days of gestation. This also applies to other countries where mifegyne has been approved. This restricted use of a potentially important drug lends urgency to further studies of misoprostol, since these could lead to evidence-based recommendations for its use in other settings than those that are currently approved.

1.2 ANATOMY AND PHYSIOLOGY OF THE UTERUS

The uterus consists of two parts, the corpus and the uterine cervix. It is located in the pelvic cavity of non-pregnant women and of pregnant women during the first trimester of pregnancy (Brody 1993). In later stages of gestation, it becomes an abdominal organ. It is situated between the bladder on its anterior surface and the rectum on its posterior surface.

1.2.1 Corpus uteri

The corpus is primarily a muscular organ, with connective tissue distributed between the muscle bundles. It is composed mainly of smooth muscle (70%) with an extracellular matrix (30%) between the cells that contains a collagen network of proteins, proteoglycans and polysaccharides (Schwalm and Dubrauszky, 1966). The extracellular matrix (ECM) provides the tissue with the necessary strength while also connecting the muscle bundles with each other to facilitate cell-cell and cell-matrix communication.

1.2.2 Uterine contractility

Compared to other smooth muscle, that of the uterus, known as the myometrium, is almost entirely under hormonal control. The most important of these hormones are the steroids known as progesterone and oestrogen. There are many factors that modulate uterine contractility, including prostaglandins (Egarter and Husslein, 1992, Word et al., 1992), oxytocin (Blanks and Thornton, 2003), endothelin, platelet activating factor (Tetta et al., 1986, Valenzuela et al., 1995), while relaxing factors include prostacyclin, corticotrophin releasing hormone and nitric oxide (Hertelendy and Zakar, 2004).

During early pregnancy, uterine contractility is suppressed by progesterone (Csapo et al., 1973a, Csapo et al., 1973b). In the before mentioned studies, luteectomy before the
luteo-placental shift and the subsequent decrease in plasma progesterone levels resulted in increased uterine contractions and spontaneous abortion. Csapo (1974) suggested that the degree of uterine activity is regulated by the balance between the intrinsic suppressor progesterone and the stimulant prostaglandin PGF2α. Progesterone also inhibits any softening of the cervix (see below). During most of pregnancy, the myometrium is refractory and its spontaneous phasic activity is suppressed by progesterone (Hertelendy and Zakar, 2004). Progesterone suppresses uterine contractions by inducing hyperpolarization of the cell membrane, which makes the myocytes less sensitive to electrical stimulation. Progesterone also regulates gap junction formation. These junctions are believed to coordinate myometrial function by conduction of electrophysiological stimulations (Egarter and Husslein, 1992). However, in the 4th month of gestation, irregular Braxton-Hicks contractions start to occur. Towards the end of the pregnancy they develop into more regular contractions in readiness for childbirth.

1.2.3 Cervix uteri
The cervix is an ingeniously constructed organ. In a nulliparous woman who is not pregnant, it is a relatively stiff cylindrical structure that is around 2.5 cm long (Cunningham, 1993). It allows the passage of a sperm, but will resist all ascending infections which might present a threat to fertility. During pregnancy, it normally remains firm and closed and withstands the pressure of the growing foetus, amnion and placenta. At the end of pregnancy, however, it softens and distends very rapidly, allowing the foetus to pass through the birth canal. The anatomical facts have been known for centuries, but the physiological changes and the fact that the cervix mainly consists of connective tissue, predominantly collagen, have only been known since 1947 (Danforth, 1947). Only 4-10% of the cervix is smooth muscle, the remainder consists of connective tissue and extracellular matrix (ECM), totalling some 85% (Schwalm and Dubrauszky, 1966).

The cervical mucosa is lined with tall columnar epithelium with large highly branched glands. The portion of the cervix that projects into the vagina, the portio vaginalis, is covered with a stratified squamous epithelium (Rooss and Romrell, 1989).
1.2.4 Extracellular matrix

The ECM consists mainly of fibrillar components – collagen and elastin. Other components of the ECM are proteoglycans, glycoproteins, hyaluronan and different cell types (Hay, 1991), as well as non-fibrillar components (proteoglycans and specialised proteins). The principal function of the ECM is to provide tissue strength and mediate cell communication.

Collagen is a protein made by fibroblasts and macrophages. Collagen fibrils are built up from tropocollagen molecules. Tropocollagen is a triple helix of three protein chains with covalent cross-links in between (Ninni, 1983). The collagen in the ECM is mainly of types I, III and IV. Types I and III are interstitial collagens, while type IV collagen is associated with smooth muscle cells and vascular basement membranes. The collagen is organized into fibrils and larger fibres that form a network. Meanwhile, the elastin in the connective tissue contributes to the viscoelasticity of the cervix. (Minamoto et al., 1987, Leppert, 1995). Matrix metalloproteinases (MMPs) are enzymes involved in the degradation of the connective tissue matrix. MMP 8 degrades fibrillar collagen and MMP 9 degrades the type IV collagen of basement membranes (Leppert, 1995, Osmers et al., 1995). Tissue inhibitors of metalloproteinases (TIMPs) are proteins that inhibit MMPs by making complexes of MMP and TIMP (Woessner, 1999). Other cell types in the cervix are the immune cells, leukocytes, macrophages, T-lymphocytes and Langerhans cells (Bokström et al., 1997, Edwards and Morris, 1985, White et al., 1997).

As mentioned above, progesterone prevents cervical softening and dilation during early pregnancy. It does so by suppression of interleukin-8 (IL-8), as well as other pro-inflammatory cytokines and nitric oxide (NO). This effect can be counteracted by antiprogesterone (Denison et al., 2000). Cervical softening at term is the result of an inflammatory reaction characterised by an influx of inflammatory cells followed by an extensive remodelling of the ECM and an increase in water content of the organ (Liggins, 1981). At term pregnancy, neutrophils, for example, are present in significantly higher numbers than during the first trimester (Bokström et al., 1997).

Despite the wide clinical use of prostaglandin analogues, there is only limited knowledge about the biochemical events involved in prostaglandin-induced cervical ripening (Kelly, 2002). Prostaglandins may have a direct effect in stimulating matrix
metalloproteinase (MMP) activity, or an indirect effect via vasodilation and influx of leukocytes containing MMPs, chemokines and cytokines (Busiek et al., 1995, Shankavaram et al., 1998, Rath et al., 1994). Prostaglandins may also enhance the effect of other mediators of cervical ripening, such as IL-8. In skin, the chemotactic action of IL-8 on neutrophils and oedema formation is greatly enhanced by prostaglandin E2 (PGE2) (Rampart et al., 1989). IL-8 also has a selective effect on stimulating the release of MMP 8-containing granules in the neutrophil cytoplasm.

1.2.5 Cervical ripening

The remodelling of the cervix at term pregnancy is an active biochemical process involving dramatic changes in the form and consistency of the cervix. Existing knowledge about cervical ripening is mainly based on research into the physiological ripening of the cervix or following pharmacological intervention at term pregnancy. The exact mechanism leading to physiological cervical ripening is however not known. The cervix becomes soft and dilated as a result of an extensive remodelling of the ECM (Uldbjerg et al., 1983, Ekman et al., 1986). When the cervix softens, the collagen fibres decrease and dissociate in a characteristic way due to oedema (Theobald et al., 1982, Minamoto et al., 1987). Simultaneously, an increased vascularisation and influx of leukocytes occur. The process has been compared to an inflammatory reaction (Liggins, 1981, Sennström et al., 2000). Indeed, during cervical ripening, there is an influx of inflammatory cells including macrophages, neutrophils, mast cells and eosinophils, into the cervical stroma. It has been proposed that these cells produce cytokines and prostaglandins that have an effect on extracellular matrix metabolism. Influx of neutrophils at term pregnancy has been shown to be associated with breakdown of collagen tissue. Active degranulation has been visualised by electron microscopy (Junqueira et al., 1980) and prostaglandins have been proposed to play an important role in cervical ripening (see below).

Numerous hormones and mediators are involved in cervical ripening – progesterone, prostaglandins, proinflammatory cytokines, NO, neuropeptides and oestrogen. Progesterone seems to play an overall role in the cervical ripening process (Chwalisz and Garfield, 1998, Neilson, 2004) and treatment with antiprogestin induces labour at term (Stenlund et al., 1999, Neilson, 2004). However, in rats and guinea pigs, physiological ripening starts at mid-trimester and independently of uterine contractions (Chwalisz and Garfield, 1998). This occurs long before the fall in progesterone levels,
indicating an additional progesterone-independent mechanism. Locally administered prostaglandins are effective in inducing cervical ripening, and prostaglandins have for some time been thought of as key mediators. Other local mediators may be important, and NO has been proposed to act as the final mediator of cervical ripening (Chwalisz and Garfield, 1998). NO donors, such as isosorbidenonitrate, may thus be effective in inducing cervical ripening (Ekerhovd et al., 2003b).

1.3 PROSTAGLANDINS

1.3.1 History

Prostaglandins (PG) are a whole family of compounds with a wide spectrum of biological effects. All prostaglandins contain 20 carbon atoms and have the same basic carbon skeleton – “prostanoic acid”. (Bergström et al., 1968). They are both autocrine (when the released PG acts via receptors on the same cell) and paracrine (when the PG acts on neighbouring cells) and are produced in virtually all cells in the human body (Speroff and Fritz, 2004). Eicosanoids include all 20-carbon-atom derivatives generated from phospholipids in response to a wide range of stimuli, and their presence has been detected in virtually every tissue in the human body. They are implicated in the control of many physiological processes and are among the most important mediators and modulators of the inflammatory reaction (Rang and Dale, 1991). The principal eicosanoids are the prostaglandins, the thromboxanes and the leukotrienes. The term “prostanoids” encompasses both prostaglandins and thromboxanes.

The first prostaglandin effects were discovered in 1930. During artificial insemination to treat sterility in women, it was observed that in a number of cases, semen that was injected into the uterine cavity was promptly expelled. When Ringer’s solution was injected however, it was retained. In some cases, there was a contraction of the uterus after installation of the semen, and it was noted that semen also caused contraction of human uterine muscle in vitro (Kurzrok and Lieb, 1930). Human seminal plasma was discovered to contain a substance that is a powerful vasodilator and can stimulate muscle activity (Goldblatt, 1935). This acid, lipid-soluble, smooth-muscle-stimulating and blood-pressure-lowering principle was named prostaglandin (v. Euler, 1935) and was shown to consist of highly active, lipid-soluble, unsaturated hydroxy acids (Bergström, 1949). Almost thirty years later, the chemical structure was elucidated and
1.3.2 Synthesis and metabolism

Prostaglandins are continuously synthesised from almost all cell membranes in the human body (Alberts et., 1989). The precursors are unsaturated essential dietary fatty acids – linoleic acid, arachidonic acid and pentanoic acid. Linoleic acid is the precursor of the PG1-series (the number refers to the number of double bonds). Arachidonic acid (AA) is the precursor of the PG2-series. PGE$_2$ is synthesised mainly in the cervix (Barclay et al., 1993), while the uterus, placental trophoblasts (Bennett et al., 1992) and amnion synthesise PGE$_2$ and PGF2$\alpha$ (Skinner and Challis, 1985, Mitchell, 1991, Kelly 2002).

Most cellular AA is derived from linoleic acid, which is converted into arachidonic acid in the liver (Zhou and Nilsson, 2001). Pentanoic acid is the precursor of the PG3-series and has 3 double bonds (Bergström et al., 1968) see figure 1.

![Prostaglandin synthesis](image)

Figure 1. Prostaglandin synthesis

The prostaglandins with the greatest biological activity, and also the most widely studied, are the 2-series derived from AA. Most of this AA originates from the fatty
acids in phospholipids, with only 1-2% in free form in plasma. The initial step in the formation of the PG2-series is the release of AA from the membranes by phospholipase A2 (PLA2) and phospholipase C (PLC). The release of AA from membrane phospholipids by PLA2 is the principal mechanism regulating the levels of free AA for the production of eicosanoids in response to a variety of extracellular stimuli (Lapertina, 1982, Hirabayashi and Shimuzu, 2000). Mammalian cells have structurally diverse forms of PLA2, including cytosolic PLA2, which is believed to play a pivotal role in the biosynthesis of free AA for eicosanoid biosynthesis (Hirabayashi and Shimuzu, 2000). It has now been established that both an increase in calcium and phosphorylation of cytosolic PLA2 are important for its full activation, which leads to AA release and eicosanoid production (Leslie, 2004). Free AA is then converted into the first true PG compounds – PGG2 and PGH2 – by the enzyme cyclooxygenase (COX). COX exists in two isoforms, COX-1 and COX-2. Prostaglandin metabolism is initiated by 15-hydroxyprostaglandin dehydrogenase (PGDH). PGDH activity is reduced by antiprogestin and cytokines such as IL-1β (Cheng et al., 1993, Brown et al., 1998) and in the presence of infection (Van Meir et al., 1996). Primary PGs are very rapidly metabolised locally and only appear in the circulation in insignificant amounts (Bygdeman, 2003).

1.4 MISOPROSTOL

1.4.1 Source
Misoprostol is approved in most countries under the brand name Cytotec® (Pfizer, NY, USA). The tablets are produced for oral use and contain either 100 or 200 µg of misoprostol. More recently 25 µg tablets for vaginal use have been approved in Brazil and Egypt for labour induction. Arthrotec (Pfizer, NY, USA is a further development of Cytotec and in addition to misoprostol, it also contains diclofenac. In some places, Arthrotec is now replacing Cytotec as the source of misoprostol. The included doses of diclofenac vary and must be considered in order not to exceed recommended maximal intake.

1.4.2 Pharmacology of misoprostol
1.4.2.1 Structure and chemistry of misoprostol
Misoprostol (C22 H38 O5, M:W.= 382.5; (11α, 13E,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oic acid methyl ester) is a synthetic prostaglandin E1 analogue developed in 1973 by Searle for the treatment and prevention of gastric ulcers The
figure included below shows the structures of both misoprostol and naturally occurring prostaglandin E1.

![Chemical structure of misoprostol](image)

Figure 2. Chemical structure of misoprostol.

The naturally occurring prostaglandin E series was discovered to inhibit gastric acid secretion in 1967 by Robert et al. However, naturally occurring prostaglandins have three drawbacks that have hindered clinical application: (1) rapid metabolism manifested as a lack of oral activity and a short duration of action when given parenterally, (2) incidence of numerous side-effects, and (3) chemical instability leading to short shelf-life (Collins et al., 1985).

Misoprostol differs structurally from prostaglandin E by the presence of a methyl ester at C-1, a methyl group at C-16 and a hydroxyl group at C-16 rather than C-15. It appears that the methyl ester at C-1 increases the anti-secretory potency and duration of action of misoprostol, while the movement of the hydroxyl group from C-15 to C-16 and the addition of a methyl group at C-16 improve oral activity, increase the duration of action and improve the safety profile of the drug compared with prostaglandin E (Collins et al., 1985). However, the compound was still chemically unstable at room temperature. This problem was solved through the dispersion of misoprostol in hydroxypropylmethylcellulosa (Sanvordeker, 1981). Misoprostol contains 2 chemical sites at which 4 different stereochemical isomers may occur. During manufacture of the drug, approximately equal amounts of the 4 isomers are formed. The 11R, 16S-isomer seems to be principally responsible for the gastric acid inhibitory activity of misoprostol, while the other 3 isomers possess minimal gastric anti-secretory activity.

1.4.2.2 Pharmacokinetics

Misoprostol is extensively absorbed and undergoes rapid de-esterification by the liver to form the free acid (MPA), which is responsible for its clinical activity. Unlike the parent compound, it is detectable in plasma (Schoenhard et al., 1985, Karim, 1987). The alpha side chain undergoes beta oxidation and the beta side chain undergoes omega
oxidation. This is followed by reduction of the ketone to produce prostaglandin F analogues (Cytotec product information).

In a multiple oral does study by Karim, no accumulation of MPA was noted following 400 µg given at 12 hour intervals over 4 days and plasma steady state was achieved within 2 days (Karim, 1987). Maximum plasma concentrations of MPA are diminished when the dose is taken with food, and the total availability of misoprostol acid is reduced by concomitant use of antacid (Karim et al., 1989). After oral administration of radio-labelled misoprostol, about 80% of detected radioactivity appears in the urine (Karim, 1987). Misoprostol does not affect the hepatic mixed function oxidase (cytochrome P-450) enzyme systems in animals (Watkinson and Akbar, 1987). Drug interaction studies of misoprostol and several non-steroidal anti-inflammatory drugs (NSAIDs) showed no effect on the kinetics of ibuprofen or diclofenac (Cytotec product information). Misoprostol has both anti-secretory (inhibiting gastric acid secretion) and mucosal protective properties (Wilson et al., 1986). NSAIDs inhibit prostaglandin synthesis, and a deficiency of prostaglandins within the gastric mucosa may diminish bicarbonate and mucus secretion and contribute to the mucosal damage caused by these agents. Because of its potent anti-secretory and cytoprotective effects on the gastric and duodenal mucosa, misoprostol is an effective drug in the treatment of gastric and duodenal ulcers. In addition, misoprostol has been shown to protect the gastro-duodenal mucosa from the damaging effects of alcohol and non-steroidal anti-inflammatory drugs.

Misoprostol does not have clinically significant effects on serum levels of prolactin, gonadotropins, thyroid stimulating hormone, growth hormone, thyroxine, cortisol, gastrointestinal hormones, creatinine or uric acid. Immunologic competence, platelet aggregation, pulmonary function and the cardiovascular system are not modified by recommended doses of misoprostol (200-800 µg/day) (Cytotec product information).

1.4.3 Different routes of administration of misoprostol
1.4.3.1 Oral route

Earlier studies focused exclusively on the pharmacokinetic properties of misoprostol after oral administration. Most of these studies reported the pharmacokinetic profile after a single dose of misoprostol. After oral administration, misoprostol is rapidly and almost completely absorbed from the gastrointestinal tract. However, the drug
undergoes extensive and rapid first-pass metabolism (de-esterification) to form MPA, the principal and active metabolite of the drug. Following a single dose of 400 µg oral misoprostol, the plasma misoprostol level increases rapidly and peaks at about 30 minutes. However, the plasma level declines rapidly by 120 minutes and remains low thereafter (Zieman et al., 1997, Tang et al., 2002a, Khan et al., 2004, Meckstroth et al., 2006). There is a high variability in plasma levels of MPA between and within the studies, although mean values after single doses show a linear relationship with dose over the range of 200–400 µg (Karim, 1987, Foote et al., 1995, Cytotec product information, Zieman et al., 1997).

1.4.3.2 Vaginal route

It was discovered and proven by clinical studies that vaginal administration was more effective than oral administration in medical abortion (El-Refaey et al., 1995, Ho et al., 1997). Zieman et al. performed the first pharmacokinetic study comparing oral and vaginal routes of administration (Zieman et al., 1997). In contrast to the oral route, the plasma concentration after vaginal administration increases gradually, reaching maximum level after 70-80 minutes. It then slowly declines, with detectable levels present up to 6 hours after administration. The peak concentration reached following oral administration is higher than that following vaginal administration, see figure 3. However, the AUC is significantly greater after vaginal than after oral administration. Thus bioavailability measured as AUC is greater following vaginal than following oral administration.

![Figure 3. Mean plasma concentrations of misoprostol acid over time with oral and vaginal administration modified from Zieman et al., 1997.](image)

The greater bioavailability of vaginal misoprostol may help to explain why vaginal administration is more effective in medical abortion. Due to its clinical efficacy,
vaginal misoprostol is widely used off label. However, the misoprostol tablet was not manufactured and developed for use by routes other than the oral one. The AUC after 400 µg vaginally administered misoprostol varies between different studies (Zieman et al., 1997, Tang et al., 2002a). The mean peak serum levels in a recent study by Meckstroth et al. (2006) were 2.7 times higher than mean peak levels in earlier studies (Zieman et al., 1997, Tang et al., 2002a, Khan et al., 2004). This means that the absorption of misoprostol when using the vaginal route is less consistent than that of the oral route (Zieman et al., 1997). It is not uncommon to identify remnants of the tablets hours after vaginal administration, indicating that absorption is variable and incomplete. This may be due to variations in the amount and pH value of the vaginal discharge in different women. Variations in the amount of bleeding during medical abortion may also affect the absorption of misoprostol through the vaginal mucosa. Numerous attempts have been made to improve the absorption of vaginal misoprostol. The addition of water to the misoprostol tablets is a common practice. However, the bioavailability of vaginal misoprostol was not improved significantly by this practice, indicating that adding water to the tablets may not in fact improve absorption (Tang et al., 2002a).

Drawbacks of vaginal administration include the fact that tablets may, as mentioned above, be found in the vagina during an examination. Studies have also shown that a majority of women prefer the oral route of administration as being more private and convenient. Alternative routes of administration have therefore been sought.

1.4.3.3 Sublingual route

The sublingual route was proposed as an alternative to the vaginal route by PC Ho (Tang et al., 2002b). The misoprostol tablet can be dissolved in 20 minutes when it is placed under the tongue. At the time that the studies included in this thesis were initiated, the clinical effect of sublingual administration of misoprostol (the tablet is held under the tongue to dissolve) was investigated in two pilot studies with promising results. Following pre-treatment with 200 mg mifepristone, 800 µg misoprostol were administered sublingually. Complete abortion occurred in 94% of treated women (Tang et al., 2002b). In the second pilot study, sublingual misoprostol was used for termination of pregnancy up to 12 weeks and showed an 86% abortion rate and 97.7% acceptability (Tang et al., 2002c). A pharmacokinetic study showed that sublingual and oral administration produce the quickest increase in MPA when
compared with vaginal administration. The systemic bioavailability as measured by the AUC was also highest following sublingual misoprostol (Tang et al., 2002a).

1.4.3.4 Buccal route
At about the same time as the studies on sublingual misoprostol were initiated, the buccal route was described. The drug is placed between the teeth and the cheek and allowed to be absorbed through the buccal mucosa. Clinical studies are limited compared to other routes. They have however shown that the buccal route is also effective for early medical abortion, cervical priming and labour induction (Middleton et al., 2005, Castleman et al., 2006, Curlan et al., 2002). The shape of the absorption curve is very similar to that of the vaginal route, although the serum drug levels attained are lower throughout the 6-hour study period (Meckstroth et al., 2006). The \( T_{\text{max}} \) is reached 75 minutes after buccal administration, which is similar to that after vaginal administration. The AUC for buccal administration is however only half that seen after vaginal administration. Another study comparing buccal to sublingual administration has also shown that the AUC for sublingual misoprostol is 4 times that seen after buccal administration (Schaff et al., 2005). The buccal route is a promising way of administering misoprostol, but more studies are required to compare it with other routes of administration.

1.4.3.5 Rectal route
Rectal administration has been studied for the management of post-partum haemorrhage. This route of administration is less commonly used for other applications, such as medical abortion, cervical priming or labour induction. The shape of the absorption curve after rectal administration is similar to that seen after vaginal administration, although the AUC for vaginal administration is 3 times that seen after rectal administration. The mean \( T_{\text{max}} \) ranges between 40 and 65 minutes (Meckstroth et al., 2006, Khan and El-Refaey, 2003).

1.4.4 Effects of misoprostol on the uterus and the cervix
The uterotonic and cervical softening effects of misoprostol in the female genital tract were originally regarded as side-effects rather than therapeutic effects when misoprostol was first introduced. However, it is precisely these effects that nowadays
make misoprostol so widely applicable and used in daily obstetric and gynaecological practice.

1.4.4.1 Uterus
The typical effect of a single dose of oral misoprostol is an increase in uterine tonus (Norman et al., 1991, Gemzell Danielsson et al., 1999). It is only following repeated treatment that regular uterine contractions appear. Thus it seems that a sustained plasma level of misoprostol is required for the development of regular contractions. Regular contractions are essential for many of the clinical effects of misoprostol in medical abortion and induction of labour. The effect of vaginal administration of a single dose of misoprostol on uterine contractility is initially similar to that of oral administration – an increase in uterine tonus. After 1-2 hours, however, regular uterine contractions appear and they last at least up to 4 hours after administration (Gemzell-Danielsson et al., 1999). The development of regular contractions after vaginal administration may explain the better clinical efficacy of vaginal administration when compared to oral administration (El-Refaey et al., 1995, Ho et al., 1997).

1.4.4.2 Cervix
It has been shown that various prostaglandin analogues can decrease the hydroxyproline content of the pregnant cervix. (Rath et al., 1982). The histochemical changes in the pregnant cervix after misoprostol administration were studied using electron microscopy and proline uptake assay. The mean proline incorporation per µg protein and collagen density, estimated by light intensity, was significantly less than in the control group. The diameter of the collagen fibres was also smaller in the misoprostol group, although the difference was not statistically significant. This indicated that the action of misoprostol appeared to focus mainly on the connective tissue stroma, with evidence of disintegration and dissolution of collagen (El-Refaey, 1994)

There are also many clinical studies that have demonstrated the cervical priming effect of misoprostol in the pregnant state. Misoprostol has been used extensively for its cervical softening effect before induction of labour and surgical evacuation of the uterus. Studies have demonstrated that less force was required for mechanical dilation of the cervix if misoprostol was applied before the procedure (El-Refaey et al., 1994, Ngai et al., 1995). While this softening effect on the cervix may be secondary to the
uterine contractions induced by misoprostol, it is more likely to be due to a direct effect of misoprostol on the cervix. Dilation of the softened cervix may thereafter increase following induction of uterine contractions.

1.4.5 Side-effects and incidence of foetal malformations

Misoprostol is a safe and well-tolerated drug. Preclinical toxicological studies indicate a safety margin of at least 500-1000-fold between lethal doses in animals and therapeutic doses in humans (Kotsonis et al., 1985). No clinically significant adverse haematological, endocrine, biochemical, immunological, respiratory, ophthalmic, platelet or cardiovascular effects have been found. Diarrhoea is the major adverse reaction to have been consistently reported for misoprostol. The diarrhoea is usually mild and self-limiting, and usually stops once misoprostol is withdrawn. Nausea and vomiting may occur and will resolve 2 to 6 hours after taking misoprostol. Fever and chills were also reported in studies using misoprostol for medical abortion. This is especially the case when the women are exposed to a high serum level of misoprostol, as in cases of oral administration.

Another concern about the use of misoprostol is the risk of uterine rupture, especially in women with previous uterine scarring. Reports of uterine rupture are rare in first trimester medical abortion (Kim et al., 2005). The risk may increase with gestational length. Evidence from the literature shows that the risk of uterine rupture is usually associated with induction of labour in the third trimester and occurs in women with previous uterine scarring and other risk factors for uterine rupture (Plaut et al., 1999).

Exposure to misoprostol in early pregnancy has however been associated with multiple congenital defects. The first case reports that misoprostol could be teratogenic came from Brazil 1991. In Brazil, where the abortion law is very restrictive, misoprostol was used for illegal termination of pregnancy. However, mutagenicity studies of misoprostol have been negative and misoprostol has been shown not to be embryotoxic, foetotoxic, teratogenic or carcinogenic (Pastuszak et al., 1998) so the reported malformations may be due to disruption of the blood supply to the developing embryo during contractions. It is estimated that the absolute risk of malformations after exposure to misoprostol is relatively low, in the region of 10 per 1000 foetuses exposed. In population registers, the incidence of abnormalities does not seem high, given that exposure to misoprostol is quite common in some populations (Orioli and
 Castilla, 2000). A wide range of defects are however possible depending on the time of exposure to misoprostol. Central nervous system and limb defects are the most commonly reported anomalies. Mobius syndrome, which is characterised by congenital facial paralysis with or without limb defects, has been associated with misoprostol exposure (Pastuszak et al., 1998). Other abnormalities, such as transverse limb defects, ring-shaped constrictions of the extremities, arthrogryposis, hydrocephalus, holoprosencephaly and exostrophy of the bladder, have also been reported (Orioli and Castilla, 2000). Foetal malformation is more commonly associated with the use of a misoprostol-only regimen for abortion, rather than a sequential regimen using mifepristone and misoprostol. It may be due to the stronger uterine contraction associated with repeated and high doses of misoprostol and less effective regimens.

1.5 INDUCED ABORTION

1.5.1 Surgical abortion

Vacuum aspiration is still the preferred method in late first- and early second-trimester termination of pregnancy. Although it is usually a very safe and effective method for termination of pregnancy, it is sometimes associated with complications such as cervical laceration, uterine perforation, incomplete abortion and haemorrhage, with risks of long-term effects on subsequent fertility. It is well known that cervical tearing and uterine perforation are directly related to the required degree of forcible mechanical dilation of the cervix required. (Moberg 1976, Schultz et al., 1983, Grimes et al., 1984). When used prior to surgical abortion, cervical priming has been shown to result in a shorter operation time, reduced blood loss and easier mechanical dilation (El-Refaey et al., 1994, Ngai et al., 1999). Cervical priming may also reduce the incidence of complications during the procedure and is therefore recommended in a number of guidelines (WHO 2003, RCOG 2004, Schultz et al., 1983, Grimes et al., 1984). A variety of procedures and agents have been studied with the aim of developing a simple, safe and less traumatic method of preparing the cervix prior to dilation and vacuum aspiration.

1.5.1.1 Priming agents

Agents for cervical dilation were described scientifically for the first time by Braxton-Hicks in 1869 (Braxton-Hicks 1869), but have been known since the time of Hippocrates (460-377 BC). They included springs, roots, dilators, screws and sponges
The methods most commonly used today are intracervical tents, prostaglandin analogues administered by various routes (mainly misoprostol but also gemeprost) and mifepristone.

1.5.1.2 Intracervical tents

The laminaria tent is an osmotic dilator derived from dried seaweed and is the classic method used to dilate the cervix. When inserted into the cervical canal, the tent will swell over a period of 6-12 hours, dilating the cervix in the process (Lauersen et al., 1982). Dilapan is a synthetic tent and Lamicel is a synthetic magnesium sulphate impregnated sponge compressed into a tent. Both dilate the cervical canal in a similar way to the natural laminaria tent, although within 3-4 hours following insertion. (Bokström and Wiqvist, 1989, Rådestad and Christensen, 1989). The disadvantages of the tents are that they need to be inserted during a gynaecological examination, and the insertion can be painful and cause vaso-vagal symptoms and bleeding, as well as possibly inducing a false passage. Moreover, a period of 8-10 hours before the surgical procedure is needed for maximal dilation.

1.5.1.3 Gemeprost

Gemeprost (Cervagem®) is a PGE1 analogue and is licensed for use in preoperative dilation prior to abortion. It is administered vaginally. A significant effect in the form of cervical ripening and dilation of the cervical canal is achieved as well as reduction in operative blood loss. (Kajanoja et al., 1984).

1.5.1.4 Misoprostol

Misoprostol has several advantages over other priming agents, such as osmotic dilators (Krishna et al., 1986, Maelisaac et al., 1999, Darwish et al., 2004), other prostaglandins (Celentano et al., 2004, El-Refaey et al., 1994, Ekerhovd, 2003a, RCOG 2004, Henry and Haukkama, 1999, Preutthipan and Herabutya, 2006) and mifepristone (Ashok et al., 2000). Misoprostol can be administered orally and, compared to gemeprost, it does not require refrigeration.

1.5.1.5 Mifepristone

Mifepristone is more effective than misoprostol for cervical priming, although it requires a longer time interval between administration of the tablets and the procedure (>24 hours). It is also more expensive than misoprostol alone (Rådestad et al., 1988,
Ashok et al., 2000). In addition, mifepristone is not widely available and therefore not legally accessible to most care providers.

### 1.5.2 Medical abortion

Medical abortion is a way of terminating pregnancy pharmacologically and is an alternative to surgical abortion. The development of medical abortion started in the 1970s when a prostaglandin injection was given used intraamniotically to induce second trimester abortion in preference to hypertonic saline (Bygdeman and Wigvist, 1974). Very soon, a vaginal prostaglandin was developed that was effective even in early pregnancy (Lundström et al., 1977), although the prostaglandins used at that time required high doses to be effective and caused a high incidence of side-effects and pain (Swahn and Bygdeman, 1990). When mifepristone became available in 1980, expectations were high that the compound would provide an effective means of terminating pregnancy.

#### 1.5.2.1 Mifepristone

Mifepristone (RU-486) is a synthetic antiprogesterone compound designed in France in 1980 (Roussel-Uclaf, Paris). It was found to bind strongly to the glucocorticoid receptor (Ulman et al., 1990). It was also found to have a great affinity for the progesterone receptor and mainly act as an antagonist of progesterone (Herrman et al., 1982). Mifepristone is a 19-norsteroid substituted at the 11β position by a p-(dimethylamino) phenyl group. A hydrophobic 17α-substituent increases the binding affinity to the progesterone receptor (PR) (Teutsch., 1985). The affinity for the PR is 2.5-5 times that of progesterone (Moguelwsky and Philibert, 1984, Lähteenmäki et al., 1987). Mifepristone acts like progesterone by entering the target cell and interacting with receptors that are largely loosely bound to the nucleus (Baulieu, 1989).

Clinical studies showed that mifepristone used alone during early pregnancy is capable of inducing complete abortion in only about 60% of patients (Bygdeman and Van Look, 1988). The breakthrough for the development of medical abortion came in 1985 when Bygdeman and Swahn discovered that mifepristone increased uterine contractility and sensitised the myometrium to prostaglandin (Swahn and Bygdeman, 1988). Pretreatment with mifepristone 36-48 hours prior to administration of a prostaglandin analogue made it possible to lower the dose of prostaglandin 5 times compared to
prostaglandin-alone regimens, thus reducing side-effects (Swahn and Bygdeman, 1988).

Medical abortion has developed over the last 20 years and has proven to be a safe and effective method (Fiala and Gemzell-Danielsson, 2006). It is now considered more effective than surgical abortion in terms of inducing complete abortion during early pregnancy (RCOG 2004).

In 1991, it was shown that misoprostol was as effective as the initially used prostaglandin analogue sulprostone in medical abortion (Aubeny and Baulieu, 1991). Further studies confirmed the efficacy of oral misoprostol given after initial treatment with mifepristone for medical abortion up to 49 days of pregnancy (McKinley et al., 1993, Ashok et al., 1998). However, it was discovered that oral misoprostol was less effective beyond 49 days of gestation and that efficacy could be improved by vaginal administration of the oral tablets (von Hertzen et al., 2003, El-Refaey et al., 1995, Ho et al., 1997). Today, the most widely used regimen is a combination using mifepristone followed by administration of misoprostol. The combined regimen can be used for all pregnancy durations provided the route and dose of misoprostol are adjusted (RCOG 2004, WHO 2003).

1.5.2.2 Misoprostol-alone regimens for medical abortion

Since mifepristone is expensive and only available in a limited number of countries, the use of misoprostol alone in medical abortion has become common. To find an appropriate method for terminating pregnancy in low-income countries, Bugalho et al. studied misoprostol for induced abortion. However, the misoprostol-alone regimens are less effective, require higher doses of misoprostol and thus result in more side-effects. Different misoprostol-alone regimens have been studied for early medical abortion (Creinin and Vittinghoff, 1994, Bugalho et al., 1996, Koopersmith and Mishell, 1996, Carbonell et al., 1997a, Carbonell et al. 1997b, Carbonell et al., 1998, Tang et al., 1999, Ngai et al., 2000, Tang et al., 2002c). The evidence based recommendations are shown in Table II.
2 AIMS OF THE STUDY

The synthetic prostaglandin E1 analogue misoprostol has become an important drug in obstetric and gynaecological practice because of its uterotonic and cervical priming actions. A better understanding of the impact of the route of administration on pharmacokinetics, uterine contractility and cervical softening would help to further improve misoprostol regimens for several indications.

The aims of this thesis were therefore to investigate the following effects of misoprostol in the first trimester of pregnancy:

- Uterine contractility following oral, vaginal and sublingual administration of misoprostol

- Intracervical accumulation of inflammatory cells, expression of matrix metalloproteinases-8 and -9 as well, as their regulators TIMP-1 and -2, following oral and vaginal administration of misoprostol.

- Uterine contractility following orally administered conventional misoprostol and two different doses of a new slow release (SR) formula of misoprostol.

- Serum levels of MPA up to 12 hours after administration of conventional misoprostol given sublingually or vaginally and oral SR misoprostol.
3 MATERIAL AND METHODS

All studies were conducted at the Department of Obstetrics and Gynaecology, Sesam and C 21, Karolinska University Hospital. The studies were approved by the ethics committee at the Karolinska Institutet and by the Swedish Medical Product Agency. All women gave their written informed consent prior to participation.

Table I. Overview of dose, route and formulation of the misoprostol administered.

<table>
<thead>
<tr>
<th>Study and topic</th>
<th>n</th>
<th>Misoprostol route, dose</th>
<th>Parity mean (range)</th>
<th>Gestational length (days) median / mean* (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>I</strong> Uterine contractility</td>
<td>10</td>
<td>Oral 400 µg</td>
<td>2.2 (1-4)</td>
<td>*9.8 (9-11)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Vaginal 400 µg</td>
<td>1.7 (1-3)</td>
<td>*9.9 (9-11)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Sublingual 200 µg</td>
<td>1.7 (0-4)</td>
<td>*9.7 (9-11)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>Sublingual 400 µg</td>
<td>1.7 (1-5)</td>
<td>*8.9 (8-10)</td>
</tr>
<tr>
<td><strong>II</strong> Cervical ripening</td>
<td>6</td>
<td>Oral 400 µg</td>
<td>0</td>
<td>60.5 (57-78)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Vaginal 400 µg</td>
<td>0</td>
<td>67.0 (58-80)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>None</td>
<td>2.0</td>
<td>57.5 (50-82)</td>
</tr>
<tr>
<td><strong>III</strong> Uterine contractility</td>
<td>10</td>
<td>Oral 400 µg</td>
<td>0.8 (0-2)</td>
<td>61.5 (48-80)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>SR 400 µg</td>
<td>1.2 (0-3)</td>
<td>60 (44-83)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>SR 800 µg</td>
<td>1.1 (0-3)</td>
<td>58 (45-75)</td>
</tr>
<tr>
<td><strong>IV</strong> Pharmacology</td>
<td>9</td>
<td>Sublingual 400 µg</td>
<td>2.0 (0-3)</td>
<td>69 (46-77)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Vaginal 400 µg</td>
<td>0.5 (0-4)</td>
<td>71.5 (53-78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SR 800 µg</td>
<td>0 (0-5)</td>
<td>65 (48-83)</td>
</tr>
</tbody>
</table>
Patients were recruited from January 2000 to August 2001 for study I, January 2001 to May 2002 for study II, February to August 2004 for study III and March to August 2006 for study IV.

3.1 PATIENTS
Healthy women aged 18-43 years, with a normal intrauterine pregnancy up to 12 weeks of gestation, who requested termination of pregnancy by vacuum aspiration, were recruited for the studies. All women were parous 0 – 5, except for the women in the treatment groups in paper II, all of whom were nulliparous. When the women called the hospital, they received the first information from a midwife and were given an appointment to see both doctor and midwife for further counselling and examination. At the first visit to the hospital, a medical history was taken, including last menstrual period. A gynaecological examination and vaginal ultrasound were performed in most cases to confirm gestational length and intrauterine pregnancy. Blood pressure was measured, blood group was identified, screening for sexually transmitted diseases was performed and an HIV-test was offered according to the clinical routine protocol. The women were also asked about their weight (kg) and length (cm). All women received counselling about contraception and were offered in-depth counselling if required at a follow-up visit.

Inclusion criteria

- Good general health.
- Viable intrauterine pregnancy ≤ 11 weeks and 6 days of gestational length.
- ≥18 years
- Requesting termination of pregnancy with vacuum aspiration
- Able to understand the information provided
- Willing and able to participate in the study

Exclusion criteria

- Asthma (on daily treatment) or any other medical contraindication to prostaglandin therapy (e.g. hypertension, earlier allergic reaction to prostaglandin)
- Any signs of local infection
- Inability to understand the information provided
• Breast-feeding

3.2 STUDY DESIGN
In study I, the women were consecutively allocated to the treatment groups. In study II, the control group consisted of untreated parous women, whereas nulliparous women were randomised to one of the two misoprostol regimens. Studies III and IV were designed as randomised controlled trials.

3.3 DAY OF SURGERY
On the day of admission, all women came to the hospital early in the morning, having fasted overnight. They received the PGE1 analogue, conventional misoprostol or SR misoprostol according to the assigned treatment group (study I) or according to randomisation (studies II-IV). The tablets for oral administration were swallowed with a small amount of water, while the tablets for vaginal treatment were inserted digitally in the vagina by the women themselves. Sublingually administered tablets were allowed to melt under the tongue.

A research nurse supervised the patients during their time at the hospital. Prophylactic pain treatment was given and any additional pain intervention or medication, type and dose were noted. Side-effects, such as pain, shivering, fever, nausea, vomiting or diarrhoea, were noted.

Pulse, blood pressure and temperature were recorded repeatedly and side-effects were monitored.

The vacuum aspiration was performed under general anaesthesia by administration of intravenous propofol (Abbot Scandinavia AB) (1.5 – 2.0 mg/kg) and fentanyl (Alpharma AB, Solna Sweden) (0.5μg/kg). Any bleeding before mechanical dilation was noted. Cervical dilation was measured with Hegar dilators.

3.3.1 Pain relief
At the same time as treatment was being given, prophylactic treatment with pain medication was given to all patients, using a suppository, Citodon® (Astra Zeneca 500
mg paracetamol and 30 mg codeine phosphate hemihydrate), which was given in studies I, II and III, while in study IV, one Voltaren® tablet (Novartis, diclofenac 50 mg) or one Voltaren® suppository (Novartis, diclofenac 50 mg) was given.

### 3.3.2 Misoprostol

Conventional misoprostol, T.Cytotec® 200 µg, Pfizer, for oral administration, was used in all studies. For dose and route of misoprostol administration, please see table I.

In studies III and IV, a new SR or sustained release formula, containing 200 µg misoprostol, was also evaluated. The chemical properties have been described by Chen and colleagues (Chen et al., 2000).

### 3.4 INTRAUTERINE PRESSURE RECORDING (PAPERS I AND III)

In study I, intrauterine contractility was recorded to evaluate the effect on uterine contractility of different routes of misoprostol administration: oral, vaginal and sublingual (2 doses). In study III, the effect on uterine contractility was evaluated after orally administered conventional misoprostol compared with SR misoprostol in two different doses.

In study I, the intrauterine pressure was monitored with a Grass polygraph (Grass Instruments, USA) (Bygdeman and Swahn, 1985, Swahn and Bygdeman 1988, Gemzell et al., 1990, Gemzell-Danielsson et al., 1993, 1999). In study III, a computer polygraph and software (Medtronic, Stockholm, Sweden) were used.

In all patients, the recording started 20-30 minutes before treatment with misoprostol was given so as to allow baseline recording. It then continued for 195-240 minutes after treatment. When the women came to the hospital in the morning, a gynaecological examination was performed by one of the investigators. A sterilised transducer was inserted extra-amniotically through the cervical canal under sterile conditions up to the uterine fundus and then withdrawn so that the tip was positioned 1-2 cm from the fundus. The transducer was secured in position by a suture in the cervix (the oral and the vaginal group in study I) or with tape on the inside of the patient’s thigh. The women remained in supine position throughout the recording procedure. Uterine tonus was measured in mm Hg, the frequency expressed by the number of contractions per 10 minutes. The uterine activity was reported in Montevideo Units (MU)( intensity x frequency) the product of contractions (Caldeyro-Barcia and Poseiro, 1959). The
intensity of each contraction was measured by the rise in pressure in mm Hg. The frequency was expressed by the number of contractions per 10 minutes.

Tonus and contractility per time unit were then calculated manually and expressed as a mean value for 30 minute intervals in paper I, while 10 minute intervals were reported in study III (Caldeyro-Barcia and Poseiro, 1959).

3.5 TISSUE COLLECTION (PAPER II)
Twelve nulliparous women with pregnancies of between 8-12 weeks and requesting termination of pregnancy by vacuum aspiration were treated with 400 µg misoprostol either orally or vaginally. Six women who had previously had at least one delivery served as controls.

Three hours after treatment with misoprostol and immediately prior to the dilation of the cervix, cervical biopsies were taken. The specimens (approximately 4 mm³) were obtained with a concotome from the anterior lip of the cervix under general anaesthesia. Each biopsy was instantaneously frozen in liquid nitrogen and later sectioned.

3.5.1 Immunohistochemistry
To investigate the effect of oral and vaginal administration of misoprostol on the local cervical inflammatory response, immunostaining was used to identify leukocyte common antigen CD 45, monocyte/macrophage cell marker CD 48 and matrix metalloproteinases MMP 8 and 9, as well as their tissue inhibitors (TIMP 1 and TIMP 2). Each biopsy was frozen in liquid nitrogen and later sectioned to 9 µm using a Reichert-Jung Cryocut 1800 (Cambridge Instruments, Nussloch, Germany). The sections were then placed on glass slides and immersed in methanol and acetone. The mounted sections were wrapped in parafilm and stored at -70°C until they were processed for immunohistochemistry. A standard immunohistochemical protocol (avidin-biotin-peroxidase) was used to visualize immunostaining and distribution. The primary and unlabelled antibody which reacts with the tissue antigen was normal horse serum or normal goat serum (Sigma, St Louis, MO, USA). The labelled secondary antibody was a biotinylated horse anti-mouse IgG or goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA, USA), which reacts with the primary antigen-antibody
complex. In order to check for primary antibody specificity, the primary antibody was replaced with non-immunoserum in an equivalent concentration from the same species. Two persons who were unaware of the identity of the slides evaluated the immunohistochemical staining using a Zeiss light microscope at x 200 magnifications. The staining was graded on a scale of absent (-;0%), weak (+; 1-30%), moderate (++; 31-60%), or strong (+++; 61-100%).

3.6 PHARMACOKINETICS AND ANALYSIS OF MISOPROSTOL ACID (PAPER IV)

To evaluate the pharmacokinetics of sublingual, vaginal and oral SR misoprostol up to 12 hours after treatment, we measured the serum levels of the active metabolite, misoprostol acid, or MPA. Thirty women who were parous 0-5 and pregnant between 7-12 weeks of gestation were included. Venous blood samples were taken before treatment, as well as 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours after treatment with misoprostol. The blood samples were immediately stored in a refrigerator for half an hour and centrifuged in a cooled centrifuge. The serum was then withdrawn and stored at minus 20°C. The samples were sent to the department of Pediatrics, Philipps University Marburg, for further analysis. The MPA was measured in serum using a modified liquid chromatography (LC-MS/MS) isotope dilution assay (Watzer et al., 2002).

3.7 STATISTICS

No placebo treatment was used. The women were not blinded to the studies. However, all analyses were performed in a blinded fashion. Investigators were blinded when evaluating the immunohistochemical staining and carrying out the MPA analysis.

**Paper I:** T-tests were used to assess the significance of differences between two means, in uterine tonus and contractility, under assumption of normal distribution. To evaluate the differences in time to increase in tonus and time to maximum tonus between the four groups, one-way analysis of variance (ANOVA) was employed. In the latter case, appropriate comparisons of the means were calculated (Snedecor and Cochran, 1973).
**Paper II:** For statistical analysis of the immunohistochemical staining, ANOVA on ranks (Kruskal-Wallis) test was used to assess the differences between the four groups, followed by Dunn’s test. The inter-observer correlation in immunoscoring was measured by calculating the Spearman correlation coefficient, r_s.

**Paper III:** Repeated measures analysis of variance was performed using the SAS software, testing to assess differences between the three treatment groups. A significant overall test was followed by the corresponding pair-wise comparisons. When overall differences between two treatments were found to be significant, differences at each time-point were tested in Stats Direct, version 2.4.1 (www.statsdirect.com) by unpaired t-tests, in order to locate where in time the most substantial differences appeared. Instead of t-tests, we also performed Mann-Whitney U-test to verify that the conclusions were not dependent on choice of statistical method. The differences between the groups in demographic data, time needed for dilation and surgery and amount of bleeding were tested using the Mann-Whitney U-test. Two-sided P-values were reported.

**Paper IV:** Kruskal–Wallis tests were applied to test the overall difference between the three groups. If the overall difference was significant, the Wilcoxon two-sample test was used to test pair-wise differences. P < 0.05 was considered statistically significant. P-values were not corrected for multiple comparisons; however, all stated differences remained significant (P < 0.05) after applying Bonferroni–Holm correction (Holm, 1979). Statistical analyses were performed by PROC NPAR1WAY (exact tests) in SAS software (SAS Institute Inc., 1997).

The limit of significance was set at P <0.05 in all sub-studies.

For further details about the statistics and statistical methods, please see the separate studies.
4 RESULTS

4.1 UTERINE CONTRACTILITY (PAPERS I AND III)

4.1.1 Tonus

Before treatment with misoprostol, the mean uterine tonus for all groups was low. Independent of dose and route of administration, the first effect of misoprostol treatment was an increase in tonus. This increase was more rapid and more pronounced following oral and sublingual treatment compared to vaginal treatment. The mean time to increase in tonus was 7.8 (±1.7) min for oral treatment, for sublingual 0.2 mg, 10.7 (±2.7) min, for 0.4 mg sublingual misoprostol 11.5 (± 4.6) min, while for vaginal misoprostol, it was 19.4 (±4.6) min, \( P < 0.001 \). The mean time to increase in tonus for oral conventional misoprostol was significantly shorter than following 400 µg SR misoprostol.

The mean time to maximum tonus was significantly shorter for oral, 39.5 (±7.6) min, and sublingual treatment with 0.2 mg, 47.1 (±4.9) min, as well as with 0.4 mg, 51.7 (±14.7) min, compared to vaginal treatment, 62.2 (±13.9) min, \( P < 0.01 \). The mean time to maximum tonus was also shorter for conventional misoprostol compared to both doses of SR misoprostol.

The maximal uterine tonus was significantly higher for sublingual treatment with 0.4 mg misoprostol compared to orally administered misoprostol. For tonus measurements, there was a significant overall difference between conventional oral misoprostol and 400 µg SR misoprostol and between conventional oral and 800 µg SR misoprostol, but not between 400 µg SR and 800 µg SR misoprostol.

4.1.2 Contractions

After 1-2 hours, the tonus began to decrease. Following vaginal and sublingual treatment, regular uterine contractions developed slowly, as they also did after the higher dose of SR. The effect on uterine contractions following conventional oral misoprostol and the lower dose of SR misoprostol varied between no contractions to only weak contractions.

Following vaginal administration, uterine activity continued to increase during the entire recording period, whereas for sublingual administration of 400 µg misoprostol,
uterine contractility tended to decrease during the last hour of recording. At 165 and 195 minutes, the difference was significant when oral treatment was compared with both doses of sublingual misoprostol ($P < 0.05$).

The effect on uterine contractility of SR misoprostol was dose-dependent. Treatment with 800 µg SR misoprostol had a more persistent and stronger effect on uterine contractility compared with 400 µg SR misoprostol. Following 800 µg SR misoprostol, there was also a significantly more pronounced increase in uterine activity when compared to conventional oral misoprostol. In contrast, treatment with 400 µg SR misoprostol resulted in less pronounced activity compared to conventional oral misoprostol.

4.2 THE EFFECT OF MISOPROSTOL ON INFLAMMATORY MEDIATORS AND CERVICAL RIPENING DURING EARLY PREGNANCY (PAPER II)

4.2.1 CD45 (leukocyte common antigen)
CD45 staining was observed in the cytoplasm and detected in all samples. In biopsies from the control group, CD45-immunopositive cells were located in the stroma beneath the surface epithelium. No significant difference was found, but there was a greater expression of subepithelial immunopositive cells and of cellular infiltrates in both treatment groups compared to controls.

4.2.2 CD68 (monocyte/macrophage cell marker)
In the untreated control group, CD68 immunostaining was present in only a few cells in four of six biopsies. The staining was located in the cytoplasm and immunopositive cells were found in the cervical stroma. In the treatment groups, the number of CD68-positive cells in the stroma was higher than in some control biopsies, while the staining was absent in the remaining biopsies.

4.2.3 MMPs and TIMPs
MMP 8-immunopositive cells were located in subepithelial stromal cells and in stromal cells located around the glands. The immunostaining was significantly greater after treatment compared to the controls and the increase was more marked following oral
misoprostol. A few MMP 8-positive cells were located in the stroma in four of the six control biopsies. Staining was also seen in intravascular cells in both treatment groups.

MMP 9 was detected and localised to the stromal compartment, in all samples. In biopsies obtained from the treatment groups, a trend was seen towards a higher number of MMP 9-positive cells in the stroma compared to the controls. In some of the biopsies obtained following treatment, MMP 9-positive cells were found in intravascular spaces.

TIMP 1 and 2 were localised to the surface and glandular epithelium and the stromal compartment in the control biopsies. The expression of TIMP 1 and 2 did not differ between the treatment groups.

4.3 PHARMACOKINETIC PROFILES UP TO 12 H AFTER ADMINISTRATION OF MISOPROSTOL USING DIFFERENT ROUTES AND FORMULATIONS (PAPER IV)

In study IV, we determined the serum concentration of MPA (Cmax), time to peak concentration (Tmax) and AUC up to 720 minutes after administration of misoprostol. Treatment with sublingual misoprostol gave not only the highest serum concentration, but also the earliest peak serum concentration of MPA (see figure 2). The AUC was significantly larger following sublingual administration compared to vaginal and SR misoprostol. Treatment with vaginal and SR misoprostol resulted in serum peak levels of MPA at 1.6-1.7 hours after administration. AUC for the vaginal compared with the SR group was significantly higher. The cumulative concentrations after SR misoprostol continued to rise after 6 hours, which was not the case after sublingual and vaginal misoprostol (see Figure 4).
Figure 4. Cumulative dose of misoprostol (pg/ml) by time curves, showing the result of slow-release administration (A), sublingual administration (B) and vaginal misoprostol (C).
5 DISCUSSION

A better understanding of the influence of misoprostol’s administration route on pharmacokinetic properties, uterine contractility and cervical softening could help to guide and further optimise misoprostol regimens, including its use for several more than the current indications.

One of the aims of this thesis has therefore been to evaluate the effects of different routes of conventional misoprostol administration, including the newly described sublingual route, as well as investigating the new SR formulation, focusing particularly on uterine contractility and the pharmacokinetic profile up 12 hours after treatment during early pregnancy. The effects of misoprostol on inflammatory mediators in the pregnant cervix have also been investigated. It is hoped that the studies will provide important new insights into the optimal use of misoprostol.

5.1 PHARMACOKINETICS

5.1.1 Oral and vaginal administration

The doses and dosage intervals of misoprostol have generally been derived empirically from clinical trials. There have only been a few previous studies comparing the absorption kinetics of the misoprostol tablet designed for oral absorption with vaginal administration of the drug (Zieman et al., 1997; Gemzell-Danielsson et al., 1999, Tang, 2002a, Khan et al., 2004, Meckstroth et al., 2006). Tang and colleagues were the only group to explore the sublingual route of administration, although Meckstroth recently investigated the newly described buccal route. In addition, previous studies have only investigated the effect of misoprostol over the period up to 6 hours following a single dose, while we continued to measure MPA for up to 12 hours after administration.

In the two earlier pharmacokinetic studies (Zieman et al., 1997, Gemzell-Danielsson et al., 1999) comparing the pharmacokinetics of vaginal and oral administration of misoprostol, the peak plasma concentration of MPA was higher and was also reached earlier after oral administration, although the detectable plasma concentrations lasted longer after vaginal administration. These results are similar to those of study IV and to those of Tang and Meckstroth. The peak plasma concentrations and the time taken to reach maximum concentration in our study were also comparable to those of previous
studies. In one of these studies (Zieman et al., 1997), the systemic bioavailability of vaginally administered misoprostol (AUC \(_{360}\)) was 3 times higher than that of orally administered misoprostol. A direct vagina-to-uterus transport route was recently described for progesterone absorption (Cicinelli et al., 2000). A similar mechanism may exist for misoprostol absorption and could also explain the more favourable clinical effects of vaginal administration. However, the study by Meckstroth of the buccal and vaginal routes indicates that this may not be the only explanation. Interestingly, the effects of the routes studied by Meckstroth on uterine contractility were similar, while the AUC for buccal misoprostol was only 50% of the AUC for vaginal misoprostol (see below).

### 5.1.2 Sublingual administration

Our findings are consistent with those of Tang and colleagues in that we found that sublingual administration leads to the highest plasma concentration when compared to all other routes of administration. See figure 5. Systemic bioavailability, as measured by the AUC, is also highest following sublingual misoprostol administration.

Figure 5. Mean concentrations of MPA over time. A: slow release administration, B: sublingual administration and C: vaginal administration.
This may be explained by the absence of first-pass metabolism in the liver after sublingual administration. Other contributing factors may be the good blood supply under the tongue and the relatively neutral pH in the buccal cavity. By contrast, it is interesting to note that in the recent study by Meckstroth et al. (Meckstroth et al., 2006), buccal administration resulted in lower plasma levels than vaginal administration, and these levels were only slightly higher than for rectal administration.

In study IV, we found that the duration of elevated MPA serum levels following sublingual misoprostol was shorter compared to vaginal administration see figure 5. A tendency towards shorter duration of elevated serum levels has also been observed previously (Tang et al., 2002a). This corresponds well with our observations on uterine contractility (study I) and clinical efficacy (see below). The vaginal route thus seems to produce a longer-lasting elevation of serum MPA compared to the oral and sublingual routes.

5.1.3 SR Misoprostol

Despite being shown to be the most effective route, vaginal administration of misoprostol may not be acceptable to all women. A review of women’s attitudes towards medical abortion has shown that women prefer oral administration of misoprostol, a shorter interval until complete abortion is diagnosed and fewer visits to the clinic (Winikoff, 1995). Other drawbacks of the vaginal route may also include fear of infection.

This thesis therefore also studied the new orally administered SR formulation of misoprostol, which serves as an alternative to vaginal administration. A slow-release or sustained-release form would in theory also allow longer duration of elevated serum
MPA levels and consequent stimulation of the myometrium. Only two previous studies have investigated the effect of SR prostaglandin analogues on induced abortion. One by Bygdeman et al. (1984) used a slow release vaginal prostaglandin analogue in second trimester abortion. Two years later, another vaginal prostaglandin released over a 24-hour period was studied for early abortion (up to 56 days of gestation) (Cameron and Baird, 1986). Both regimens were used without pre-treatment with mifepristone.

If an oral slow-release preparation of misoprostol is effective within a reasonable period of time, it is likely to be more acceptable to women than vaginal administration, and may even be preferred to the sublingual or buccal routes. A slower release of misoprostol following mifepristone may also result in a lower total dose of prostaglandin being needed, and thus in fewer side-effects compared to oral and sublingual administration. A slow release form of misoprostol was recently synthesised and its chemical properties described (Chen et al., 2000). In vitro data on SR misoprostol showed that ≤40% of misoprostol is released after 4 hours in a water solution with a pH of 1.2 (Chen et al., 2000). Pharmacokinetic studies (Fiala et al., 2005) indicate that intake of this form of SR misoprostol may result in a longer duration of elevated plasma concentrations of active misoprostol free acid. This was confirmed in study IV. Assuming that it is the duration of elevated serum MPA levels rather than their height that is crucial to clinical effect and efficacy, SR misoprostol may allow the development of an orally administered form of misoprostol that can be given as a single dose together with mifepristone. This would also reduce the number of visits to the clinic.

The doses used in studies III and IV were the standard dose of 400 µg conventional misoprostol. This was then compared to two doses of SR misoprostol, 400 and 800 µg. The dose of SR misoprostol was based on previous in vitro and in vivo data (Chen et al., 2000, Fiala et al., 2005). The AUC520 was significantly lower following SR misoprostol compared to the sublingual and vaginal routes (study IV). Interestingly, however, it was noted that SR misoprostol resulted in a longer lasting elevation of serum levels of MPA compared to the other routes. Starting 5-6 hours after misoprostol administration, serum levels of MPA following 800 µg SR misoprostol also exceeded those in the other groups. Furthermore, the cumulative AUC for the sublingual and vaginal routes did not continue to rise beyond 6 hours after treatment.
5.1.4 Pharmacokinetics in human breast milk

Nursing mothers may need misoprostol for a variety of reasons. There have only been a few studies of the pharmacokinetics of oral misoprostol in breast milk. In these, misoprostol was detected in breast milk within 30 minutes of administration, while peak concentration was attained after 1 hour, which is slightly longer than it took to achieve the peak plasma level (30 minutes). The level in breast milk then drops rapidly to become undetectable 4-5 hours after ingestion. The misoprostol acid level in milk is only one third of that in plasma (Vogel et al., 2004, Abdel-Aleem et al., 2003). So far, there is no data on the pharmacokinetics of misoprostol in breast milk when other routes of administration are used (ongoing study). However, it is to be expected that breast milk concentrations after vaginal administration will be lower than after oral administration, although they may last longer. Meanwhile, levels following sublingual administration may be both higher and longer-lasting. Although effects on the infant are probably negligible, it may be prudent, following vaginal or sublingual administration of misoprostol, to discard the mother’s breast milk for a period of 6 hours following the last dose so as to preclude any possibility of inducing colic or diarrhoea in the infant.

5.2 UTERINE CONTRACTILITY

The higher efficacy of vaginal administration can most probably be explained by the higher plasma levels, which also remain elevated for a longer period of time than after oral administration, resulting in a more intense and longer lasting stimulation of uterine contractility (Zieman et al., 1997, Gemzell-Danielsson et al., 1999, study I, see figure 6). The aim of the first paper of this thesis was to evaluate whether the then newly described sublingual route of administration could be as effective as the vaginal route in stimulating uterine contractions.

Typically, the effect of a single oral dose of misoprostol is an increase in tonus (Gemzell-Danielsson et al., 1999, Norman et al., 1991). In the study by Norman et al., (1991), doses ranging from 200 to 600 µg of oral misoprostol resulted in a dose-dependent increase in uterine tonus. Irrespective of dose, however, the effect on contractions was the same – the response was weak and there was no development of regular contractions.
Figure 6. Uterine activity in MU after 0.4 mg vaginal and oral administration and 0.2 and 0.4 mg sublingual administration of misoprostol.

The effect of vaginal administration of misoprostol is initially similar to that of oral administration with an increase in uterine tonus. After 1-2 hours, however, regular uterine contractions appear (study I). Similar results have been reported earlier (Norman et al 1991, Gemzell-Danielsson et al., 1999). These uterine contractions last for a minimum of 4-5 hours after the start of the treatment (study I, study III, Meckstroth et al., 2006). The duration of contractions following a single vaginal dose of misoprostol is not known. At the end of the recording, 4 hours after administration of misoprostol, contractions still seemed to be increasing, see figure 6 (study I).

The time from start of treatment to start of effect (increase in tonus) was the same following both oral and sublingual administration (between 7.8 and 11.5 min), although it was significantly longer following vaginal administration. The time to maximum tonus elevation was also the same following oral and sublingual administration, and significantly shorter than following vaginal administration of misoprostol. The increase in tonus was most pronounced following sublingual administration of misoprostol and significantly higher than that following oral administration, which is in accordance with the pharmacokinetic results (study IV, Tang et al., 2002a).

In contrast to the effect on uterine tonus, the effect of sublingual administration on uterine contractions was similar to that of vaginal treatment, as shown in Fig 2. Compared with vaginal administration, sublingual misoprostol resulted in a faster onset
of contractions, although the effect seemed to be shorter lasting and uterine contractions tended to decrease at 2.5 hour.

A shorter lasting effect for the sublingual route is in agreement with clinical data. When misoprostol was used alone to induce second-trimester abortion, the effect seemed to be more pronounced following vaginal administration as compared with sublingual administration (Tang et al., 2004). In a large multicentre trial conducted by WHO on misoprostol regimens for termination of pregnancy up to 63 days of gestation, women were randomised to 800 µg misoprostol administered sublingually or vaginally using a 3 or 12 hours interval (von Hertzen et al., 2007). The complete abortion rates at the two-week follow-up showed a higher efficacy for the sublingual group with a 3 hours interval (84%) compared to the group with a 12 hours interval (78%). For the vaginal group, the rates were 85 and 83% respectively. These results are consistent with our data on pharmacokinetics and uterine activity.

In study III, we also investigated the effect of orally administered SR misoprostol on uterine contractility. The time interval between administration of SR misoprostol and the initial increase in uterine tonus was significantly longer than following conventional oral misoprostol. SR misoprostol had a less pronounced effect on uterine tonus compared to oral administration of conventional misoprostol, and its effect was similar to that of vaginal administration of conventional misoprostol. The findings in the present study show that the effects of orally administered SR misoprostol on uterine tonus and contractility were similar to those of vaginally administered conventional misoprostol (Gemzell-Danielsson et al., 1999, study I).

The uterine effect of buccal and rectal administration was recently studied by Meckstroth et al. (2006). The study showed that the uterine tonus and contractility pattern seen after buccal administration is very similar to that seen after vaginal administration, although the AUC was 2 times smaller. Rectal administration, which has the lowest AUC, also displays the lowest uterine activity in terms of tonus and contractility.

Based on the results of studies I, III and IV, as well as our previous study (Fiala et al., 2005), it appears that a certain plasma level threshold, which has yet to be identified, must be reached before uterine contractions can be initiated. Following administration
of the lowest dose of 400 µg SR misoprostol, MPA reached very low serum levels and had only a minor effect on uterine contractility, in contrast to the higher dose of 800 µg. Furthermore, the same doses of buccal and vaginal misoprostol result in different maximum serum levels and AUC, although they have a similar effect on uterine contractility (Meckstroth et al., 2006). The duration of elevation of serum levels above this potential and as yet unknown threshold (that is the duration of myometrial stimulation) seems to be more important than either the absolute level of MPA (level of increase in plasma MPA), Tmax or the AUC. The administration route would thus appear to be crucial to the development of uterine contractions, while the dose has a greater effect on the increase in tonus (level of increase in plasma MPA, Tmax).

Whether the pharmacokinetic data and the effects on uterine contractility of SR misoprostol correspond to high clinical efficacy remains to be shown. To date, there is no SR misoprostol dosage form available on the market. The optimal dose of the SR formula needs further assessment and should be followed by assessment of feasibility, efficacy and acceptability in clinical studies, where it should be compared to the standard misoprostol formula.

5.3 EFFECTS OF MISOPROSTOL ON CERVICAL RIPENING

In addition to uterine contraction, the softening effect of misoprostol on the cervix is an important part of the clinical picture.

The biochemical events that have been implicated in cervical ripening are: (1) a decrease in total collagen content, (2) an increase in collagen solubility, and (3) an increase in collagenolytic activity. Existing knowledge about cervical ripening is mainly based on research into the physiological ripening of the cervix or following pharmacological intervention at term pregnancy. Prostaglandins have been implicated and probably play an important role in cervical ripening. It has been shown that various prostaglandin analogues could decrease the hydroxyproline content of the pregnant cervix. (Rath et al., 1982). The histochemical changes in the pregnant cervix after misoprostol administration have been studied using electron microscopy and proline uptake assay. The mean proline incorporation per µg protein and collagen density, estimated by light intensity, was significantly less than in the control group. The diameter of the collagen fibres was also smaller in the misoprostol group, although the
difference was not statistically significant. This indicates that the action of misoprostol appears to be mainly on the connective tissue stroma, with evidence of disintegration and dissolution of collagen. (El Refaey et al., 1994)

In study II, it was shown that the cervical priming effect of misoprostol may be mediated through an inflammatory response. It was demonstrated that the administration of misoprostol mimics the cervical ripening at term pregnancy via a possible influx and activation of inflammatory cells, increasing matrix metalloproteinases and thereby leading to the degradation of collagen and ultimately to cervical softening. This supports the hypothesis that prostaglandins increase vasopermeability and facilitate the influx of inflammatory cells, such as neutrophils and macrophages, into the cervix during the first trimester.

Interestingly, we observed no dramatic differences in the effect on inflammatory cells or MMP 8 or MMP 9 depending on the route of administration of misoprostol, despite the described differences in pharmacokinetic properties. These differences have been shown to lead to pronounced differences in uterine contractility, and this is believed to explain the observed differences in the clinical efficacy of misoprostol when used for medical abortion. In contrast, the importance of the administration route seems to be less pronounced for misoprostol when used to induce cervical ripening. Vaginal and oral misoprostol, 400 μg 3 hours before surgical evacuation, have been proven to be equally effective for cervical priming (Ngai et al., 1999). More recent studies have however shown that the vaginal route is superior to the oral route, although the difference is small. In line with this, we were able to show that sublingual misoprostol was slightly more effective in terms of cervical priming than oral administration (Aronsson et al., 2004). It has also been shown that sublingual misoprostol was just as effective as vaginal misoprostol when used for cervical priming. (Hamoda et al., 2004a, 2004b, Saxena et al., 2004).

Sublingual misoprostol has a number of advantages, in that there is no need for an additional vaginal examination for application of the drug, which may be an important factor in a busy day-surgery setting (when the misoprostol is not applied by the women themselves). It also avoids any oral intake of water, which may be advantageous if general anaesthesia is required for surgical evacuation.
In study II, oral misoprostol seemed to have a more pronounced effect on MMP 8. This could be due to a faster onset of changes in the connective tissue. Cervical dilation is the result not only of tissue remodelling, but also of the effect of uterine contractions. This may lead to a similar net effect for the oral and vaginal routes. Based on the pharmacokinetic effect of sublingual misoprostol and its effect on uterine contractility, it is likely that this route will result in the most rapid cervical effect. This is currently being evaluated in an ongoing study.

In closing, it should also be pointed out that most of the studies of uterine contractility and cervical softening after misoprostol administration (including study II) have been performed on pregnant women. There is, however, evidence to suggest that the same effects also occur in the non-pregnant uterus. Some non-pregnant women experience uterine cramps after misoprostol administration and misoprostol has also been shown to have a cervical priming effect even in the non-pregnant uterus (Sääv et al., 2007).

5.4 SIDE-EFFECTS AND ACCEPTABILITY

Misoprostol is licensed for oral use for the treatment and prevention of peptic ulcers or for medical abortion together with mifepristone. The vaginal and sublingual routes are not licensed ways of administering the drug. It is thus extremely important to look at the side-effects and safety of these routes of misoprostol administration. Since work on this thesis began, there have been a large number of reports concerning the use of sublingual misoprostol for medical abortion, cervical priming in pregnant and non-pregnant women, management of silent miscarriage, labour induction and management of post-partum haemorrhage (Wagaarachchi et al, 2001; Aronsson et al., 2004; Hamoda et al., 2003 and 2004; Saxena et al., 2003 and 2004; Bisharah et al., 2003; Shetty et al., 2002a and b). Many studies have also been added to the already large number of studies of the vaginal route. There have been no reports of serious complications or adverse events associated with misoprostol use. This may be due to careful selection of subjects and exclusion of women with medical diseases from the studies, although it is more likely to reflect the safety of the drug itself.

Based on the pharmacokinetic findings and the results of clinical trials, it is possible to speculate as to whether the incidence of side-effects is associated with high serum levels of MPA. Conventional oral misoprostol results in a rapid and short-lived serum
peak after intake. This is even more pronounced for the sublingual route (Tang et al.,
2002a, Hamoda et al., 2004). Oral and particularly sublingual misoprostol also lead to
the highest reported incidence of side-effects (El-Refaey et al., 1995, Hamoda et al,
2004). It has previously been reported that women prefer oral to vaginal misoprostol
administration for medical abortion (Ho et al., 1997). However, the acceptability of
sublingual misoprostol for cervical priming before surgical abortion has also been
compared to that of vaginal misoprostol (Hamoda et al., 2004), and this study found no
difference between the two routes in terms of acceptability to patients. On the other
hand, most of the clinical staff recommended the sublingual route, since it precludes the
need for a vaginal examination, thereby shortening the admission time for each patient.
One possible alternative might also be to allow women to administer the tablets
themselves.

5.5 METHODOLOGICAL CONSIDERATIONS
The present studies were too small to allow for proper comparison of the side-effects
of misoprostol. In addition, the women were not blinded to the route of
administration, and this may have introduced an element of bias regarding
experienced side-effects.

It is currently not known whether removal of the vaginal tablet remnant affects
absorption of misoprostol. Nor is it clear at which point in time absorption may be
regarded as complete. In the present pharmacokinetic study, the time between
insertion of the tablets and vacuum aspiration was kept constant at 3 hours. No
vaginal cleansing was performed prior to the vacuum aspiration, in accordance with
clinical routines. However, it cannot be excluded that the vaginal intervention has at
least a theoretical effect on absorption after 3 hours.

In study IV, we only measured serum levels of misoprostol acid after the
administration of a single dose of misoprostol. The effect of multiple dosing is
unknown. It is especially difficult to predict in the case of vaginal administration,
given its slow and less predictable absorption and elimination.

The present studies only involved women in the first trimester of pregnancy and
cautions needed when results are extrapolated to cases of more advanced gestation.
This is especially true when the use of sublingual misoprostol is extended to the third trimester for labour induction and cervical priming. Given the current data on the pharmacokinetics of oral, vaginal and sublingual misoprostol and their effect on uterine contractility, the dose of sublingual misoprostol may need to be further reduced for indications such as cervical priming. For SR misoprostol, the dose may instead need to be increased. More data on the safety of SR misoprostol should also be available before its clinical application is considered.

**Clinical applications and recommended regimens**, TABLE II. Following our initial study of the effect on uterine contractility of oral, vaginal and sublingual administration of misoprostol, as well as the pharmacokinetic study by Tang et al. (2002), several clinical trials comparing these routes for various indications have been performed. For some of the indications, the number of studies is small and the evidence on which the recommended regimen is based is weak, while others are now well established. An overview of the current recommendations for misoprostol regimens for a number of indications in pregnant women is provided in TABLE II. The recommendations are the result of a recent workshop supported by WHO, Ipas and Gynuity. More details can be found at [www.misoprostol.org](http://www.misoprostol.org).
6 CONCLUSIONS

Taken together, the results of this thesis indicate that while peak MPA serum levels seem to correlate with uterine tonus and, when considered together with clinical data, with the incidence of side-effects as well. In contrast the duration of these elevated serum levels that seems to correlate best with the effect on uterine contractility. A certain threshold level of MPA is needed to induce contractions, but once this level is reached, the duration of elevated MPA above this critical threshold seems to be the most important parameter. Prolonged stimulation of the uterus seems to be needed to overcome the progesterone block, which normally prevents uterine contractions (Csapo, 1956). It is thus possible that the prolonged duration of stimulation caused by elevated plasma levels following vaginal, sublingual and buccal conventional misoprostol or oral SR misoprostol is able to overcome this block, which otherwise prevents regular activity in the myometrium.

While the effect of misoprostol on uterine contractility depends largely on the route of administration, the effect on cervical ripening is less dependent on the administration route. Both oral and vaginal administration of misoprostol result in a similar inflammatory-type response in the cervical tissue.

The findings regarding the pharmacokinetic properties and the effect on uterine contractility of different routes of administration will hopefully help clinicians design optimal regimens for various clinical applications. Sublingual misoprostol, which has the shortest $T_{\text{max}}$, is perhaps most useful for clinical applications that require a fast onset of clinical action, such as post-partum haemorrhage or cervical priming before surgical evacuation, or indeed early medical abortion using a regimen that combines mifepristone and misoprostol. Vaginal misoprostol, on the other hand, which has a high bioavailability and sustained serum levels, is probably most useful for indications that require longer for the manifestation of clinical effects, such as medical abortion beyond 49 to 56 days, or a misoprostol-only regimen for first-trimester medical abortion, or the management of silent miscarriage, and especially when repeated doses are required. The plasma level may accumulate with repeated doses and the plasma level may be higher in the vaginal group compared to the sublingual group. In addition, the absorption kinetics can also explain why some
routes of administration are associated with a higher incidence of side-effects. Sublingual administration, which produces the highest $C_{\text{max}}$, is thus associated with the highest incidence of side-effects when compared to other routes of administration.

The results so far indicate that SR oral misoprostol could be an effective alternative to other routes, leading to elevated serum levels up to at least 12 h after administration. Whether the pharmacokinetic data and the effects on uterine contractility correspond to high clinical efficacy remains to be shown. The optimal dose of the SR formula needs further assessment and should be evaluated by performing clinical studies of feasibility, efficacy and acceptability in which comparisons are made with the standard misoprostol formulation.
7 ACKNOWLEDGEMENTS

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Any omissions from this list are entirely unintentional. Should anyone feel left out, this is entirely the responsibility of the author and should be ascribed to her poor memory and to the pressures of time!

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www.misoprostol.org

www.developmentgoals.org/Maternal_Health.htm


**TABLE II. Misoprostol Regimens for Reproductive Health**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Dose and route of misoprostol</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced abortion (0-12 weeks)</td>
<td>800μg vaginally 6-hrly x 3</td>
<td>Only if mifepristone is not available</td>
</tr>
<tr>
<td>Missed abortion (0-12 weeks)</td>
<td>800μg vaginally 3-hrly x 2 alt. 600μg sublingually x 2</td>
<td>Leave to work for 1-2 weeks (unless heavy bleeding or infection)</td>
</tr>
<tr>
<td>Incomplete abortion (0-12 weeks)</td>
<td>600μg orally alt 400μg sublingually stat.</td>
<td>Leave to work for 2 weeks (unless heavy bleeding or infection).</td>
</tr>
<tr>
<td>Induced abortion (13-22 weeks)</td>
<td>400μg vaginally 3-hrly (max. 5 doses) 7.1.1</td>
<td>Use 200μg in women with caesarean scar. Only if mifepristone is not available.</td>
</tr>
<tr>
<td>Intrauterine fetal death (&gt;24 weeks)</td>
<td>13-17 wks: 200μg 6-hrly vag. 18-26 wks: 100μg 6-hrly vag. 27-43 wks: 25-50μg 4-hrly vag.</td>
<td>Reduce doses in women with previous caesarean section</td>
</tr>
<tr>
<td>Induction of labour (live fetus &gt;24 weeks)</td>
<td>vaginal 25μg 4-hrly (max x 6) oral 50μg 4-hrly (max. x 6) oral solution 20μg 2-hrly (max. x 12)</td>
<td>Do not use if previous caesarean section.</td>
</tr>
<tr>
<td>PPH prophylaxis</td>
<td>600μg orally or sublingually stat.</td>
<td>Not as effective as oxytocin or ergometrine. Exclude second twin before administration. Do not repeat within 2 hours.</td>
</tr>
<tr>
<td>PPH treatment</td>
<td>600μg orally or sublingually stat.</td>
<td>Limited evidence for benefit – use conventional oxytocics first</td>
</tr>
<tr>
<td>Cervical ripening prior to instrumentation</td>
<td>400μg vaginally or sublingually 3hrs before procedure</td>
<td>Use for insertion of intrauterine device, surgical termination of pregnancy, dilatation and curettage, hysteroscopy</td>
</tr>
</tbody>
</table>

stat = single dose taken immediately, PPH = postpartum haemorrhage. For more details see www.misoprostol.org