NITRIC OXIDE DONORS IN LABOR MANAGEMENT

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Göteborg 2007

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2007

Cover:

“Fetus” after **Soranus of Ephesus** (98 - 138 A.D.)

Illustrated in*: La commare o riccoglitrice dell'eccmo. sr. Scipion Mercurii*, Venice 1601.

Original size: 19.5 x 14 cm.

The University Library, Lund, Sweden

ISBN-978-91-628-7113-0

In memory of my dearest sister,

Helena

ABSTRACT

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Background: Nitric oxide (NO), a free radical with ultra-short half-life synthesized from L-arginine by the enzyme nitric oxide synthase (NOS), is in the human involved in many physiological and pathophysiological processes, including different reproductive processes. During pregnancy NO is produced endogenously in the human uterine cervix and placenta. Different effects of NO can be studied by administering NO donors.
Aims and methods: The aims of the thesis were to perform experimental and clinical studies on late pregnant women to examine the effects of NO donors on cervical ripening and labor induction and to evaluate possible effects of NO on the management of retained placenta. In Paper I the effects of the NO donor isosorbide mononitrate (IMN) administered vaginally were examined by measuring cervical distensibility using a cervical tonometer. In addition, maternal and fetal side effects of the medication were evaluated. In Paper II the efficiency of vaginally adminstered IMN to induce cervical ripening and labor induction in an outpatient setting was examined. The safety and side effects of this treatment were also evaluated.
In Paper III cervical biopsies were obtained prior to elective caesarean section following vaginal administration of IMN. Western blotting was used for semiquantitative measurements of cyclooxygenase-1 and -2 (COX-1 and COX-2), enzymes involved in prostaglandin synthesis as well as cervical ripening. Immunohistochemistry was used for localizing these enzymes within the cervical tissue. Paper IV describes the efficiency of sequential treatment with oxytocin and nitroglycerin for management of retained placenta.
Results: Treatment with IMN resulted in a significantly increased cervical distensibility. Headache and palpitations of little to moderate intensity were common maternal side effects. A significant decrease in maternal blood pressure and increase in pulse rate were registered. However, the effects were modest and not of clinical importance. No fetal side effects were observed according to CTG, Doppler ultrasound, Apgar score and umbilical pH. Vaginally administered IMN seemed to be effective in promoting labor induction within 24 hours (22 patients compared to 8 in the placebo group). There were no differences in fetal side effects and the rate of caesarean section between women treated with IMN and women in the placebo group. Semiquantitative measurements by immunoblotting revealed increased expression of COX-2, but not of COX-1 in the NO donor group compared to placebo group. Immunohistchemistry showed similar localizations of COX-1 and COX-2 in the two groups. Sublingually administered nitroglycerin in combination with oxytocin resulted in successfully delivered placenta among all 12 women compared to only 1 woman in the control group. Maternal hemodynamic effects were mild to moderate. Blood loss was increased in women who needed manual removal of placenta.
Conclusion: The data suggest that IMN administered vaginally at term and postterm for cervical ripening and labor induction seems to be effective and safe. Combined treatment with oxytocin and nitroglycerin seems to promote detachment of retained placenta.
Key Words: cervical ripening, labor induction, nitric oxide donor, retained placenta.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

Paper I.

Ekerhovd E, Bullarbo M, Andersch B and Norström A.

Vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening at term: A randomized controlled study.

Am J Obstet Gynecol 2003; 189: 1692-1697

Paper II.

Bullarbo M, Eriksson-Orrskog M, Andersch B, Granström L, Norström A and Ekerhovd E.

Outpatient vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening and labor induction postterm: A randomized controlled study.

Am J Obstet Gynecol 2007; 196: 50.e1-e5

Paper III.

Bullarbo M, Norström A, Andersch B and Ekerhovd E.

Isosorbide mononitrate induces increased cervical expression of cyclooxygenase-2, but not of cyclooxygenase-1, at term.

Eur J Obstet Gynecol 2007; 130: 160-164

Paper IV.

Bullarbo M, Tjugum J and Ekerhovd E.

Sublingual nitroglycerin for management of retained placenta.

Int J Gynecol Obstet 2005; 91: 228-232

ABBREVIATIONS

AP-1	activator protein-1
BS	Bishop score
Ca	calcium
cGMP	cyclic guanosine monophosphate
CI	confidence interval
COX	cyclooxygenase
CRH	corticotrophin releasing hormone
CTG	cardiotocography
CS	caesarean section
EDRF	endothelium dependent relaxing factor
eNOS	endothelial nitric oxide synthase
GTN	glyceryl trinitrate
hACTH	human adrenocorticotropin hormone
hCG	human chorionic gonadotropin
hCS	human chorionic somatomammotropin
hCT	human chorionic thyrotropin
hPL	human placental lactogen
IgG	immunoglobulin G
IL	interleukin
IMN	isosorbide mononitrate
iNOS	inducible nitric oxide synthase
IHC	immunohistochemistry
IU	international unit
LHRH	luteinizing hormone releasing hormone
min	minutes
mm	millimeters
MMP	matrix metalloproteinase
MROP	manual removal of placenta
N	number of women
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NFκB	nuclear factor kappa B
NG	nitroglycerin

NICU	neonatal intensive care unit
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
NS	not significant
PBS	phosphate-buffered saline
PG	prostaglandin
PGDH	prostaglandin dehydrogenase
PGE_2	prostaglandin E_2
PGI_2	prostaglandin I_2
PI	pulsatility index
PPH	postpartum hemorrhage
PTL	preterm labour
RCOG	Royal College of Obstetricians and Gynaecologists
RI	resistance index
RT-PCR	reverse transcriptase polymerase chain reaction
SD	standard deviation
SNP	sodium nitroprusside
TNF_α	tumor necrosis factor alpha
VAS	visual analogue scale
WHO	World Health Organization

CONTENTS

INTRODUCTION

Labor is one of the most exciting events in life. Most often the process follows a normal pattern and results in delivery of a healthy child. Many steps are normally involved from spontaneous start of labor at term to partus followed by delivery of placenta. This thesis is focused on problems concerning onset of labor and delivery of placenta as well as possible effects of nitric oxide (NO) donors for management of problems related to these stages of labor.

There may be situations when induction of labor is necessary, as it could be hazardous to wait for spontaneous onset of labor, e.g. preeclampsia, severe hypertension, postterm pregnancy, diabetes mellitus, multiple pregnancy, intrauterine growth retardation or oligohydramniosis. If the cervix is ripe the most common methods of labor induction are amniotomy and/or oxytocin infusion. In cases the cervix is unripe it is necessary to use a local medical or mechanical treatment to achieve cervical ripening. The most common medical agent is prostaglandin (PG) administered vaginally or intracervically. However, PGs may be associated with side effects. Thus, in approximately 5% of treated women uterine hyperstimulation is registered. Furthermore, the treatment is not always effective and often it has to be repeated. In these cases the risk of failed induction is increased.

It is desirable to find a treatment that is more effective and not associated with hyperstimulation. Nitric oxide, a free radical with extremely short half-life, is endogenously produced in the human uterine cervix (Tschugguel *et al*, 1999) and has been shown to be up-regulated at term pregnancy (Ledingham *et al,* 2000; Väisänen-Tommiska *et al,* 2003). Thus, NO is thought to play a role in cervical ripening (Chwalisz, 1997; 1998) and several studies on vaginally administered NO donors have shown ripening effects of the treatment (Thomson *et al,* 1997; Facchinetti *et al*, 2000). The present study aims to give some aspects on the role of NO donors on cervical ripening and labor induction.

The third stage is defined as the interval from birth of the infant to delivery of the placenta. It normally lasts for approximately 6 minutes. In about 3% of all deliveries the placenta does not detach spontaneously. In such cases, a variety of medical treatments to avoid manual extraction in regional or general anaesthesia may be applied. Oxytocin as well as PGs, both

promoting uterine contractions, are the most common agents for management of retained placenta. However, administration of these agents is not always effective. Treatment with NO donors, such as nitroglycerin, has in some trials shown to have effect on management of retained placenta. Few studies have been carried out in order to identify the mechanisms of retained placenta. Herman and co-workers, carrying out ultrasonographic examinations, concluded that it is not until the retroplacental part of the uterine body contracts that the placenta detaches. This procedure seems to be independent of oxytocin administration (Herman *et al*, 1993). On the other hand, NO is upregulated in the amniotic membranes during labor (Ticconi *et al*, 2004), a mechanism that could play a role in the third stage of labor. In this thesis the possible effect of nitroglycerin for management of retained placenta when administered sublingually in combination with oxytocin was investigated.

BACKGROUND-review of the literature

NITRIC OXIDE

The nitric oxide molecule

Nitric oxide (NO) is one of the 10 smallest molecules in nature, detected by Joseph Priestly more than 200 years ago. The chemical formula is NO. It is polar, heavier than air and paramagnetic due to an unpaired electron. Such substances are usually called free radicals. Its melting point is at -164ºC while its boiling point is at -152ºC. It is colorless, highly reactive with an ultrashort half-life of 4-8 seconds.

Figure 1. The nitric oxide molecule, also called nitrogen monoxide.

Nitric oxide has for years been known as a nuisance. It is a poisonous gas with adverse effects on the environment. Produced in internal combustion engines and electrical generating stations NO has been implicated in depletion of the ozone layer, formation of photochemical smog, and acid rain. In 1916 Mitchell and co-workers observed that oxides of nitrogen were produced by mammals. Twelve years later, in 1928, Tannenbaum and co-workers confirmed that nitrite and nitrate are formed by endogenous synthesis in the human intestine. Despite these discoveries NO was for many decades commonly regarded by chemists and environmentalists as a very toxic molecule with only negative effects. In 1978 Murad and co-workers demonstrated that the vasodilatory effect of nitroglycerin and several other nitrates was mediated by NO. Furthermore, in 1980 Furchgott and Zawadzki provided evidence that acetylcholine-induced relaxation of vascular rings was mediated by a non-prostanoid, endothelium dependent relaxing factor (EDRF). Five years later, Stuehr and Marletta discovered that macrophages synthesize nitrite/nitrate. Independently and simultaneously,

Palmer and co-workers and Ignarro and co-workers in 1987 provided evidence that EDRF is NO. Since then the research on the biology and functions of NO has substantially increased. In 1998 Furchgott, Ignarro and Murad were rewarded with the Nobel Prize in Medicine for their discoveries regarding NO. The discovery that NO can penetrate cell membranes and regulate the function of other cells was an entirely new principle for signalling in the human organism.

Synthesis of nitric oxide

Nitric oxide is synthesized intracellularily from the amino acid L-arginine (Palmer *et al,* 1988). This reaction is catalyzed by a family of enzymes, known as nitric oxide synthases (NOS) (Palmer and Moncada, 1989). These enzymes are structurally complex haemproteins with properties similar to cytochrome P-450 reductase (Marletta, 1994). NOS require a number of cofactors, such as tightly bound flavoproteins and tetrahydrobiopterin, while the production of NO itself requires the co-substrate oxygen and nicotinamide adenine dinucleotide phosphate (NAPDH) (Zapol *et al*, 1994). The reaction goes through a two-step conversion of L-arginine to NO and L-citrulline via N^{ω}-hydroxy-L-arginine as an intermediate product (Aktan, 2004) (Figure 2).

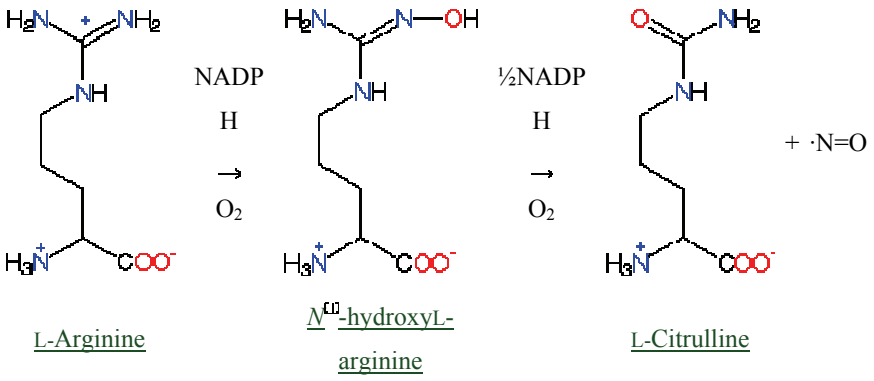


Figure 2. Biosynthesis of NO from L-arginine through a two-step conversion to L-citrulline.

Three major isoforms of NOS have been identified. These isoforms are encoded by separate genes, located on chromosomes 7, 12 and 17 (Knowles *et al,* 1989; Lamas *et al,* 1992; Löwenstein *et al,* 1992; Knowles and Moncada, 1994). They are neuronal NOS (nNOS, type

1) and endothelial NOS (eNOS, type 3), which both are expressed constitutively. In addition, a macrophage-derived form, inducible NOS (iNOS, type 2) that is induced by endotoxin and inflammatory mediators, such as cytokines and lipopolysaccharides has been identified (Liu, 1993; Morris and Billiar, 1994). Both nNOS and eNOS require calcium/calmodulin for activation (Griffith and Stuehr, 1995; Snyder, 1995). Although iNOS is said to be calmodulin independent, calmodulin is tightly bound to each subunit of the isoform (Cho *et al*, 1992). This classification might be a simplification since other isoforms of NOS also have been described. Thus, reports of calcium-dependent isoforms that are inducible as well as calcium-independent isoforms that are constitutive have been published (Radomski *et al,* 1991; Palmer *et al,* 1992). Inducible NOS was initially cloned from activated macrophages (Xie *et al,* 1992) and later described in human macrophages (Moilanen *et al,* 1997; Aktan, 2004) (Table 1). Nitric oxide production under the influence of iNOS occurs with a delay of 6-8 hours after stimulation. Once induced, iNOS is active for hours or even days and produces NO in 1000-fold larger quantities than the constitutive forms (Moncada and Higgs, 1993; Beck *et al,* 1999).

	nNOS constitutive	**eNOS** constitutive	**iNOS** inducible
quantity of NO release	10^{-12} (pmol)	10^{-12} (pmol)	10^{-9} (nmol)
location chromosome number	12	7	17
cellular compartment	cytosolic	membrane-bound	cytosolic
celltype	neurons	endothelium platelets endocardium myocardium	macrophages smooth muscle cells epithelial cells endocardium myocardium hepatocytes astrocytes fibroblasts chondrocytes osteoblasts/-clasts
target organs	nerves	vascular smooth muscle	microbes
activation	ca-dependent	ca-dependent	ca-independent
expression	constitutive	constitutive	induction by LPS/cytokines
activators	sex hormones, cytokines, stress, physical exercise	acetylcholine, bradykinin, sex hormones, mechanical pressure, physical exercise	inflammatory mediators, cytokines, kinases, LPS, PG

Table 1. Properties of constitutive and inducible isoforms of NOS.

Nitric oxide and its mechanisms of biological action

Nitric oxide is a highly reactive molecule with a half-life of 4-8 seconds. It is soluble both in water and lipid and is therefore highly diffucible through membranes. It is the first example of a completely new signalling molecule quite different from the classical mediator concept (Nathan, 1992; Snyder, 1992). Whereas NO is a simple radical gas which forms covalent bonds fairly easily, classical mediators have complex structures and depend for their action on a complementary fit to a specific receptor.

Nitric oxide has several targets of action, which can be divided into at least following major groups:

- 1) Through cyclic guanosine monophosphate (cGMP).
- 2) Interaction through cGMP-independent mechanisms.
- 3) Interaction with free radicals.

1) Cyclic GMP. The physiologically most relevant action of NO is the activation of soluble guanylatcyclase by nitrosation of its haem moiety (Ignarro, 1990). Cyclic GMP regulates intracellular calcium concentrations, which mediate physiological functions of NO smooth muscle relaxation and platelet aggregation. The subsequent increase in cGMP levels alters the activity of three main target proteins: cGMP-regulated ion channels, cGMP-regulated phosphodiesterases and cGMP-dependent protein kinases (Schmidt *et al,* 1993).

2) Interaction through cGMP-independent mechanisms. One of the most important of these involves the nitrosylation of the target proteins. In this way NO can influence other enzymes such as cytochrome oxidase (Brown, 1997), ribonucleotide reductase (Lepoivre *et al*, 1990) and cyclooxygenase (Salvemini, 1997) and hence influence cellular respiration, DNA synthesis and inflammatory and immune responses. Endogenous NO enhances PG production in inflamed tissues (Salvemini, 1994). Nitric oxide may also affect smooth muscle relaxation, cell signalling and phagocytosis by their direct activation of gene transcription (Nathan, 1992; Umansky *et al,* 1988). Furthermore, there is increasing evidence that NO can directly regulate gene expression by modulating the activity of transcription factors such as NFκB and the activator protein 1 (AP-1) (Sen and Packer, 1996). Since NFκB inhibits progesterone receptor action via protein-protein interaction (Kalkhoven *et al*, 1996) NO may therefore modulate progesterone responses in the reproductive tract. The list of genes under the regulatory control

of NO is expanding, including those involved in the extracellular matrix protein (MMP) synthesis and their degrading enzymes (Chatziantoniu *et al*, 1998; Sasaki *et al*, 1998), cytokines, and chemokines, such as IL-8 (Villarete and Remick, 1995). However, higher concentrations of NO may also interact directly with MMPs (Trachtman *et al,* 1996). Finally, at high concentrations NO plays a role in apoptotic cell death. An increased apoptosis following exogenous application of NO donors or iNOS induction has been described in different celltypes, such as macrophages and mesangial cells (Brüne *et al*, 1998).

3) Interaction with free radicals. Nitric oxide can interact with free radicals such as superoxide anion and free thiols to mediate its effect. The reaction with superoxide anion results in the formation of toxic hydroxyl radicals and peroxynitrite that are involved in host defence responses. Interactions between thiols and NO result in the formation of S-nitrosothiol derivatives that are more stable and prolong the effects of NO *in vivo* (Clancy *et al*, 1994; Jia *et al*, 1996).

Detection of nitric oxide

As a consequence of the short half-life of NO its detection is difficult both *in vivo* and *in vitro*. Nitric oxide was first quantified in 1987 by Palmer and co-workers who used a chemiluminescence assay. *In vitro*, detection of NO has been possible by using a rapid response chemiluminescence analyzer (Lee *et al,* 2000) or by using NO specific microelectrodes (Tsukahara *et al,* 1993). Indirect measurement of NO can be done by measuring the conversion of radiolabeled arginine to citrulline or by measuring the formation of cGMP (Ogden and Moore, 1995). Another method of measuring NO production is by detecting positive NADPH diaphorase activity (Rosselli et al, 1996; Ekerhovd et al, 1997). *In vivo*, it is even more challenging to assess NO production. Among experimental studies on endothelial vasomotor function, indirectly measuring NO release, pletysmography and pulsewave analysis have been used (Benjamin *et al,* 1995; Wilkinson *et al,* 2002). Stefansson and colleagues have described a new method for monitoring NO production *in vivo* using Teflon membrane microdialysis (electron spin resonance). This Teflon membrane allows only gaseous molecules (NO) to penetrate and the technique has been used in renal studies. The expression of NOS can be assessed by semiquantitative measurements like Western blotting or reverse transcriptase-polymerase chain reaction (RT-PCR). Immunohistochemical studies can also be used for expression of NO metabolites and NOS (Tschugguel *et al,* 1999;

Alderton *et al,* 2001; Aktan, 2004; Törnblom *et al,* 2005). The NO metabolites nitrite and nitrate can be assessed by the use of the Griess reaction (Nakatsuka *et al*, 2000; Väisänen-Tommiska *et al,* 2003; 2004). Griess reagent forms azo dye with nitrite, which can be spectrophotometrically measured (Green *et al,* 1982). In studies on NO donors and their effects on different enzymes, such as cyclooxygenases and MMPs, Western blotting, RT-PCR, radioimmunoassay, ELISA, and immunohistochemistry can be used for analysis (Salvemini *et al, 1993*; Ledingham *et al,* 1999b; Stygar *et al*, 2002).

General effects of nitric oxide

Nitric oxide is an important physiological and pathophysiological factor in cardiovascular, nervous and immunological systems (Moncada and Higgs, 1993; Alderton *et al*, 2001; Aktan, 2004). It reduces blood pressure by vascular dilatation and smooth muscle relaxation and inhibits platelet aggregation. It stimulates angiogenesis and seems to play a role in placental trophoblast villi (DiIulio *et al,* 1995; Ramsay *et al*, 1996). The wavelike motions of the gastrointestinal tract are aided by the relaxing effect of NO on the smooth muscle in its walls (Vanderwinden, 1994). It is an important neurotransmitter. Nitric oxide has also been associated with processes of learning, sleeping, pain as well as neurological disorders. Nitric oxide is involved in the physiological regulation of renal function (Haynes *et al*, 1997). It is involved in wound healing (Schaffer *et al,* 1996) and normal lung function (Lehtimäki *et al,* 2005) as well as being a toxic agent in malignant and anti-inflammatory disorders (Moncada and Higgs, 1993; Alderton *et al*, 2001). Nitric oxide affects secretion from several endocrine glands such as hypothalamus (Rettori *et al,* 1992), pancreas (Yago *et al,* 2001) and adrenal medulla (Lai *et al,* 2005).

Nitric oxide in the reproductive system

Nitric oxide is involved in most processes of human reproduction (Figure 3). There is convincing evidence that NO, both ovarian cell-derived and vascular endothelian cell-derived, plays an important role in the physiology and biology of the ovary with regard to regulation of folliculogenesis and ovulation (Shukovski and Tsafriri, 1994; Zackrisson *et al,* 1996; Ekerhovd *et al,* 2001). Nitric oxide mediates contractile activity in the Fallopian tube (Rosselli *et al*, 1994; Ekerhovd *et al*, 1997). It regulates endometrial receptivity, implantation and menstruation (Shi *et al*, 2003; Sun *et al,* 2003*;* Mörlin and Hammarström, 2005) and promotes embryonal implantation (Zhang *et al,* 2005). Nitric oxide has been shown to play a

role in regulating smooth muscle cell contractility, contributing to uterine quiescence during pregnancy (Norman *et al,* 1997; Ekerhovd *et al,* 1999). In the human myometrium all three isoforms of NOS are present. Their physiological role during pregnancy and labor has been studied in detail (Buhimschi *et al,* 1996). In the uterine body it has been demonstrated that these enzymes are up-regulated during pregnancy but down-regulated during labor. Hence, NO seems to play an important role for maintaining pregnancy. While NOS enzymes of the uterine body decrease towards term, an up-regulation of NOS enzymes takes place within the uterine cervix at term before onset of labor. The mechanisms that regulate NOS activity during pregnancy are still not known. However, it has been suggested that cytokines may play a role (Das *et al,* 1992; Bry and Hallman, 1993). In the rat uterus, interactions between cyclooxygenase, NO and cytokines have been described (Dong and Yallampalli, 1996). Similar interactions may exist in the human uterine body.

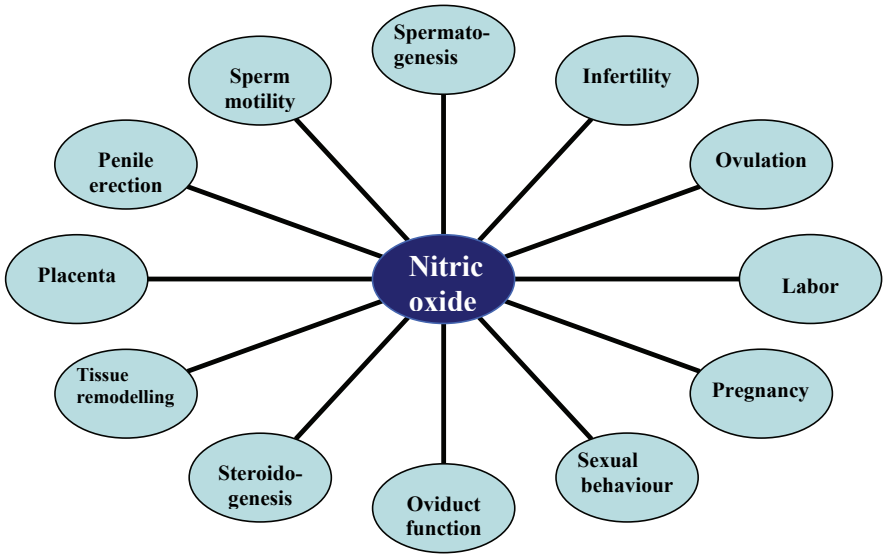


Figure 3. Various reproductive processes regulated by nitric oxide.

Nitric oxide also plays an important role in the male reproductive system. In 1990 Ignarro and co-workers demonstrated that electrical stimulation for penile erection promoted endogenous formation and release of NO. Nitric oxide synthase activity has been localized in penile neurons (Burnett *et al*, 1992) and in the endothelium of penile vasculature and sinusoidal

endothelium within the corpora cavernosa (Burnett *et al*, 1996). It has been shown that the NO-cGMP pathway is largely responsible for mediating penile erection (Melis *et al,* 1996; Penson *et al,* 1996; Zvara *et al*, 1997). By inhibiting the degradation of cGMP penile erection can be achieved. Presence of NOS activity has also been demonstrated in other male urogenital organs (Ehren *et al*, 1994; Burnett *et al*, 1995). It has been established that the NO-cGMP pathway is present in testicular cells. This pathway may participate in the regulation of testicular function, for instance spermatogenesis (Zini *et al*, 1996) and steroidogenesis (Adams *et al*, 1994). Nitric oxide also regulates sperm motility (Hellström *et al*, 1994). In addition, LHRH not only triggers the synthesis of gonadotrophins and gonal steroids but also participates in regulating mating behaviour in vertebrates (Moss and McCann, 1973; Sakuma and Pfaff, 1983). Studies have indicated that NO-induced mating is LHRH mediated (Mani *et al*, 1994). Nitric oxide seems to be regulating both male and female sexual behaviour (Hull *et al*, 1994; Rachman *et al*, 1996). It has also been shown that oxytocin induces LHRH release via NO generation, suggesting that sexual effects of oxytocin are NO mediated (Rettori *et al*, 1997).

CERVICAL RIPENING

Normal anatomy and physiology

The uterus consists of two basic parts: the corpus (uterine body) and the cervix. The most caudal part of the cervix, portio cervicis uteri, protrudes into the vagina and is approximately 2 cm long (Danforth, 1947; Leppert *et al*, 1986). The entire uterus is composed mainly of smooth muscle and extracellular matrix. The cervix, however, consists mainly of connective tissue (85-90%), smooth muscle constituting only 10-15% of the tissue (Danforth, 1983). The extracellular matrix consists of connective tissue, mainly collagen bundles, type I and III (Leppert, 1995; Kelly, 2002). Type IV collagen is also present in smooth muscle cells and blood vessel walls (Minamoto *et al,* 1987). Water, glycosaminoglycans, and proteoglycans are important constituents of the extracellular matrix, especially dermatan sulphate, hyaluronic acid and heparin sulphate (Golichowski *et al*, 1980; Leppert, 1995). Fibronectin, laminin and elastic fibers are other constituents of the extracellular matrix. Towards term a fundamental reorganization of the extracellular matrix occurs, comprising changes in the composition of proteoglycan complexes, collagen breakdown and water contents. These biochemical events

underly the overt clinical changes in the consistency, dilatation and effacement of the cervix, as evaluated by the classical Bishop score. Parturition can be divided into two major phases: 1) Conditioning (preparatory) phase and 2) irreversible active labor phase (Chwalisz and Garfield, 1997). Cervical ripening is an integral part of the conditioning phase of parturition. It occurs independently of uterine contractions (Leppert, 1995; Chwalisz and Garfield, 1998). It is an active biochemical process similar to an inflammatory reaction, involving infiltration of leukocytes, activation of degradative enzymes (MMP, LPS) leading to rearrangement of extracellular matrix proteins and glycoproteins (Leppert, 1995; Maul *et al*, 2003; Sennström *et al*, 2003).

Control

Progesterone appears to have a dominant role in cervical ripening since antiprogestins are effective agents in inducing cervical ripening (Chwalisz, 1998; Neilson, 2004). Treatment with antiprogestin has been shown to induce labor at term (Stenlund *et al*, 1999; Neilson, 2004). In animals, the progesterone agonist R5020 completely blocked onapristone induced cervical ripening, indicating that the process was mediated via the progesterone receptor (Chwalisz, 1994; Garfield *et al,* 2001). However, it has been shown that cervical ripening starts long before the decrease in serum progesterone, indicating an additional progesterone-independent mechanism (Shi *et al*, 1996). Oestrogen has also been attributed a role in cervical ripening since vaginally applied oestrogen has been shown to promote cervical ripening (Trumans *et al,* 1979; Keirse and Van Oppen, 1989) and oestradiol attenuated onapristone-induced cervical ripening (Chwalisz *et al,* 1995). In addition, relaxin, an ovarian and placental hormone, stimulates cervical ripening (Leppert, 1992). The corticotropin releasing hormone (CRH) is also considered to play a role in cervical ripening. This hormone is produced by the placenta and myometrium during preganancy (Berkowitz *et al,* 1996; Challis, 2000). It is believed to contribute to the up-regulation of iNOS. It stimulates PG production and is thought to act synergistically with oxytocin during labor. Parturition is also associated with an increase in different interleukins in the cervix, as well as in the chorio-decidua and the amnion (Sennström *et al*, 2000; Osman *et al*, 2003; Sakamoto *et al*, 2004). The levels of IL-8 correlate with the expression of collagenases (Garcia-Valesco and Aric, 1999) and increase at term vaginal delivery (Sennström *et al*, 1997; Osman *et al*, 2003). Furthermore, tumor necrosis factor alpha (TNF-α) seems to be involved in cervical ripening (Chwalisz, 1994). It is produced in decidual and amniotic cells and stimulates COX-2 and subsequent PG formation

(Casey *et al*, 1989; Keelan *et al*, 2003). These inflammatory events result in vascular dilatation and extravasation of leukocytes (Winkler and Rath, 1999), considered as the main source for release of collagen degradating matrix metalloproteinases (MMPs), a family of at least 17 enzymes (Hulboy *et al,* 1997) capable of degrading collagen as well as other extracellular matrix components. Especially MMP-8 has been found to play a central role in this process (Sennström *et al,* 2003; Aronsson *et al,* 2005). It has also been suggested that MMP-1 and MMP-3 are involved in the ripening process (Sennström *et al,* 2003).

Role of prostaglandins, cyclooxygenases and NO in cervical ripening

Prostaglandins, especially PGE_2, have for a long time been thought of as key mediators of cervical ripening (Kelly, 1994) by causing dilatation of cervical vessels and extravasation of leukocytes (Winkler and Rath, 1999). Synthesis of PGs has been described in the amnion, decidua, chorion, myometrium, placenta and cervix with the human cervix synthesizing primarily PGE_2 (Ekman *et al*, 1983; North *et al,* 1991; Barcley *et al,* 1993; Zakar *et al,* 1996; Kayem *et al,* 2003; Korita *et al,* 2004). PGE_2 is thought to act principally as a vasoactive agent. It facilitates infiltration by inflammatory cells and also regulates the release of cytokines. It has also been reported to stimulate collagenase activity (Goshowaki *et al,* 1988). $PGF_2\alpha$ is known to increase hexosamine, a constituent of glycosaminoglycans, and to increase hyaluronic synthetase activity (Rath *et al,* 1987). In the uterus the contractile effect of PGs is well known. However, their role in the cervical ripening process has been questioned, as this process is independent of uterine contractions. COX is the rate-limiting enzyme in the biosynthesis of PGs. In addition to the well characterized constitutive form of COX (COX-1) (deWitt, 1991), an inducible isoform of COX (COX-2) is found in endothelial cells (Maier *et al,* 1990), fibroblasts (Raz *et al,* 1988) and macrophages (Fu *et al,* 1990; Masferrer *et al,* 1990; 1992). COX-2 is typically undetectable in most tissues under normal physiological conditions but can be expressed at high levels following stimulation. A recent study has shown that there is an increase in cervical COX-1 and COX-2 at parturition (Stjernholm-Vladic *et al,* 2004). Moreover, from several studies conclusions have been made that PGs can stimulate the release of NO (Maul *et al,* 2003). The role of 15-OH prostaglandin dehydrogenase (PGDH), an enzyme responsible for the metabolism of PG to inactive metabolites, in preterm labor (PTL) is not known. Studies have shown that PGDH activity is lower in the chorion at PTL (van Meir *et al,* 1997) and that the activity is decreased by

antiprogestines and cortisol (Patel *et al,* 1999). Thus, decreasing PGDH activity at term may have a role in cervical ripening.

Nitric oxide is involved in regulating many factors in the inflammatory process of cervical ripening. All three isoforms of NOS are present in the human uterine cervix (nNOS, iNOS, eNOS) (Tschugguel *et al,* 1999; Ledingham *et al,* 2000; Bao *et al,* 2001). Inducible NOS has been localized in the epithelial cells and stromal spindle cells (Tschugguel *et al,* 1999) and has been demonstrated by immunostaining in uterine cervix at term (Ekerhovd *et al,* 2000). Furthermore, iNOS has been shown to be up-regulated in human uterine cervix during delivery (Tschugguel *et al,* 1999; Ledingham *et al,* 2000). The metabolites of NO in cervical fluid are increased at term compared to preterm (Väisänen-Tommiska *et al*, 2003). The iNOS isoform can be induced by cytokines, TNF-α, interferon-λ or endotoxins (lipopolysaccharides) in a calcium-independent manner as has been described earlier. Nitric oxide may exert its effect through stimulation of endogenous PG synthesis through COX-activation (Salvemini *et al,* 1993) or through stimulation of at least one MMP, MMP-1 (Yoshida *et al*, 2001). Animal studies, though, have shown that NO has an effect on several MMPs in other human and animal tissues (Murrell *et al,* 1995; Trachtman *et al,* 1996; Sasaki *et al,* 1998).

An alteration in the glycosaminoglycan composition of the cervix occurs during late pregnancy, which may be important in the ripening process. The amount of hyaluronic acid present in the cervix increases at term. These changes bring about an alteration in the binding affinity to collagen thus altering the tissue hydration and hence cervical extensibility (Rechberger *et al,* 1996). Nitric oxide suppresses proteoglycan synthesis (Hauselmann *et al,* 1998). In addition, NO may also promote apoptosis which has been described in smooth muscle cells (Romero *et al*, 1990) and fibroblasts (Leppert, 1998) during cervical ripening. Several lines of evidence from studies of other tissues suggest that this process is stimulated by NO (Nicotera *et al,* 1997; Brüne *et al,* 1998). Finally, cervix constitutes to 10-15% of smooth muscle cells, the role of which has only to some extent been examined. Relaxing effects of cervical smooth muscle following administration of NO donors have been demonstrated *in vitro* in cervical tissue specimens from early pregnant as well as term women (Ekerhovd *et al,* 1998; Ekerhovd *et al,* 2000).

Taken together, NO has been proposed to be the final mediator of cervical ripening, according to Chwalisz and Garfield (Figure 4).

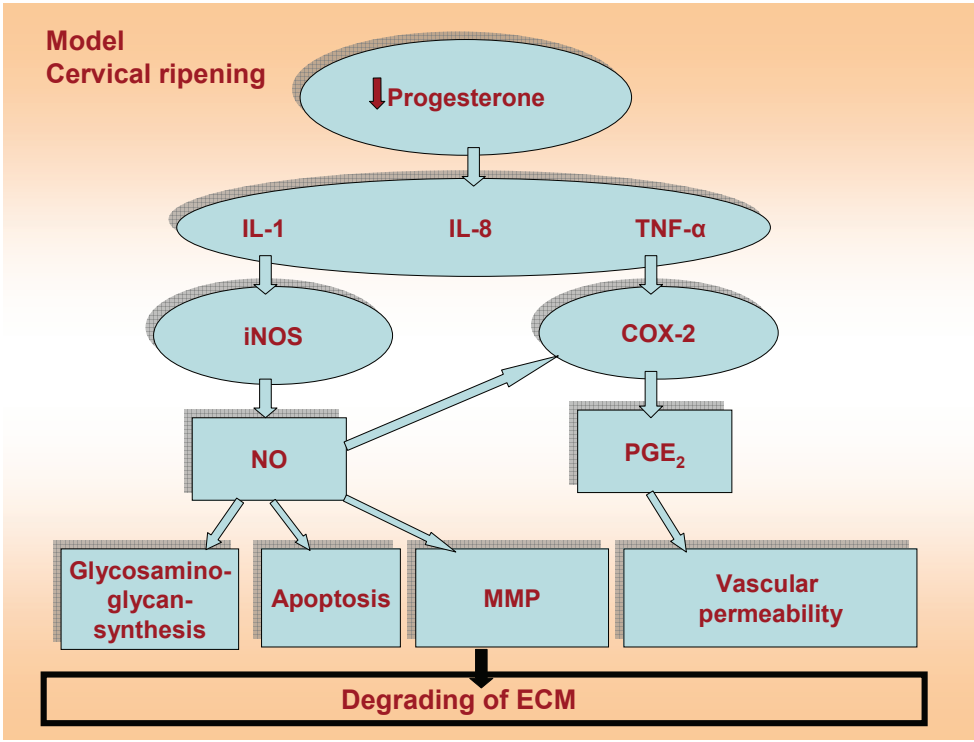


Figure 4. Model of cervical ripening. ECM=extracellular matrix.

Cervical ripening and induction of labor

Induction of labor can be defined as an intervention designed to artificially initiate uterine contractions leading to progressive dilatation and effacement of the cervix and birth of the infant. (Royal College of Obstetricians and Gynaecologists (RCOG), *Induction of Labour*, Guideline Nr 9). This includes both women with intact membranes and women with spontaneous rupture of membranes who are not in labor. As with any other intervention, induction of labor may have unwanted effects, but is indicated when it is agreed that the mother or the fetus will benefit from a higher probability of a healthy outcome than if birth is delayed. Naturally, the process of induction of labor should only be considered when vaginal delivery is felt to be the appropriate route of delivery. Induction of labor is a common procedure: about 20% of pregnant women will have labor induced for a variety of reasons according to RCOG. In Sweden, however, the induction rate is lower, approximately 10% according to local statistics. Induction does not usually involve just a single intervention but a

complex set of interventions and, as such, presents challenges for both clinicians and mothers. Figure 5 shows a schematic model of different methods of induction according to membrane and cervical status.

Cervical status can predict the success of induction and duration of labor (Jackson and Regan, 1997). The most common method for assessment of cervical status is by using the modified Bishop score (Bishop, 1964; Calder *et al,* 1977; Fuentes and Williams, 1995; Laube, 1997). This score is based on five factors: cervical dilatation, effacement, station, consistency and position. Each factor is graded from 0-2 giving a maximum score of 10 points. A total score of 4-8 is according to the literature regarded as an indication of a ripe cervix (RCOG, 2001). Amniotomy is feasible for induction of labor when the score is ≥6. The optimal method for inducing labor should be efficient but should not cause uterine hyperstimulation or other major side effects. If the membranes are ruptured and cervix is considered ripe, the method of choice is stimulation by oxytocin infusion. Oxytocin can be administered intravenously to cause uterine contractions and dilatation of cervix. The effect of oxytocin depends more on the number of receptors on the uterine myometrial cells than on the actual local hormone concentration. If the cervix is found to be unripe both oxytocin and PGs can be used. However, according to RCOG PGs are recommended for preinduction cervical ripening for better labor outcome. When the membranes are intact and cervix is ripe it is most common to induce labor with either amniotomy or oxytocin, or the combination of both. If the cervix is unripe different methods can be used for inducing cervical ripening before onset of contractions. Prostaglandins can be administered orally, sublingually, rectally, vaginally or intracervically. Numerous studies have been performed to find the optimal dose, drug and administration form. However, PGs are associated with side effects, such as uterine hyperstimulation, nausea and abdominal pain (Keirse, 1994). The most common PGs used today for cervical ripening are PGE_2- and PGE_1- analogues. Bygdeman and co-workers have also concluded that during early pregnacy the combination of mifepristone and PG is effective for termination of pregnancy (Bygdeman and Swahn, 1989). In the second trimester pretreatment with the antiprogestin mifepristone will significantly reduce the duration of labor, dose of PG, and the frequency of side effects (Bygdeman *et al,* 2000). Beside antiprogestin effects mifepristone also has anti-glucocorticoid and estrogen-realated properties (Olive, 2002). Mifepristone induces uterine contractions by blocking progesterone and by inducing COX activity (Hapangama *et al,* 2002), but it is not known how it promotes cervical ripening. Furthermore, Stenlund and co-workers have performed a study using mifepristone for labor induction. Thirty-six postterm pregnant women with an unripe cervix

were given either 400 mg mifepristone (n=24) or placebo (n=12). During the first 48 hours following treatment, 79% of the women treated with mifepristone compared to 16% of the women who received placebo tablets went into labor (Stenlund *et al,* 1999). Neilson has later confirmed that mifepristone can be used for termination of late pregnancy (Neilson, 2004). Mechanical treatment with an intracervical Foley catheter to achieve cervical dilatation prior to labor induction represents an alternative to medical treatment. The technique was described in 1967 (Embrey and Mollison, 1967). The use of the transcervical Foley catheter has been demonstrated to be both safe and effective for preinduction cervical ripening (Leiberman *et al,* 1977; James *et al,* 1994; Levy *et al,* 2004). However, because of its mechanical effects on the cervix one must consider possible damaging effects on the cervix and the risk of premature labor in future pregnancies (Gelber and Sciscione, 2006). After the Foley catheter is removed patients generally require other methods for further labor induction. In cases medical treatment for cervical ripening has failed the use of a balloon catheter could be an alternative to caesarean section.

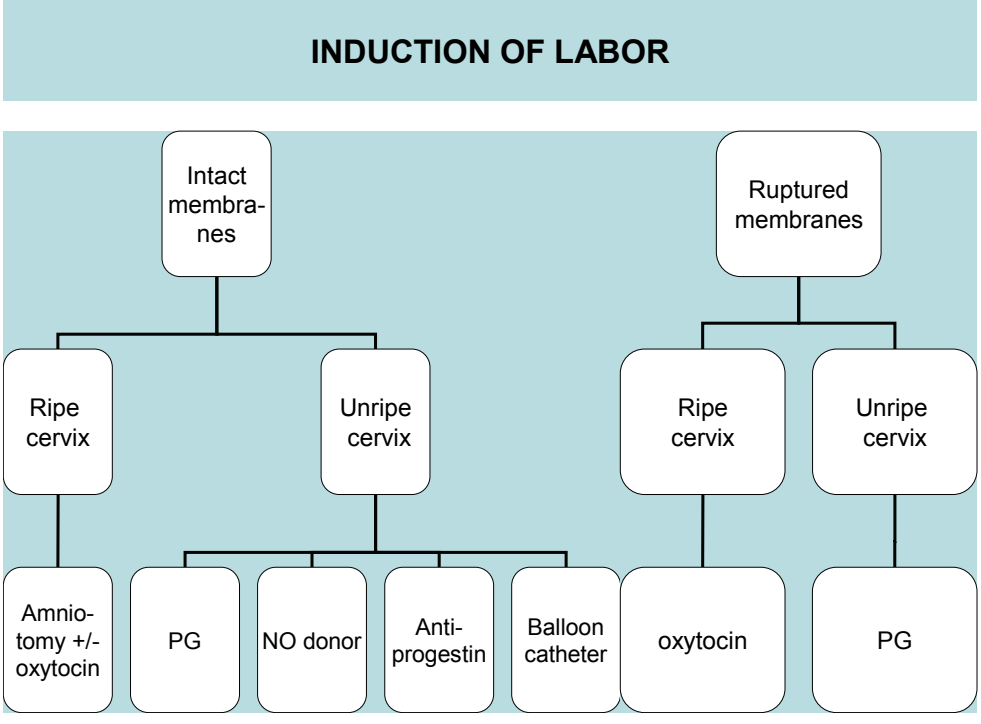


Figure 5. Methods of induction of labor.

Nitric oxide donors for cervical ripening

It is well established that NO donors have a cervical ripening effect (Chwalisz *et al*, 1994; Chwalisz and Garfield, 1997; Shi *et al,* 2000). Sodium nitroprusside (SNP), isosorbide mononitrate (IMN) and glyceryl trinitrate (GTN) are NO donors that have been administered vaginally in the first and second trimester before termination of pregnancy, and have shown to have ripening effects on the cervix (Thomson *et al,* 1997; Facchinetti *et al,* 2000; Ledingham *et al,* 2001; Eppel *et al,* 2005). The mechanism of action of NO donors has been addressed by a number of *in vivo* and *in vitro* studies. A complex interaction between NO and PGs, COX-enzymes, cytokines, glycoproteoglycans, MMPs, apoptosis, as well as smooth muscle cells seems to exist. The advantage of using NO donors for cervical ripening is its relaxing effect on uterine contractions, thus probably decreasing the risk of fetal side effects. Since 2000 several clinical studies have been performed using NO donors for cervical ripening at term pregnancy. Before NO donors can be generally applied in clinical practice it is of importance to identify possible maternal and fetal side effects. In one study maternal blood pressure and pulse rate were significantly affected following treatment with IMN, but no side effects of clinical importance were registered (Nicoll *et al*, 2001). Table 2 summarizes the results from clinical studies on NO donors for cervical ripening and labor induction in pregnant women at term. No difference in the rate of caesarean section was seen. However, the ripening effect of PGs seems to be more efficient than NO donors according to Bishop score and cervical distensibilty measured by cervical tonometer (Thomson *et al*, 1998). Of importance is the fact that no women treated with NO donors even after up to 3 consecutive doses suffered from uterine hypertonus. Thus, the possibility to induce labor in an outpatient setting by means of NO donors seems evident. Another aspect is the fact that clinical studies have shown that NO donors not only ripen the cervix but also seem to induce labor (Chanrachakul *et al,* 2000a; 2000b; 2002). According to these studies, 27-39% of the women went into labor within 24 hours following vaginal administration of NO donors. This could indicate that a vaginally applied NO donor initiates a cascade of events that not only leads to ripening of the cervix but also effective labor.

Author	N	Medical agent & dose	Comparison agent	Exposure time (hours)	Cervical Ripening
Chanrachakul *et al* 2000	110	GTN 0.5mg	Dinoprost 3mg	6	GTN<Dinoprost CS 35% vs 35%
Nicoll *et al* 2001	36	IMN 20mg IMN 40mg	Vaginal examination Vaginal examination	6 6	IMN=vaginal examination CS 46% vs 33% IMN=vaginal examination CS 18% vs 33%
Chanrachakul *et al* 2002	107	IMN 40mg Three doses	Misoprostol 0.05mg Three doses	6	IMN<Misoprostol CS 36% vs 31%
Sharma *et al* 2005	65	GTN 0.5mg GTN 0.5mg	Misoprostol 0.05mg Dinoprost 3 mg	6 6	GTN<Misoprostol CS 43% vs 48% GTN<Dinoprost CS 43% vs 53%
Osman *et al* 2006	400	IMN 40mg, Two doses	PGE_2 2mg, Two doses	12	IMN<PGE_2 CS 33% vs 31%
Wölfler *et al* 2006	120	IMN 40mg + Dinoprost 3 mg 2doses/day up to 2 days	Placebo + Dinoprost 3mg 2doses/day up to 2 days	6	IMN+Dinoprost= Placebo+Dinoprost CS 33% vs 25%
Nunes *et al* 2006	196	GTN 0.5mg + Dinoprostone 2 mg	Placebo + Dinoprostone 2mg	6	IMN+Dinoprost= Placebo+Dinoprost IMN group shorter time to delivery CS 32% vs 34%

Table 2. Randomized controlled trials on NO donors for cervical ripening at term. IMN: isosorbide mononitrate; GTN: glyceryl trinitrate; SNP: sodium nitroprusside; CS: caesarean section, N: number of women.

THE PLACENTA

Normal anatomy

After conception the development of embryo and placenta is divided into several events from mitotic divisions as blastomeres to morula after 3 days, after 4 days to blastocyst consisting of inner cell mass (embryoblast) and outer ring of trophoblast cells. The outer layer proliferates and differentiates to cytotrophoblasts and syncytiotrophoblasts. The hypoblast is then formed from inner cell mass later giving rise to a loosely arranged tissue named the extraembryonic mesoderm. Trophoblasts and the extraembryotic mesoderm later form the chorion. At its final stage the human placenta consists of two components: 1) The chorion, including the syncytiotrophoblast, the cytotrophoblast and the extraembryotic mesoderm, and 2) the allantois, containing three umbilical vessels (2 arteries and 1 vein). The amniotic cavity takes form at the embryonic pole, a layer from the inner cell mass which differentiates into a thin membrane that separates the new cavity (amnion) from the cytotrophoblasts. The fetal trophoblast invades the endometrium, brakes down endometrial blood vessels into lacunae and forms villi. Thus, the placenta becomes a materno-fetal unit with the fetal portion formed by the chorion (chorionic plate) and the maternal portion formed by the decidua basalis (basal plate). Chorionic villi begin to develop with extensions of cytotrophoblasts, covered with syncytiotrophoblasts, growing out into the lacunae, called primary stem villi. The villi undergo further development to secondary (intermediate) and tertiary (terminal) villi, the last ones constituting of anchoring villi, which reach the maternal endometrium. After implantation all but the deepest layer of the endometrium proliferates. These proliferating cells constitute the decidua. Stromal cells under the influence of invading trophoblasts, differentiate into large, rounded, glycogenfilled decidual cells. There are three regions of the decidua: 1) decidua basalis, underlying the implantation site, 2) the decidua capsularis, a thin portion between the implantation site and the uterine lumen surrounding the chorion, and 3) the decidua parietalis, including the remaining endometrium. As the embryo grows, the amnion rapidly expands until the chorionic cavity is obliterated and its surrounding decidua capsularis eventually fuses with the decidua parietalis, thereby obliterating the uterine cavity by the third month.

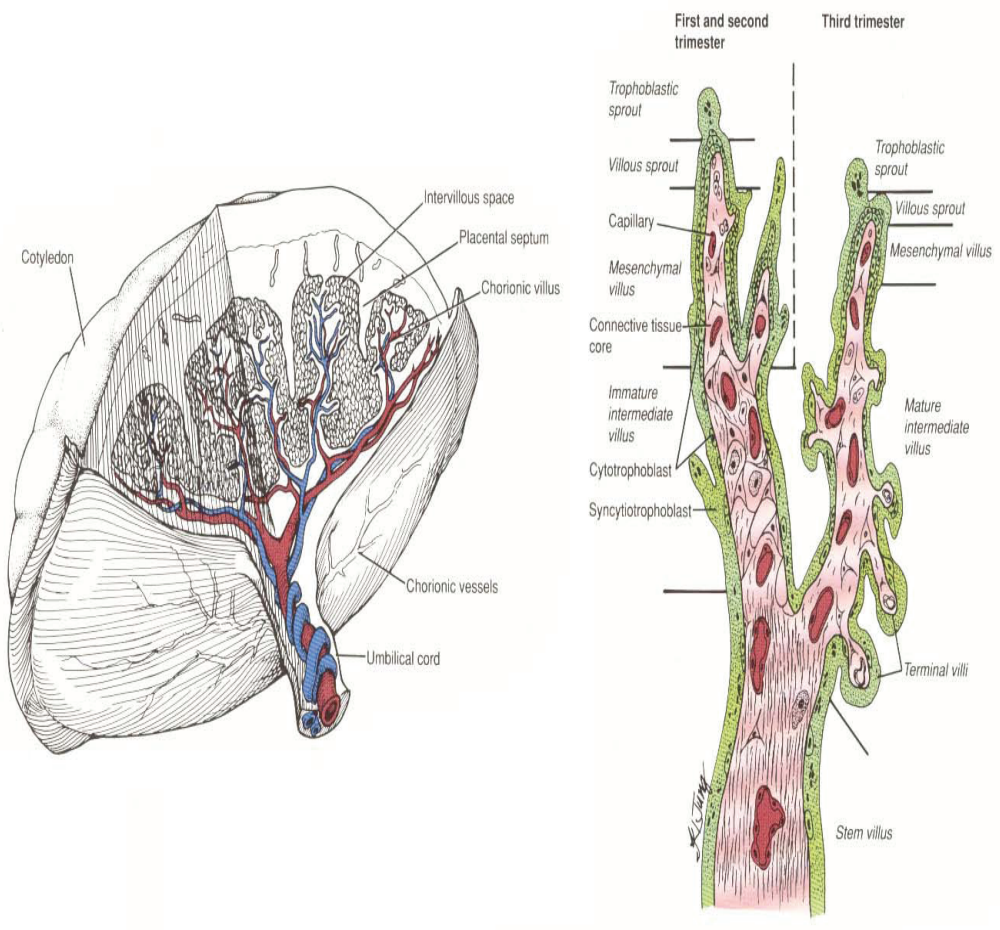


Figure 6. Placenta to the left and a placental stem villus to the right.

Normal physiology

Circulation through the embryo and the villi starts at about day 21 with the intervillous space as the site of exchange of nutrients and waste products between the maternal and fetal circulatory system. Fetal and maternal blood circulations do not mix since they are separated by the placental barrier which is derived from fetal tissue. The placenta has three main functions: 1) metabolism, 2) transport of substances and 3) endocrine secretion.

Metabolism. During early pregnancy the placenta synthesizes glycogen, cholesterol and fatty acids, which serve as sources of nutrients and energy for the growing embryo.

Transport. The placenta has a very large surface area which facilitates exchange of substances. At 28 weeks of gestation the surface area is 5 square meters, and at term almost 11 square meters. Approximately 5-10% of this surface area is extremely thin, measuring only a few microns. Deoxygenated fetal blood enters the placenta via two umbilical arteries and runs through capillaries in the villi, where gases and metabolites are exchanged across the placental barrier with maternal blood supplied by spiral endometrial arteries. Oxygenated fetal blood returns from the placenta via the umbilical vein. Oxygen, water, carbon dioxide, hormones, vitamins and antibodies can all cross the placental barrier. The exchange of gases occurs via diffusion. The placenta is also highly permeable to glucose, but less permeable to disaccharides. Amino acids are transported through specific receptors. Proteins are transferred slowly through the placenta, mainly via pinocytosis. The transfer of maternal antibodies, mainly IgG, is important in providing passive immunity to the infant. Another maternal protein, transferrin, carries iron to the placental surface from where it is actively transported to fetal tissues. Steroid hormones easily cross the placental barrier, while protein hormones are not as easily transported across the barrier. Placenta is also permeable to alcohol and other drugs and to some viruses.

Endocrine secretion. The syncytiotrophoblast is an important endocrine organ for maintaining pregnancy. It produces both proteins and steroid hormones. The major placental hormones are human chorionic gonadotropin (hCG), estrogens, progesterone, human placental lactogen (hPL), human chorionic somatomammotropin (hCS), human placental growth hormone, human chorionic thyrotropin (hCT), human chorionic adrenocorticotropin (hACTH), insulin-like growth factors, endothelial growth factors and relaxin. In addition, the placenta produces dozens of proteins that have been identified immunologically but whose function is poorly understood.

Placental detachment

Labor can be divided into three stages: first, second and third stage of labor. The first stage of labor refers to the interval between the onset of regular contractions and full dilatation of the cervix. The second stage of labor starts at full cervical dilatation and ends with the delivery of the infant. The third stage of labor is the interval between the delivery of the infant and the delivery of the placenta. This interval has a mean duration of 6.8 minutes (Combs, 1991). There is a six-fold increase in the risk for postpartum hemorrhage (PPH) if the third stage of labor is prolonged to over 30 minutes (Combs, 1991). Little is known about the physiology and pathophysiology of the third stage of labor. Historically, there have been given different explanations to why placenta detaches. Brandt (Brandt, 1933) suggested it was detached due to retroplacentar hematoma, while Dieckmann and co-workers (Dieckmann *et al*, 1947) stressed the importance of slow delivery of the fetus so that the uterine wall was given time to contract and retract. Ultrasound imaging of the third stage of labor led Herman and co-workers (1993) to divide the third stage of labor into four phases: the latent phase, the contraction phase, the detachment phase, and the expulsion phase. In the latent phase, which immediately follows the delivery of the infant, all of the myometrium except that behind the placenta contracts. In the contraction phase this retro-placental myometrium contracts. This leads to the detachment phase where the non-elastic placenta is torn from the decidua. The placenta is expelled from the uterus due to uterine contractions in the expulsion phase (Herman *et al*, 1993; Weeks, 2001).

Etiologies behind retained placenta. Retained placenta, a major cause of PPH, is a failure of the placenta to separate from the uterus. Retained placenta occurs in 2.0-3.3% of all vaginal deliveries and is the cause of 6-7% of all cases of PPH (Combs and Laros, 1991; Dombrowski *et al*, 1995). The incidence increases with decreasing gestational length (Combs, 1991; Dombrowski, 1995). The pathophysiology behind retained placenta is still unknown and little research has been carried out to understand its etiology. Dynamic ultrasonographic imaging of the third stage of labor has demonstrated that retro-placental myometrial contractions are necessary for placental detachment and that lack of retro-placental contractions resulted in retained placenta (Herman *et al,* 1993). Recently, Farley and co-workers demonstrated that human chorionic villi are able to contract and relax along their longitudinal axes and that this ability is under the control of NO (Farley *et al,* 2004). As early as 1906 smooth muscle-like spindle-shaped cells were observed in the chorionic plate of the term human placenta. Then in

1916 Iizuka confirmed that the spindle-shaped cells were smooth muscle cells (Krantz and Parker, 1963). Also noted at that time was that the muscle cells were denser at the center of the placenta where the umbilical cord is inserted and more sparse toward the periphery. In 1922 Naujoks proposed the hypothesis that the human placenta is capable of contraction. Krantz and Arey later demonstrated that these cells in the placenta have all the morphological characteristics of smooth muscle cells (Kranz and Parker, 1963). More recently, Graf and co-workers confirmed the presence of myofibroblasts in stem villi by demonstrating the immunolocalization of vimentin, desmin, α-actin, γ-actin, and myosin in these cells (Graf *et al,* 1994). They also suggested that the change in villus length is an active process, something that later was confirmed by Farley (2004). This mechanism, that anchoring villi have contractile properties was suggested to underlie placental separation.

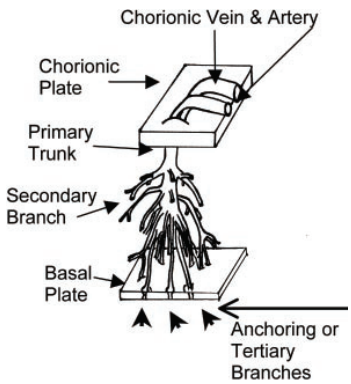


Figure 7. Schematic drawing of anchoring villi attached to the basal plate in the decidua. These anchoring villi have relaxing properties, which can be stimulated by NO.

The placental function is an important determinant of the onset of labor, and therefore it is also likely that placenta does not detach during pregnancy and first stage of labor due to an inhibitory factor. Potential candidates for this inhibitory factor have been suggested to be progesterone and/or NO (Garfield *et al*, 1998). Pathologically adherent placenta is caused by abnormal placentation and is generally classified into two groups depending on how far into the myometrium the chorionic villi have penetrated. Placenta accreta incompasses superficial penetration of the villi into the myometrium, and placenta percreta includes deeper penetration, in some cases through to the serosa, which may lead to uterine rupture. Risk factors for retained placenta are in descending order of significance: a history of retained

placenta, previous uterine surgery, preterm delivery, maternal age >35 years, placental weight less than 601 g, pethidine (an opioid analgesic) used during labor, labor induction, and parity of more than five (Soltan and Khashoggi, 1997).

Treatment of retained placenta

There are several different techniques for the management of a retained placenta (Figure 8). The techniques vary in several aspects, from time of intervention to method of treatment (pharmaceutical, invasive). Active management of the third stage of labor may result in a reduction in blood loss as well as a decrease in the average length of the third stage of labor. However, there is inconsistency in the results from studies concerning active management and its effect on retained placenta (Prendiville *et al,* 2000). The treatments summarized in this section are manual removal of placenta (MROP), and the use of various medications: oxytocin, PGs and nitroglycerin.

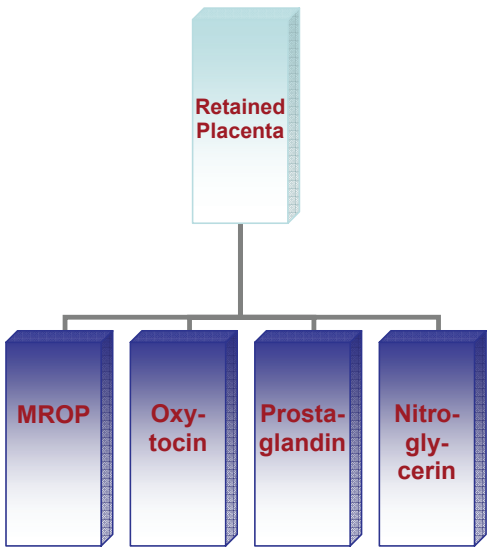


Figure 8. Different methods for management of retained placenta.
MROP: manual removal of placenta.

Manual Removal of Placenta

Manual removal of placenta (MROP) is the traditional management of retained placenta. It requires some form of hospital setting. This involves the use of anaesthetic (either regional or

general) and possibly the use of a further uterine relaxant to facilitate the manual removal of the placenta. Apart from the inherent risks associated with anaesthesia, MROP increases the risk of hemorrhage, infection due to the introduction of vaginal bacteria, rupture of the uterus, and trauma to the cervix or vagina (Carroli, 1991). General anaesthesia also carries the psychological impact of separating the mother from her family and newborn. A method for management of retained placenta that would reduce the need for manual removal in a hospital setting would be of major benefit to women worldwide.

Oxytocin

Oxytocin is currently only used in obstetrics for labor induction and stimulation of myometrial contractions. It has to be administered parenterally due to its short half-life and susceptibility to proteolytic cleavage (Mycek, 2000). Oxytocin may act as a labor promoting substance in multiple ways. It promotes myometrial contractility by increasing free intracellular calcium (Brucker, 2001). Furthermore, oxytocin exerts a stimulatory effect on the release of PGs, leukotrienes, cytokines, and NO (Ticconi *et al,* 2004), thereby possibly and indirectly contributing to degradation of extracellular matrix within both the uterine body and the cervix. The use of oxytocin has come under widespread debate over the years due to mixed results in several studies. Studies have also shown that the mean duration for third stage of labor is approximately 6 minutes, and that this interval is not shortened by administration of oxytocin (Combs and Laros, 1991; Herman *et al,* 1993). It has been suggested that results may be so varied due to the differences in dosage and method of administration (Carroli, 2001). The methods include intramuscular, intravenous or intraumbilical injections. The latter method involves direct delivery of an oxytocic agent via umbilical injection to the retro-placental myometrium, which is the area of uterine muscle failing to contract. The efficacy of this method has been questioned (Makkonen *et al*, 1995). One study, however, resulted in a success rate of 93% in delivering retained placentas using this method (Chauhan, 2000). A Cochrane review, analysing twelve trials, concluded that the umbilical vein injection of oxytocin and saline solution appeared to be effective in the management of retained placenta, but that further research was needed (Carroli, 2001).

Prostaglandins

Like oxytocin PGs increase intracellular free calcium, thus promoting uterine contractions (Brucker, 2001). The advantage of PGs is that they can be administered in different forms, either vaginally, rectally, orally, intravenously or intraumbilically. Misoprostol (a stable

analogue of PGE_1), sulprostone (a PGE_2 analogue), as well as carpoprost ($PGF_2\alpha$) have been used in studies for the management of retained placenta (Bider *et al,* 1995; Li *et al,* 2001; van Beekhuizen *et al,* 2005). Intravenous administration of 250 μg of sulprostone for thirty minutes in women with retained placenta reduced the need for MROP by 49% (van Beekhuizen *et al,* 2005). Bider and co-workers showed that intraumbilical venous administration of $PGF_2\alpha$ was an effective treatment of retained placenta with negligible side effects. Li and colleagues (2001) found in an uncontrolled study that rectal administration of 800 μg of misoprostol for treatment of retained placenta resulted in a 100% successful rate. Furthermore, rectally administered misoprostol compared to placebo has been shown to be effective (Darney, 2001; El-Refaey, 2002; O´Brien *et al*, 2002; Shannon and Winikoff, 2002). Ng and co-workers found that there were significantly fewer retained placentas following the use of oral misoprostol as a prophylactic agent compared to syntometrine (oxytocin plus ergometrine) (Ng *et al*, 2001). However, according to an analysis by WHO intravenous or intramuscular oxytocin is preferable over oral misoprostol in the active management of the third stage of labor (Gülmezoglu *et al*, 2001). This conclusion could be questionable, as WHO based it on a 1% difference in results (the incidence of major PPH during treatment with misoprostol and oxytocin were 4% vs 3%, respectively).

Nitric oxide donors

Several reports have indicated that intravenously administered nitroglycerin is effective for successful delivery of retained placenta (DeSimone, 1990; Dufour *et al*, 1997; Axemo *et al*, 1998; Chedraui and Insuasti, 2003). Nitroglycerin is a well known NO donor. *In vitro,* NO donors, including nitroglycerin, induce immediate relaxation of the term pregnant myometrium as well as the uterine cervix (Buhimschi *et al*, 1995; Norman *et al*, 1997; Axemo *et al*, 1998; Ekerhovd *et al*, 2000). Nitroglycerin administered intravenously causes uterine relaxation within 45-60 seconds. The relaxation normally lasts for two minutes (Axemo *et al,* 1998). If administered sublingually plasma concentration reaches its maximum within five minutes with a half-life of about three minutes. Nitroglycerin is known to affect blood pressure because of its vasodilating properties, but no study on nitroglycerin has reported serious decrease in blood pressure or uncontrolled bleeding after treatment of retained placenta with intravenous nitroglycerin. Only one case report has previously been published where sublingual administration of an NO donor, isosorbide dinitrate, was successfully used for manual removal of retained placenta (Okawa *et al,* 2002). In a recently published study it was demonstrated that oxytocin exerts an overall stimulating effect on NO release by fetal

membranes at term and that this stimulating effect is more marked after labor than before labor (Ticconi *et al*, 2004). An interaction between oxytocin and NO could play a role in promoting placental separation.

AIMS OF THE STUDY

The overall aim of this study was to determine in clinical and experimental studies whether NO donors have an influence on cervical ripening and labor induction at term or postterm pregnancy, and whether nitroglycerin in a sequential treatment with oxytocin has an effect on the management of retained placenta. In this context the following questions have been addressed:

- Are NO donors effective in inducing cervical ripening when administered intravaginally at term?
- Do NO donors, administered intravaginally, have an effect on labor induction?
- Do NO donors cause maternal and fetal side effects?
- Is it safe to administer NO donors for labor induction in an outpatient setting?
- Are cervical COX enzymes affected by locally administered NO donors?
- Is the sequential combination of oxytocin and nitroglycerin for management of retained placenta effective and safe?

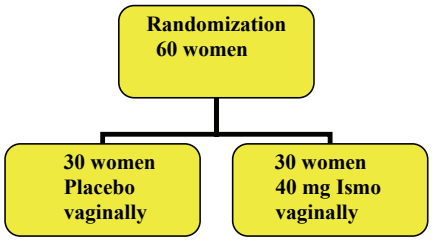
SUBJECTS AND METHODS

The women (N = 308) included in the present thesis were carefully informed about the purpose and nature of the studies before giving their consent to participate. Women between 19 and 43 years of age who were not on any medication were asked to participate. All women were in late pregnancy (papers I-III) or had just delivered an infant (paper IV) and none had suffered from any pregnancy complication. All studies were approved by the local Human Ethics Committee.

Paper I – A clinical randomized controlled study

In this study 60 patients were included (Figure 9). The primary purpose of the study was to evaluate the ripening effect of vaginally administered IMN to term pregnant women by measuring intracervical distensibility. Secondarily, possible maternal or fetal adverse effects were examined. The women were all scheduled for elective caesarean section at term due to breech presentation or for psychological reason. Inclusion criteria were nulliparity, gestational age of at least 37 weeks, uncomplicated singleton pregnancy, normal CTG, normal umbilical Doppler indices and immature cervical status (Bishop score <6 and cervical length ≥30 mm). Randomization was performed by means of numbered, sealed envelopes prepared with random-number tablets. Both the IMN (two 20mg tablets of Ismo®, Boehringer Mannheim, Mannheim, Germany) and the two placebo tablets were of similar design and were placed into the posterior vaginal fornix by a research nurse who was unaware of the agent administered. Transvaginal ultrasound of the uterine cervix was conducted for measurement of cervical length. Ultrasonography was performed by an obstetrician, who was unaware of the agent administered. Before being transported to the operating theatre, the women were asked to complete a symptom questionnaire concerning following symptoms: headache, hot flushes, palpitations, dizziness, abdominal pain, pelvic pain and nausea.

0 min: CTG, cervical status,
Maternal blood pressure
Maternal pulse
Umbilical resistance index (RI)
Umbilical pulsatility index (PI)



60 min: CTG

120 min: CTG

180 min: Maternal blood pressure
Maternal pulse
Umbilical PI
Umbilical RI

210 min: CTG
Maternal side effects according to VAS-scales

240-270 min: Cervical distensibility measured by cervical tonometer

250-280 min: Apgar score
pH in the umbilical artery
Maternal blood loss

Figure 9. Flow chart showing the procedure from randomization to delivery by caesarean section.

At the operating theatre regional anaesthesia was given and the patient placed into lithotomy position. Cervical distensibility was measured by a cervical tonometer (Figure 10). The peak force (in Newton) required to dilate the cervix from 5 to 10 mm with a cervical dilatator connected to the cervical tonometer was registered. Caesarean section was then performed according to routine praxis and blood loss was recorded as well as pH in the umbilical artery and Apgar scores at 1, 5 and 10 minutes.

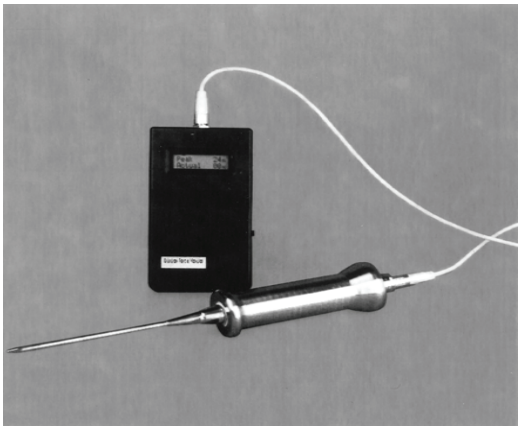


Figure 10. A cervical tonometer used for measuring cervical distensibility. Cumulative force (Newton) to dilate the uterine cervix to 10 mm was registered by means of dilators with a diameter of 5 to 10 mm connected to the tonometer.

Statistical analysis

A sample size of 60 women in two groups, estimated by Altman nomogram (a graphic method to calculate sample size and power), were calculated to yield 80% power at the 5% significance level when assuming 0.75 as a standardized difference in the cumulative force to dilate the cervix. Standardized difference is based on the ratio of the difference of interest to the standard deviation of the observations. This means that the larger the ratio is the smaller sample size is required. For comparison between groups Mann-Whitney nonparametric *U*- test was used for continuous variables. All tests were two-tailed and conducted at a 5% significance level.

Paper II – A double-blind randomized controlled clinical trial.

The main aim of the study was to investigate the efficacy of 40 mg of IMN administered vaginally for labor induction in an outpatient setting and to evaluate the safety with the treatment. The study was conducted at Sahlgrenska University Hospital, Sweden and Borås Hospital, Sweden. The women had all come to routine postterm control and were asked to participate if the following inclusion criteria were met: Uncomplicated singleton pregnancy, cephalic presentation, gestational age of at least 42 weeks, Bishop score <6, normal amniotic fluid index >5cm, reactive fetal heart pattern, and intact membranes. Examination of the cervix included an assessment of cervical status according to Bishop score and closed cervical length performed by transvaginal ultrasonography. Two hundred women fulfilled the inclusion criteria and agreed to participate (Figure 11). They were randomized to receive either 40 mg (two 20 mg tablets) of IMN or 2 placebo tablets of a similar design. The tablets were inserted into the posterior vaginal fornix by an obstetrician. Primary outcome was start of labor while secondary outcomes were cervical ripening and fetal and maternal side effects. Neither the woman nor the obstetrician knew which agent was being administered. However, both the participating women and the obstetrician who administered the agent, due to possible side effects of the NO donor, could guess which agent had been given.

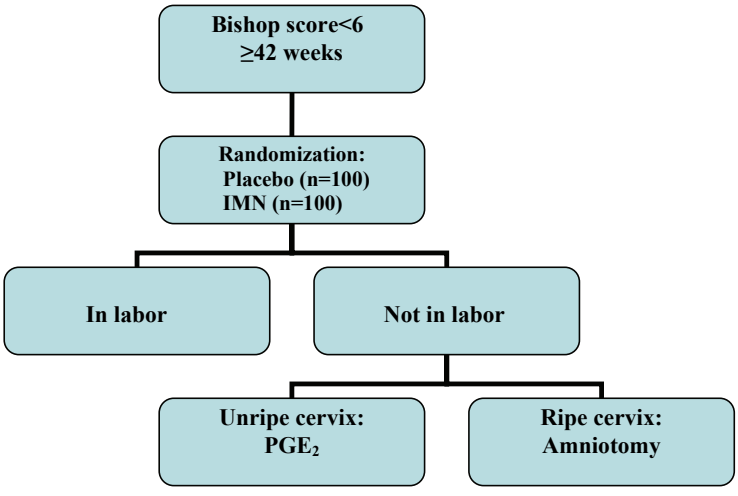


Figure 11. Flow chart of labor induction. IMN=isosorbide mononitrate.

Before returning home each woman was given an appointment for the next day and asked to complete a symptom questionnaire about side effects (headache, hot flushes, palpitations,

dizziness, nausea, and abdominal pain) during the priming interval. To assess the intensity of symptoms, a visual analogue scale graded from 0 (no symptoms) to 10 (symptom of maximum intensity) was used. If onset of labor occurred before the next day, the women were instructed to contact the labor and delivery unit. Onset of labor was defined as regular painful contractions (with or without rupture of membranes) combined with cervical length <1cm and cervical opening ≥3cm. If regular contractions had not been established within the next day, cardiotocography was performed and the cervix was assessed. Labor was induced according to the local protocol. Women with a Bishop score ≥6 underwent amniotomy. If the Bishop score was <6 or if amniotomy was not feasible, 1 mg of PGE_2 was administered vaginally for further cervical ripening.

Statistical analysis

According to audits in our labor units approximately 5-10 out of 100 postterm women would go into labor within 24 hours. Previous studies have indicated that vaginal treatment with IMN results in labor among 27-39% women within 24 hours. However, these positive results are less likely to be achieved among postterm women, and therefore, the expected number of women in labor was calculated to be 20-25. A power of 80% at the 5% significance level was reached if 22 out of 100 women went into labor within 24 hours compared to 7 out of 100 women in the placebo group. Thus, a total number of 200 women were included in the study. Fisher exact test was used for assessment of the efficacy of IMN for induction of labor. Changes in Bishop score and cervical length were analyzed using two sample *t*-test, while Mann-Whitney *U*-test was used for assessment of maternal side effects. All categorical outcomes in the two groups were compared using Fisher exact test. Logistic regression was used to assess the effect of IMN, parity, baseline cervical length, and baseline Bishop score on spontaneous labor outcome. All significance tests were two tailed. A $p < 0.05$ was considered to be statistically significant.

Paper III – An experimental randomized study

The aim of this study was to examine the relationship between NO and the two enzymes COX-1 and COX-2 in the cervical ripening process. Previous studies have demonstrated that COX-enzymes are present within the cervix at term pregnancy (Stjernholm-Vladic *et al,* 2004). The purpose of this investigation was to evaluate the effect of IMN on cervical COX-expression and to localize these enzymes within the cervical tissue. The techniques used for analysis were immunoblotting and immunohistochemistry (IHC). Twenty-four women scheduled for elective caesarean section were recruited to the study. Inclusion criteria were at least 37 weeks of gestation, cervical Bishop score <6, singleton pregnancy, no former vaginal delivery and uncomplicated pregnancy. All women included were 1–4 days before caesarean section informed of the nature and scope of the study, as well as the potential side effects of IMN. The women were randomized to receive either 40 mg of IMN (Ismo®, 2 tablets á 20 mg, Boehringer-Mannheim, Mannheim, Germany) or 2 placebo tablets of similar design administered intravaginally in the posterior fornix by an obstetrician 4 hours prior to elective caesarean section. After regional anaesthesia, the women were placed in the lithotomy position. Cervical tissue specimens were obtained transvaginally from the anterior lip of the cervix by use of a 14 gauge Tru-Cut biopsy needle (Allegiance Healthcare Corporation, McGore Park, IL, USA) (Figure 12). Three cylindrical biopsies of approximately 1.5 x 15 mm size (wet weight 25–40 mg) were obtained from each woman. Tissue specimens were immediately transferred to liquid nitrogen and frozen at -70ºC until analysis.

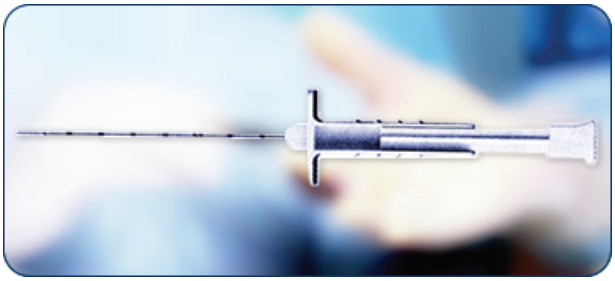


Figure 12. A Tru-cut® biopsy needle.

Immunoblotting

Immunoblotting is an immunologic method used for detecting or quantifying immunoreactive substances. This process involves the characterization of a factor based on the molecular weight and ability of antibody to bind to it. It is used to identify and characterize antigens

from a complex mixture. The antigen samples are resolved by separation in an analytical gel such as sodium dodecyl sulphate, peptide mapping gels or isoelectric focusing gels. The resolved molecules are transferred electrophoretically to a nitrocellulose membrane in a blotting tank. The blot is then treated with a monoclonal or polyclonal antibody to the specific antigen in question, then washed and treated with a radiolabeled conjugate, which detects the bound antibodies. After washing, the membrane is exposed to an X-ray film to produce an autoradiograph. The bands of antigen that have been bound to the antibodies are thereby made visible and can be quantified. The immunoblotting procedure for assessment of COX-1 and -2 expressions has previously been described in detail (Ekerhovd *et al,* 2000). In our study a monoclonal antibody raised against purified ovine COX-1 (Cayman Chemical Company, Ann Arbor, MI, USA), diluted 1:1000, was used for measurements of COX-1 expression. For determination of COX-2, a monoclonal antibody raised against a synthetic peptide from the human COX-2 580–599 amino acids sequence (Cayman Chemical Company), diluted 1:1000, was used. As positive controls commercially available standards, human COX-1 electrophoresis standard and human COX-2 electrophoresis standard (Cayman Chemical Company), were used.

Immunohistochemistry

Immunohistochemistry is used for localizing substances in tissue sections exploiting the principle of antibodies binding specifically to antigens. Visualizing an antibody-antigen complex can be accomplished in a number of ways. Most commonly, an antibody is conjugated to an enzyme, such as peroxidase, that catalyzes a color-producing reaction. Alternatively, the antibody can also be tagged to a fluorophore. The antibodies used for specific detection can be polyclonal or monoclonal. Monoclonal antibodies are generally considered to exhibit greater specificity as compared with polyclonal antibodies. However, the risk of using monoclonal antibodies is that the epitope can be damaged. Polyclonal antibodies are a heterogeneous mix of antibodies that recognize several epitopes. There are two strategies used for the immmunohistochemical detection of antigens in a tissue, the *direct* method (Figure 13) and the *indirect* method (Figure 14).

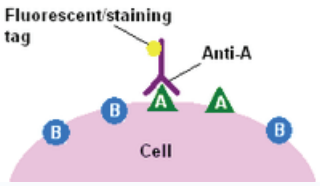


Figure 13. The direct method of immunohistochemical staining uses one labeled antibody, which binds directly to the antigen being stained for.

The *direct method* is a one-step staining method. It involves a labeled antibody reacting directly with the antigen in tissue sections. This technique utilizes only one antibody and the procedure is therefore simple and rapid. However, in some cases it may lack sensitivity due to little signal amplification and it is less common than the indirect method.

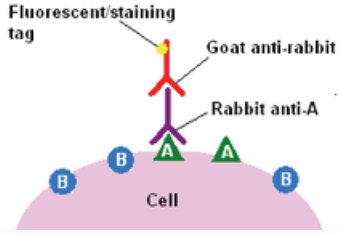


Figure 14. The indirect method of immunohistochemical staining uses one antibody against the antigen being probed for, and a second, labeled, antibody against the primary antigen-antibody complex.

The *indirect method* involves an unlabeled primary antibody (first layer) which reacts with tissue antigen, and a labeled secondary antibody (second layer) which reacts with the primary antigen-antibody complex. This method is more sensitive due to signal amplification through several secondary antibody reactions with different antigenic sites on the primary antibody. The second layer antibody can be labeled with a fluorescent dye or an enzyme. It is possible to control the sensitivity of the test either by leaving out the primary antibody or by preabsorption. Preabsorption is a procedure which aims to eliminate cross-activity of the antibody. Antibodies that bind non-specifically to different proteins are eliminated and antibodies that react with the protein of interest will remain.

In this study the indirect method, peroxidase staining and monoclonal antibodies were used. Tissue specimens for immunohistochemistry were embedded in OCT (Sakura Finetek, Zoeterwoude, The Netherlands). Sections, 6 μm thick, were rinsed in phosphate-buffered saline (PBS), treated with 0.3% hydrogen peroxide in methanol for 30 min, and then exposed to normal horse serum (Vector Laboratories Inc., Burlingame, CA, USA) for 20 min at room temperature. They were then incubated with the primary monoclonal antibody against either COX-1 or COX-2 (Cayman Chemical Company) diluted 1:100 in 1% bovine serum albumin/PBS at 4ºC overnight. The sections were rinsed in 0.05% Triton-X (Sigma Chemical Company, St. Louis, MO, USA) in PBS and then incubated with biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories) as secondary antibody for 30 min followed by the addition of avidin–biotin peroxidase complex (Vector Laboratories) for 60 min. To visualize immunoreactivity the specimens were exposed to 0.05% 3´, 3´-diaminobenzidine-tetrahydrochloride (Sigma Chemical Company) in PBS containing 0.03% hydrogen peroxide for 7 min. Two sections prepared from different levels of the cervical specimens were prepared from each of the women in the IMN and the placebo group, respectively. Negative controls were treated with PBS without any monoclonal antibody. Sections of epithelial ovarian carcinoma were used as positive controls.

Statistical analysis

Statistical analysis for immunoblotting was performed using the Mann–Whitney *U*-test. The results were expressed as median and range. A $p < 0.05$ was considered to be statistically significant. For immunohistochemical analysis, the slides were examined by two independent observers using light microscopy. Localization of positive staining was carried out, but no attempt to quantify staining by objective methods was performed.

Paper IV – A double-blind randomized controlled clinical trial

This investigation was conducted as a small pilot study where 24 patients were included. The primary outcome was the effect of the combination of oxytocin (the common agent used for treatment of retained placenta) and nitroglycerin on retained placenta. Secondary outcomes were maternal and fetal side effects and maternal hemodynamic effects. The trial was designed as a prospective, double blind, randomized controlled study. Inclusion criteria were uncomplicated singleton pregnancy with spontaneous vertex delivery of a healthy child. Exclusion criteria were gestation less than 37 weeks, postpartum hemorrhage requiring immediate intervention, uterine malformation, intolerance to nitroglycerin, maternal age less than 18 years, suspected placental accretism, and serious maternal disease, defined as daily use of medication.

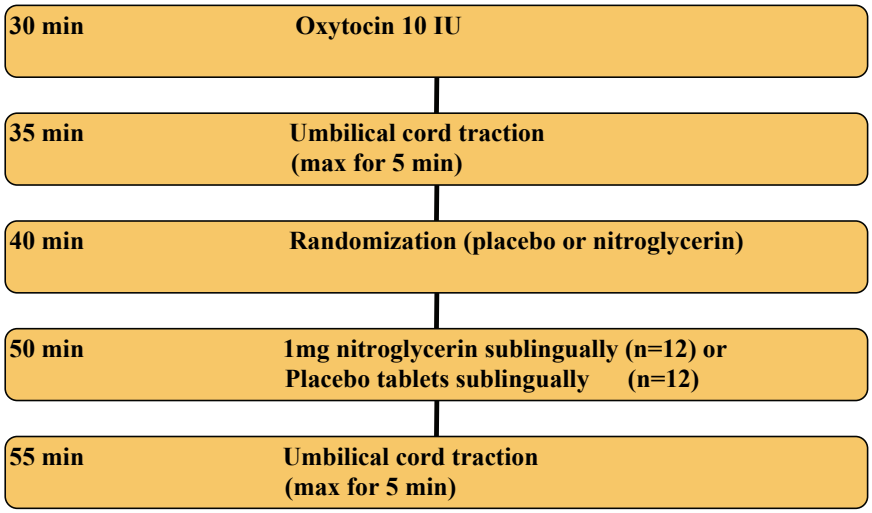


Figure 15. Schematic procedure of retained placenta from time of delivery of infant.

During the recruitment period, all women who underwent vaginal delivery had standard active management of the third stage of labor according to written hospital protocols, including intravenous or intramuscular administration of 5 IU oxytocin (Syntocinon®, Novartis, Täby, Sweden) within minutes after delivery of the neonate. Maternal effort was encouraged to expel the placenta. If this was insufficient, controlled umbilical cord traction was used. This procedure involved applying steady, gentle traction to the cord with a Kelly clamp while

providing counter tracking against the uterine fundus. If the placenta still remained undelivered 30 min after delivery of the neonate an additional dose of 10 IU oxytocin was given intravenously to induce more efficient uterine contractions and thus promote placental separation. Five minutes later a new active attempt to deliver placenta was performed in a similar way. If placenta remained undelivered after 40 minutes the women were asked to be included in the study and randomization was carried out by means of numbered opaque, sealed envelopes prepared with random-number tables. This procedure was performed by a research nurse. Once the diagnosis of retained placenta had been made, intravenous infusion was initiated and all women were hemodynamically monitored. Figure 15 shows the whole procedure as a flow chart from delivery of the infant to placental separation. It is important to emphasize the interval of at least five minutes between medication and umbilical cord traction since the half-life of sublingual nitroglycerin is approximately three minutes. The sublingual administration was chosen instead of intravenous administration just to simplify the method. Neither the participating women nor the obstetrician on duty were aware of the agent administered. If placenta was not expelled within 60 minutes, manual removal of the placenta was performed at the operating theatre under regional or general anaesthesia. To reduce postoperative hemorrhage, 5 IU of oxytocin was routinely administered intravenously at the end of the operative manual removal. Maternal blood pressure and pulse rate were measured immediately prior to the sublingual administration of either nitroglycerin or placebo tablets. These measurements were repeated 15 min later for assessment of possible hemodynamic effects caused by the nitroglycerin tablets. Blood loss during the third stage of delivery was registered in each case. In addition, all the women completed a questionnaire about possible side effects of nitroglycerin.

Statistical analysis

A sample size of 24 women (12 in each group) was calculated to yield a power of 90% at the 5% significance level when assuming a success rate of 80% in the treated group compared to a 20% success rate in the placebo group. Mean±SD and range were calculated for descriptive purposes. For statistical comparison the Student's *t*-test was used for continuous variables and the Chi squared test was used for dichotomous variables.

RESULTS AND COMMENTS

Paper I

Results

All 60 women included in the study underwent uncomplicated delivery by caesarean section. No serious maternal complications or signs of fetal distress resulting in specific medication or emergency caesarean section. All infants were healthy and had full Apgar scores (9-10) at 5 minutes, normal pH-values in the umbilical artery and none needed specific neonatal surveillance. There were no complications associated with the measurement of cervical distensibility. Table 3 shows the results of primary outcome in the study.

Results - efficacy

	IMN (n=30)	Placebo (n=30)	p-value
Cervical length (mm)			
0 min	36.3±3.3 36 (30-42)	35.1±2.7 35 (30-41)	ns
210 min	36.1±3.1 36 (30-42)	35.1±2.7 35 (30-41)	ns
Cervical distensibility (N) (cumulative force to dilate cervix from 5 till 10 mm)	2.7±1.2 2.5 (1-5)	6.4±2.1 6 (3-10)	<0.0001

Data are shown as mean±SD, median och range (min-max), ns=not significant

Table 3. Effect on cervical distensibility measured by a cervical tonometer following 4 hours of intravaginal treatment with 40mg of IMN. N=Newton.

There was a clear significant effect on cervical distensibility. The average distensibility measured in Newton in the treated group was approximately half of the distensibility in the

placebo group (p <0.0001). However, no change in cervical length could be measured by transvaginal ultrasonography approximately 210 minutes after administration of the NO donor. There were no significant differences in mean age, gestational age, pretreatment Bishop score, and treatment interval between the two groups.

Secondary outcome measures were maternal and fetal side effects.

Safety - mother

		IMN (n=30)	Placebo (n=30)	p-value
Maternal systolic blood pressure (mmHg)				
	initially	112±6.9	110.7±7.5	ns
		110 (95-125)	110 (100-130)	
	at 180 min	105.5±5.5	116.0±6.5	<0.001
		105 (90-120)	115 (100-130)	
Maternal diastolic blood pressure				
	initially	74.5±4.4	73.0±4.8	ns
		75 (70-80)	70 (65-80)	
	at 180 min	64.3±6.0	74.0±4.6	<0.0001
		65 (55-75)	75 (65-85)	
Maternal pulse rate	initially	73.2±8.3	71.7±7.4	ns
		72 (55-85)	72 (60-85)	
	at 180 min	84.0±8.4	76.5±8.3	<0.01
		85 (65-95)	75 (60-90)	
Intraoperative blood loss		346.7±140.7	360.0±122.1	ns
		300 (200-600)	400 (200-600)	

Data are shown as mean±SD, median and range, ns=not significant

Table 4. Maternal hemodynamic effects following vaginal treatment with 40 mg of IMN.

Isosorbide mononitrate is a known vasodilatator. Therefore, it is important to evaluate possible effects of the treatment on maternal blood pressure and pulse rate. In the IMN treated group there were significant effects in decrease of both systolic and diastolic blood pressure. The mean maternal pulse rate in the group 180 minutes after intravaginal administration was 84.0 beats/min. In the placebo group, the mean maternal pulse rate at this time point was 76.5 beats/min. Thus, a significant difference of 7.5 beats/min in mean maternal pulse rate was measured (p <0.01). However, as can be seen in Table 4, none of the women suffered from a clinically important decrease in blood pressure. Thus, no treatment for low blood pressure or

high pulse rate was needed. The lowest systolic blood pressure measured was 90 in the treated group and the lowest value in diastolic blood pressure was 55. No difference in total blood loss was registered between the groups and no woman needed blood transfusion. The mean blood loss in the treated group was 300 ml compared to 400 ml in the placebo group (ns).

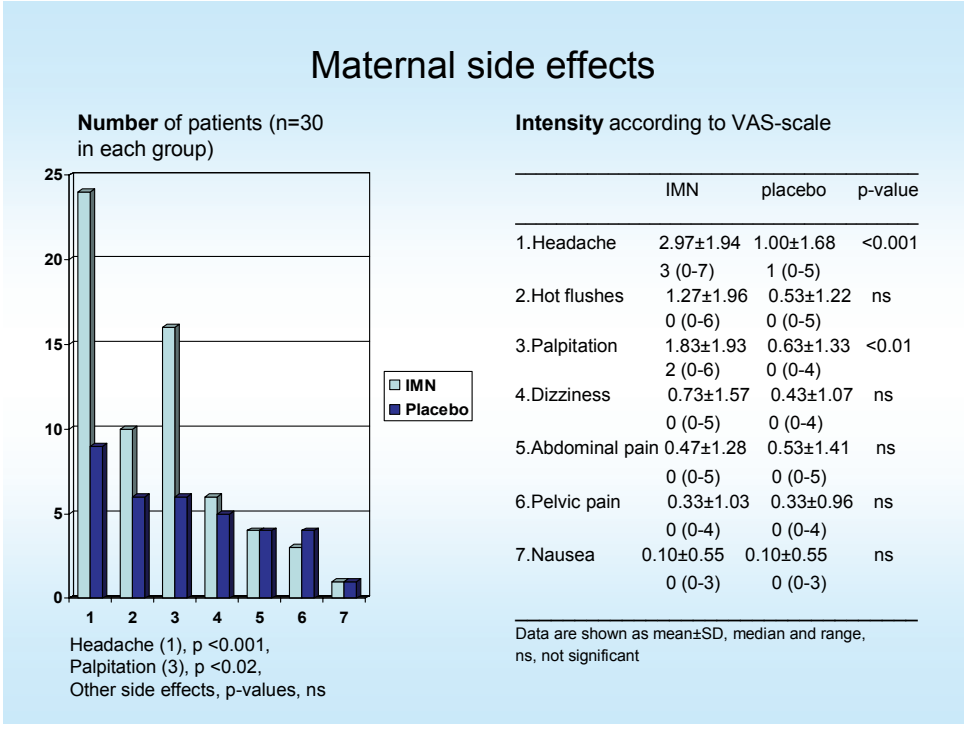


Table 5. Maternal side effects of IMN treatment.

The most frequent symptomatic side effect in the IMN group was headache, experienced by 24 of 30 women (Table 5). In the placebo group, 9 of 30 women reported headache immediately before the start of caesarean delivery ($p < 0.001$). In addition, a significant increase ($p < 0.02$) in frequency of palpitations was registered in women treated with IMN at this time point. There was no statistically significant difference in reported symptoms regarding hot flushes, dizziness, abdominal pain, pelvic pain, and nausea between the two groups. According to the results of the questionnaire, the side effects were not regarded as serious. None of the women required medical treatment due to the side effects.

Neither the umbilical artery resistance index (RI) nor the pulsatility index (PI) was influenced by IMN 180 minutes after intravaginal administration (Table 6). All CTGs intermittently performed during the preoperative observational period were normal and without any signs of fetal distress. Thus, fetal heart rate was within the normal range (110-150 beats/min) in all patients examined. No significant difference in fetal heart rate was registered between the IMN group and the placebo group 210 minutes after intravaginal administration. Umbilical arterial pH and Apgar score did not differ between the two groups.

Safety - infant

	IMN (n=30)	placebo (n=30)	p-value
Pulsatility index			
initially	0.82±0.06	0.81±0.07	ns
	0.82 (0.68-0.94)	0.80 (0.70-0.96)	
at 180 min	0.79±0.06	0.80±0.06	ns
	0.80 (0.66-0.92)	**0.80** (0.66-0.92)	
Resistance index			
initially	0.58±0.05	0.57±0.05	ns
	0.58 (0.48-0.67)	0.57 (0.48-0.66)	
at 180 min	0.57±0.05	0.57±0.06	ns
	0.56 (0.49-0.68)	**0.56** (0.50-0.68)	
pH in a.umbilicalis	7.34±0.03	7.33±0.02	ns
	7.34 (7.28-7.38)	**7.34** (7.29-7.38)	
Fetal heart rate			
initially	135.5±9.5	135.0±10.4	ns
	135 (115-150)	135 (115-150)	
at 210 min	135.3±8.2	135.8±7.6	ns
	135 (120-150)	**135** (115-145)	
Apgar score at 5 min	9.8±0.4	9.9±0.3	ns
	10 (9-10)	**10** (9-10)	

Data are shown as mean±SD, median and range, ns=not significant

Table 6. Registered fetal parameters in the IMN group and the placebo group.

Comments

This study demonstrated that 40 mg of vaginally administered IMN increases cervical distensibility following 4 hours of treatment in pregnant women at term. Thus, one can conclude that the time interval of 4 hours and the dosage seems to be sufficient to promote cervical ripening. The result is in accordance with previous reports on the effect of 40 mg of IMN on human cervical distensibility in first trimester pregnancy (Thomson *et al*, 1997). In the current study, no change in cervical length was observed by transvaginal ultrasonography

210 minutes after intravaginal application of IMN. A cervical response characterized by an increased distensibility and no change in cervical length seems to be typical for NO donors. This effect may partly be due to the fact that NO does not induce uterine contractions. A similar effect on the cervix has previously been demonstrated after local treatment with the NO donor sodium nitroprusside in guinea pigs during advanced pregnancy (Chwalisz *et al*, 1997).

When introducing a medical agent for a new indication, as is the case for NO donors as cervical ripeners, it is important not only to evaluate the efficacy of the agent but also possible side effects. When using NO donors for cervical ripening at term one has to consider possible adverse effects on both the mother and fetus. Nitric oxide donors have previously been used in obstetrics for uterine relaxing purposes at caesarean section, at vaginal breech delivery or to relieve intrapartum fetal distress related to uterine hyperactivity (Wessen *et al,* 1995; Mercier *et al,* 1997). They have also been used for treatment of preterm cervical dilatation (Rowlands *et al*, 1996). A recent study has also shown that transdermal nitroglycerin may reduce neonatal morbidity and mortality as a result of decreased risk of birth before 28 weeks (Smith *et al,* 2007). Intravenous nitroglycerin is known as a rapid relaxant with effect already within 60 seconds. The vasodilating effect of NO, causing a decrease in blood pressure, must always be considered. However, in previous reports no serious maternal or fetal complications have been reported (DeSimone, 1990; Dufour *et al*, 1997; Chedraui and Insuasti, 2003). Isosorbide mononitrate is known to be a slow-releasing NO donor. When administered into the vagina in the late third trimester peak serum levels of IMN were still not achieved at 6 hours (Bates *et al*, 2003). Of importance is also the fact that when administered vaginally serum levels of IMN are significantly lower than when administered orally or intravenously, probably due to first passage through cervix and uterus. With lower serum levels of IMN when vaginally administered the risk of maternal and fetal hemodynamic effects of IMN may decrease, but must still be taken into consideration. Prior to the present study, only one report on maternal and fetal hemodynamics had been published (Nicoll *et al,* 2001). The results of our study are consistant with this previous study.

Neither the umbilical artery resistance index nor the pulsatility index was influenced by 40 mg of IMN 180 minutes after intravaginal administration. All CTG registrations intermittently performed during the preoperative observational period were normal and without any signs of fetal distress, fetal heart rate being within the normal range (110-150 beats/min) in all patients

examined. Nicoll and co-workers observed a difference in fetal heart rate at 60 minutes (a slight increase in the IMN group), but only at this time point during registration. This observation could not be verified in our study. No significant difference in fetal heart rate was registered between the IMN group and the placebo group 210 minutes after intravaginal administration. Umbilical arterial pH and Apgar score did not differ between the two groups.

The results of maternal hemodynamic and side effects reveal a significant decrease in maternal blood pressure and increase in pulse rate, but seem to be of no clinical importance. Headache and palpitations were common side effects, but only one woman described any of the symptoms as having an intensity of 7 on a visual analogue scale between 0 and 10. Thus, almost all reported symptoms were of moderate intensity. None of the participating women needed medical treatment of the symptoms.

In summary, the results from this study indicate that 40 mg of IMN administered vaginally within 4 hours has an effect on cervical ripening without causing uterine contractions within this period of time. The number of women in this study is too small to make any conclusive statements regarding the safety of vaginally administered IMN. Headache and palpitations, experienced by the majority of treated women, may not be considered as minor side effect. Likewise, the reduction of systolic and diastolic blood pressures might be regarded as an important adverse reaction. Nevertheless, the current study indicates that intravaginal treatment with IMN is well tolerated by the women as well as the newborn infants. In addition, no adverse effects as to the infant were registered by CTG, Doppler ultrasound, umbilical arterial pH, and Apgar scoring. Later studies on vaginally administered IMN for cervical ripening at term have confirmed that IMN treatment compared to placebo or PG does not increase the risk of fetal distress. The present study indicated that IMN can be used clinically for cervical ripening.

Paper II

Results

All 200 women included in the study fulfilled the inclusion criteria and completed the study. There were no significant differences between the two groups with respect to maternal age, parity, and gestational age. The main purpose of this investigation was to evaluate the number of postterm women who went into labor within 16-24 hours after application of 40 mg of IMN in an outpatient setting. The results revealed that 22 out of 100 treated women compared to 8 women in the placebo group went into labor (p =0.01) before the return appointment the following day (table 7). Two women who went into labor following treatment with IMN underwent emergency caesarean section due to fetal distress. The other women who went into labor within 24 h had vaginal deliveries, except for one woman in the placebo group who had a caesarean section due to failure of progress. Mean time from vaginal administration of tablets to the first painful contractions was 14.0 h in the IMN group and 15.3 h in the placebo group, respectively (ns).

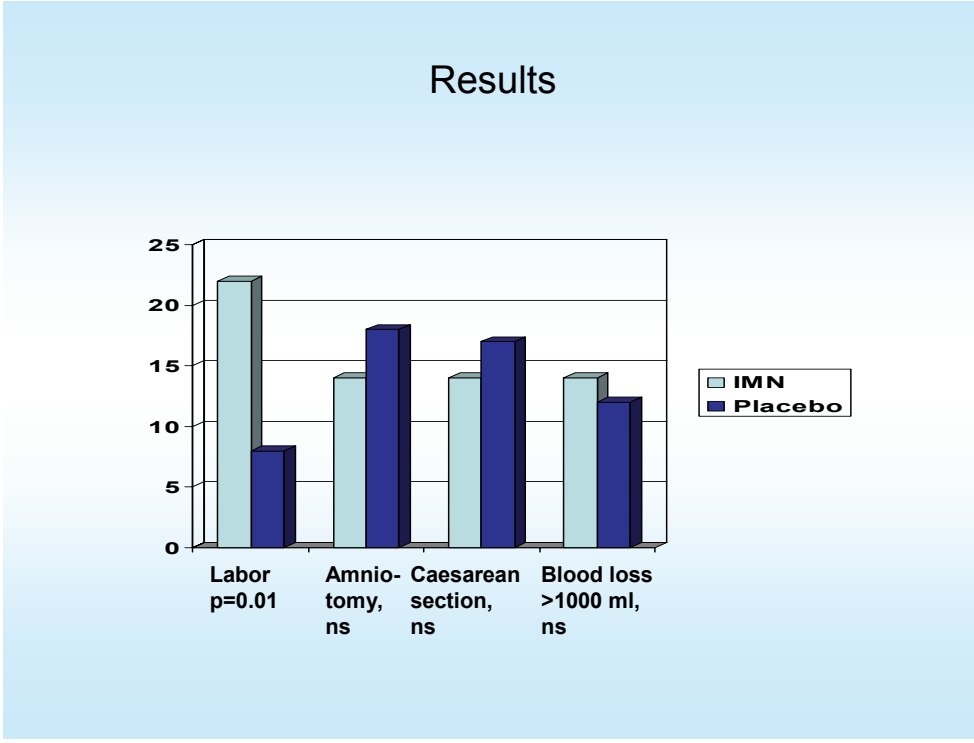


Table 7. Illustration of the results regarding labor, amniotomy, caesarean section, and blood loss.

The 95% confidence interval (CI) for the difference between the proportions of women who went into labor was 0.04-0.24. Regression analysis with spontaneous labor as dependent variable and case-control status, parity, baseline cervical length and baseline Bishop score as independent variables, showed that case-control status and baseline Bishop score remained significant (p=0.003 and p=0.001, respectively). The odds ratio for going into labor was 4.0 (95% CI 1.6-9.8) for the treatment group compared to the placebo group, and 2.0 (95% CI 1.3-2.9) for each unit increase in baseline Bishop score.

Cervical ripening assessed as cervical length measured by transvaginal ultrasound and modified Bishop score was only possible to evaluate among women who did not go into labor. Therefore, the women are divided into 2 groups: women who went into labor and women who did not (Table 8).

Clinical data - mother

Total number of women	IMN (n=100)	Placebo (n=100)	p-value
Women in labor	**n=22**	**n=8**	0.01
Bishop score at baseline	2.5±1.0 (1-4)	3.1±1.0 (1-4)	ns
Cervical length at baseline (mm)	28.9±8.6 (15-51)	28.2±8.4 (17-42)	ns
Parity			
nulliparous	12	6	ns
multiparous	10	2	ns
Treatment interval to contractions (hours)	14.0±5.0 (4-23)	15.3±4.2 (10-22)	ns
Women not in labor	**n=78**	**n=92**	
Bishop score at baseline	1.8±1.2 (0-5)	2.0±1.1 (0-5)	ns
at return appointment	1.7±1.4 (0-5)	1.3±1.5 (1-5)	ns
Cervical length at baseline	31.2±9.4 (10-55)	31.1±8.2 (9-47)	ns
at return appointment	-6.0±8.8 (5-31)	-5.2±10.2 (10-40)	ns
Parity			
nulliparous	60	68	ns
multiparous	18	24	ns

Data are shown as mean±SD and range (min-max), ns= not significant

Table 8. Clinical data of women who went into labor compared to women who did not go into labor.

There was no statistical difference between the groups in cervical length or Bishop score among the women who did not go into labor. Sixty-four women initially treated with IMN and 74 women in the placebo group had a Bishop score < 6 (ns). These women were given 1 mg of PGE_2 vaginally for further cervical ripening. In fourteen women in the IMN group and in eighteen women in the placebo group Bishop score was ≥6 (ns) and amniotomy was carried out for induction of labor.

Side effects and maternal satisfaction

	IMN (n=94)	Placebo (n=98)	p-value
Positive experience	42/88	31/94	0.01[a)]
Negative experience	7/88	12/94	
Recommendation of same treatment to other women	81/91 (89%)	71/90 (79%)	ns[b)]
VAS-scales			
Headache (after 6 hours)	88 (94%) 3.9±2.2* (0-9)	8 (8%) 0.1±0.6* (0-4)	<0.0001[a)]
Nausea (6 hours)	19 (20%) 0.5±1.2* (0-6)	5 (5%) 0.1±0.4* (0-2)	0.001[a)]
Uterine contractions (after 6 hours)	50 (53%) 1.2±1.5* (0-7)	48 (48%) 1.1±1.6* (0-8)	ns[a)]

*mean±SD, range (min-max), a) Mann-Whitney *U*-test, b) Fisher exact test, ns= not significant

Table 9. Maternal side effects and satisfaction.

The most common side effect in women treated with IMN was headache, experienced by 88 women (88%) (Table 9). The intensity of headache was reported to be moderate in most cases with a median value of 4.0 (range 0-9) on the visual analogue scale. In the placebo group eight women (8%) reported headache of mild or moderate intensity. Nausea of mild intensity was reported by 19 women (19%) in the IMN group and by five women (5%) in the placebo group. All other reported side effects were infrequent.

Ninety-four women (94%) in the IMN group and 99 women (99%) in the placebo group returned the questionnaire regarding how they had experienced the outpatient procedure. The vast majority of women in both groups were either positive or very positive to the treatment. Eighty-nine of the women (94.7%) in the IMN group and 93 of the women (93.9%) in the placebo group reported that they would recommend the procedure.

Neonatal outcome

	IMN (n=100)	Placebo (n=100)	p-value
Birth weight (g)	3867±476* (2505-5590)	3955±484* (2990-5865)	ns[a)]
Apgar score (5 min)	9.5±1.1* (2-10)	9.6±0.9* (6-10)	ns[b)]
pH i a.umb	7.2±0.1* (6.84-7.43)	7.2±0.1* (6.97-7.37)	ns[a)]
Apgar score <7 (5 min)	2	1	ns[c)]
pH in a.umb <7.05 (n)	4	8	ns[c)]
Admission to NICU (n)	13**	9***	ns[c)]

*mean±SD, range. a) independent samples t-test b)Mann-Whiney *U*-test
c) Fisher exact test, ns=not significant
**cause: infection(4), general seizures(2), heart disease(2), meconium aspiration(3), high bilirubin levels(1), hypoglycemia(1)
***cause:infection(4), heart disease(1), meconium aspiration(1), nutritional problems(2), hypoglycemia(1)

Table 10. Clinical data on neonatal outcome.

In the IMN group two infants suffered from general seizures. These infants were both delivered instrumentally by vacuum extraction due to threatening asphyxia. Both had normal pH values in the umbilical artery, but according to the medical records both vacuum extractions were difficult to perform. Both infants had general seizures of short duration, but subsequent examination with EEG showed normal patterns. Both infants have later developed normally and have no medication at one year of age. pH in the umbilical artery was <7.05 among eight infants in the placebo group compared to four infants in the IMN group. A total of three infants had Apgar score <5 after 5 minutes, but all these infants had pH >7.05 in the umbilical artery. To conclude, neonatal outcome was similar in both groups (Table 10).

Comments

Chanrachakul and co-workers were the first authors to suggest that NO donors administered vaginally at term could have an effect on both cervical ripening and labor induction. They studied the effect of both IMN and GTN (NO donors) in three different trials on cervical ripening and labor induction and reported onset of labor in 27-39% (Chanrachakul *et al,* 2000a; 2000b; 2003). In one study 40 mg of IMN was repeated vaginally up to three times every sixth hour and no hyperstimulation of the uterus with secondary fetal distress was observed. Our study confirms that 40 mg of IMN seems to be efficacious for cervical ripening and labor induction at term. The results also indicate that outpatient IMN induced cervical ripening is likely to be a safe procedure, since neither fetal nor maternal side effects of clinical importance were registered.

In our study the primary outcome (onset of labor) was not as promising as earlier studies, as only 22 % went into labor. Isosorbide mononitrate is a slow releasing agent especially when administered vaginally. Bates and co-workers studied serum levels of IMN after vaginal administration of 40 mg of IMN. They observed that peak levels were not reached before six hours. It is therefore plausible that the effect of the treatment lasted longer than 16-24 hours, which was the cut off time for evaluation in our study, and that more women would have gone into labor if the return appointment time had been postponed. It is also important to stress that the women were postterm. A previous study has shown that cervical expression of NO metabolites was seen among only 60% of women postterm compared to 87% among women at term pregnancy (Väisänen-Tommiska *et al*, 2004). Thus, it has been suggested that reduced cervical NO production may contribute to prolonged pregnancy. Vaginal administration of an NO donor, as performed in the present study, could result in sufficient cervical levels of NO to promote efficacious cervical ripening and induction of labor. It could also be that repeated administration of IMN, which was the case in one of the cited studies, is likely to be more efficacious. In one study commencement of uterine contractions was found to be associated with increased levels of cervical NO metabolites (Väisänen-Tommiska *et al*, 2003). It has also been demonstrated that IMN induces cervical production of COX-2, an enzyme that is involved in PG synthesis (Ekerhovd *et al*, 2002). Prostaglandins, stimulating both cervical ripening as well as uterine contractile activity, may mediate the NO associated effects on labor induction.

The study was designed as a prospective double-blind randomized study in an outpatient setting. The women were not observed during the time of treatment until onset of labor or time for return appointment. This procedure was considered to be safe for several reasons. All women were carefully informed to return to the labor ward if onset of painful contractions or unforseen side effects of the treatment were experienced. In a previous study, single as well as repeated treatment with 40 mg of IMN for 24 hours had shown no adverse effects on the fetus. Furthermore, only women with normal pregnancy, normal fetal heart pattern and normal amniotic fluid index were included. When analyzing the results concerning fetal side effects one can conclude that there was no difference in neonatal outcome between the groups. Relatively many infants in the present study needed NICU care (n=22). This could be due to the fact that postterm pregnancies are associated with a higher fetal morbidity than term pregnancies. Comparing the two groups, more infants in the treated group needed NICU care (13 versus 9). However, the difference was not statistically significant. On the other hand, more infants in the placebo group (8 versus 4) had an umbilical artery pH <7.05. This study has not revealed differences in fetal outcome according to Apgar score, umbilical pH and need of NICU care. However, larger studies of similar design are necessary before final conclusions can be made.

Paper III

Results

Analyses were based on cervical biopsies obtained from 24 women. Twelve women had been treated with 40 mg of IMN for 4 hours, while the other 12 women had been given placebo tablets. Western blotting revealed significant increase in COX-2, but not COX-1, following vaginal administration of 40 mg IMN. In the IMN group the median for COX-2 was 16.8 compared to 1.5 in the placebo group, but the medians for COX-1 were similar in both groups (Table 11).

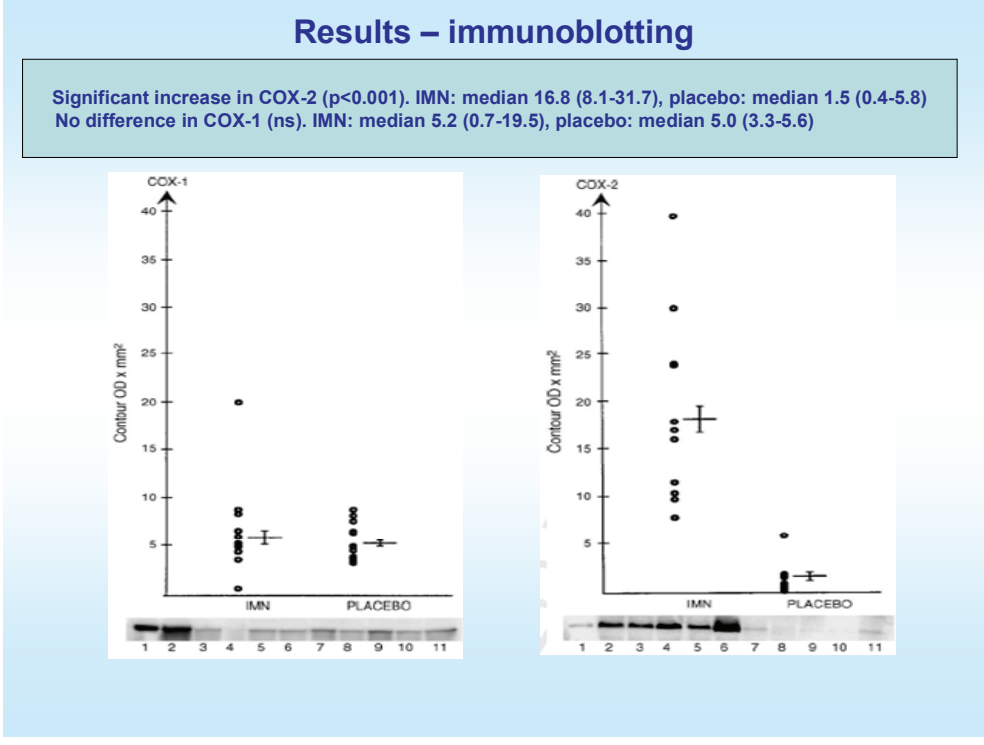


Table 11. Results on cervical expression of COX enzymes.

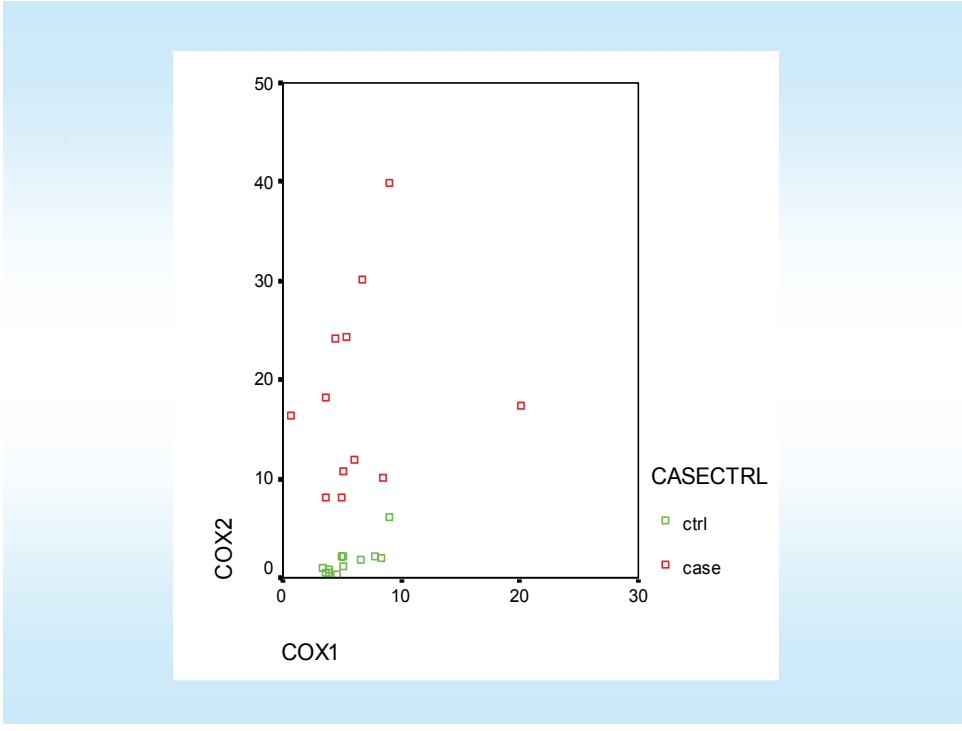


Table 12. **Illustration of correlation between case and control for COX-1 and COX-2. Case=treated women, ctrl=control group.**

Table 12 describes the correlation between cases and controls for the two enzymes. It shows that the correlation is strong for COX-1, but not for COX-2.

Positive immunostaining for both COX-1 and -2 was found in smooth muscle of the vessel wall, in bundles of stromal smooth muscle, and in glandular and squamous epithelium. In addition, immunopositive cells, probably representing inflammatory cells, were observed as isolated cells or as clusters of cells. No difference in the distribution of COX-1 positive cells or staining intensity of these cells was observed between the two groups. Although the number of immunopositive cells per visual area was too low to make any statistical calculation there was an overall impression by the two independent observers that the staining for COX-2, especially in the ‘‘inflammatory’’ stromal cells, was stronger in women treated with IMN as compared with women given placebo tablets. In sections where the squamous epithelium was included, staining intensity was found to be stronger for COX-1 than for COX-2 (Figure 16).

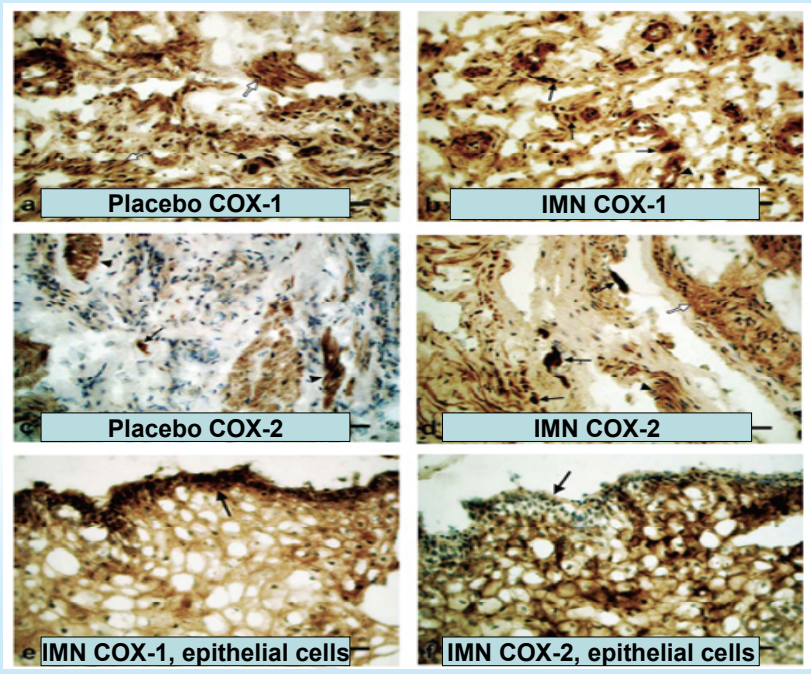


Figure 16. Immunohistochemical staining for COX-1 and -2 in cervical specimens obtained from women treated with IMN and women given placebo tablets. Scale bars = 50 µm. (a) Placebo. Positive staining for COX-1 is seen in smooth muscle of blood vessels (arrowhead), stromal smooth muscle (open arrow), and in stromal interstitial cells (arrow). (b) IMN treatment. Cervical tissue where positive staining for COX-1 is seen in smooth muscle of blood vessels (arrowhead) and in clusters of interstitial cells in the perivascular space (arrow). (c) Placebo. Positive staining for COX-2 is seen in smooth muscle (arrowhead) and in an isolated stromal cell (arrow). (d) IMN treatment. Positive staining for COX-2 is seen in smooth muscle of blood vessels (open arrow), stromal smooth muscle (arrowhead) and in perivascular and stromal cells (arrow). (e) IMN treatment. Positive staining for COX-1 is seen in epithelial cells (arrow). (f) IMN treatment. Positive staining for COX-2 is seen in epithelial cells (arrow).

Comments

Immunohistochemistry did not reveal any difference in cell types that turned out to be COX immunopositive between the two groups of women. Nevertheless, it was an overall impression that the staining for COX-2, especially in interstitial cells, was more intense in women treated with IMN as compared to women in the placebo group. The aim of immunohistochemistry was only to describe the distribution of COX enzymes within the tissue, not to quantitatively measure the expression of COX enzymes. The results are in agreement with a study performed by Stjernholm-Vladic and co-workers (2004), who described the distribution of COX enzymes within cervical tissue at term parturition.

The results indicate that NO donors administered vaginally stimulate endogenous COX-2 production in cervical tissue. However, there was no increase in expression of COX-1. COX-2 is typically undetectable in most tissues under normal physiological conditions but can be expressed at high levels following stimulation. Cervical ripening has been described as an inflammatory action as a result of influx of macrophages and inflammatory mediators. Therefore, the increased expression of COX-2, which is involved in inflammatory processes, but not of COX-1 (the constitutive isoform), following stimulation by IMN appears logical. Besides, several studies have demonstrated that NO can induce the expression of COX-2, thereby increasing PG synthesis (Salvemini *et al,* 1993; 1994; 1999; Davidge *et al*, 1995; Maul *et al*, 2003). However, a recent study has shown that there is an increase in both cervical COX-1 and COX-2 at parturition (Stjernholm-Vladic *et al*, 2004). The present study could not verify that the expression of COX-1 was increased following NO stimulation. It is possible, though, that COX-1 increases gradually over a longer period of time. In our study cervical biopsies were obtained already within 4 hours after administration of NO donor, which could explain why COX-1 was not increased. Alternatively, NO preferentially has a stimulatory effect on COX-2. When immunoblotting is carried out to assess COX expression it is important to bear in mind that the binding to COX-antibodies comprises both active and inactive enzymes. Consequently, the semiquantitative estimation of the enzymes does not directly relate to enzyme activity. This means that one can only presume that the increase in COX-2 expression was due to new enzyme synthesis. As COX- enzymes catalyze the PG synthesis, our results indicate that an interaction between NO and PG synthesis is present in the uterine cervix at term. Nitric oxide exerts its effects either via cGMP as a second messenger or via cGMP-independent pathways. In cervical tissue specimens obtained from

women before surgical termination of pregnancy in the first trimester, levels of cGMP as well as COX-2 are increased following intravaginal administration of IMN (Ekerhovd *et al,* 2002). However, Salvemini and co-workers (1993) presented evidence that NO mediated stimulation on PGE_2 synthesis is cGMP independent. The pathway leading to COX activation by NO is unknown, but it may involve an interaction at the iron-heme center of the enzyme. It is known that COX contains an iron-heme center at the active site (de Groot *et al*, 1975) and that NO interacts with iron-containing enzymes (Karthein *et al,* 1987; Moncada *et al,* 1991). There is increasing evidence that there is a link between COX and NOS pathways. In a recent study, it was reported that misoprostol, a PGE_1 analogue, stimulates cervical NO release in pregnant women and that the sensitivity of NO synthesis to misoprostol was enhanced at term (Väisänen-Tommiska *et al,* 2005). On the other hand, it has also been shown that NO stimulates PGE_2 release from cervical tissue explants and that cultured cervical tissue after *in vivo* treatment with IMN increases $PGF_{2\alpha}$ release significantly (Denison *et al,* 1999; Ledingham *et al,* 1999a) Thus, a chain reaction where the initial NO stimulation caused by PG is followed by an endogenous release of PG triggered by NO has been suggested (Väisänen-Tommiska *et al,* 2005). The interaction between NO and PG synthesis indicates a possible joint action of the two substances which may be of importance for optimal cervical ripening at term.

Paper IV

Results

In all women treated with combined oxytocin – nitroglycerin all placentas detached and were delivered as compared with just one placenta in women treated with oxytocin only (Table 13).

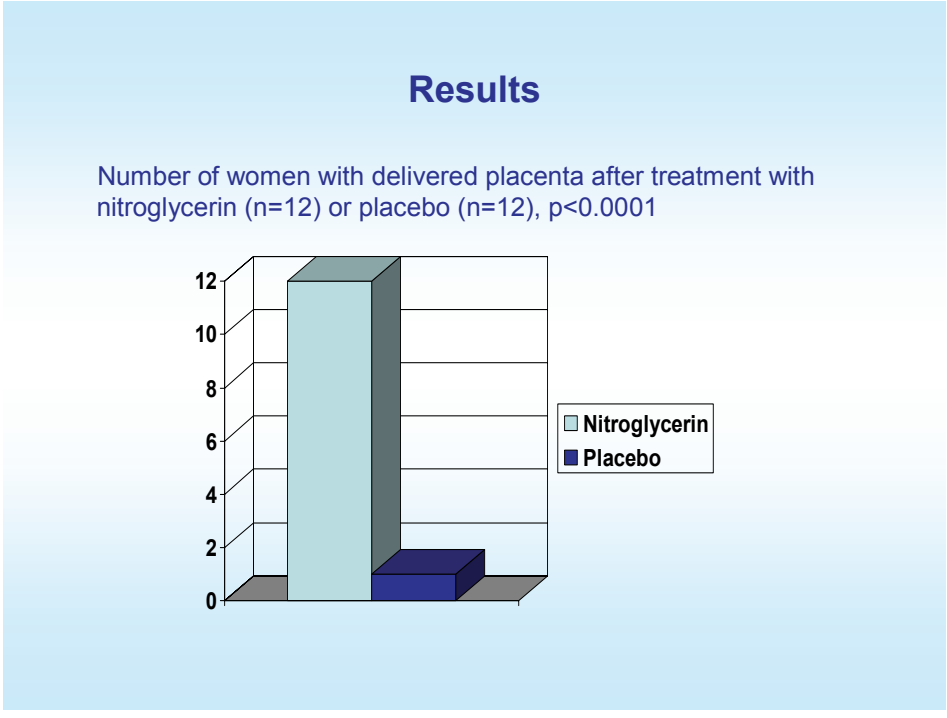


Table 13. All 12 women treated with nitroglycerin had successful delivery of placenta, compared to only one out of 12 women in the placebo group.

Hemodynamic results

	Before NG (n=12)	15 min after NG	p-value
Systolic BP	119.2±7.3 (110,130)	112.9±7.8 (100,125)	0.003
Diastolic BP	76.2±5.7 (70,85)	71.2±4.3 (65,80)	0.001
Pulse	76.7±5.4 (70,86)	78.7±4.2 (74,86)	NS
	Before placebo (n=12)	15 min after placebo	
Systolic BP	117.1±7.2 (105,130)	114.6±6.2 (105,120)	0.026
Diastolic BP	72.9±7.5 (60,85)	71.7±6.5 (60,80)	NS
Pulse	75.5±5.7 (66,86)	71.5±3.7 (62,76)	0.004
	Before and 15 min after NG	Before and 15 min after placebo	
Difference in syst BP	-6.2±5.7 (-15,5)	-2.5±3.4 (-10,0)	NS
Difference in diast BP	-5.0±3.7 (-10,0)	-1.2±3.1 (-5,5)	0.013
Difference in pulse	2.0±3.5 (-4,6)	-4.0±3.8 (-10, 2)	0.001

Data are shown as mean±SD, range. NS=not significant. BP=blood pressure

Table 14. Effect of the treatment on blood pressure and pulse rate. NG=nitroglycerin.

In women treated with nitroglycerin there were hemodynamic side effects, as a decrease in systolic and diastolic blood pressure as well as an increase in pulse rate were registered. A decrease in systolic blood pressure in the placebo group was also measured. The differences between the two groups in diastolic blood pressure and pulse rate were statistically significant. No woman needed treatment for NO induced hemodynamic effects or other side effects. The blood loss was significantly less in the treatment group ($p < 0.0001$). Headache was experienced by four women in this group. Two of the women reported headache of moderate intensity, while the other two women reported headache of mild intensity.

Comments

The aim of this study was to rule out whether combined treatment with oxytocin and nitroglycerin is effective in women with retained placenta. To our knowledge, sublingual nitroglycerin has never been used in combination with oxytocin or as a single treatment of retained placenta. Previous reports on nitroglycerin administered intravenously have shown to be effective in the management of retained placenta and with no serious effects on maternal

hemodynamics. This study confirms previous results on hemodynamic measurements, but of importance is to note, that no woman needed medical treatment for decreased blood pressure. Until more knowledge has been achieved in this matter it is recommendable that a running intravenous infusion always is given, and that only hemodynamically stable women with no excessive bleeding can be subjects for this treatment. The maternal side effects with headache proved to be of minor importance, but additional studies are needed before further conclusions can be made. One explanation to the encouraging results may be that the treatment was a sequential combination of oxytocin and nitroglycerin, both known to have an effect on retained placenta *per se*. However, it is interesting that these two agents seem to be efficacious, although they have different effects on uterine contractility. Oxytocin is known to cause uterine contractions, whereas nitroglycerin has a relaxing effect on the uterine body. As previously discussed, oxytocin has been used for many years for management of the third stage of labor. However, there are no conclusive data supporting that oxytocin shortens the third stage of labor (Combs and Laros, 1991). On the other hand, it seems as the use of oxytocin in the third stage of labor results in less blood loss (Rogers *et al*, 1998). It is important to note that in the present study no differentiation was made to identify the causes of retainment of placenta. The placenta can either be detached but trapped because of cervical spasm, or it can be completely or partially attached to the decidual wall. Ultrasonographic examination was not performed to differentiate between these circumstances. However, based on the clinical examination the placenta was assumed to be attached to the decidual wall.

When placenta is retained the most common method to separate it from the uterine wall is by inducing uterine contractions either manually or by medication. Dynamic ultrasonographic imaging has demonstrated that retro-placental myometrial contractions are necessary for placental separation and that lack of retro-placental contractions results in retained placenta (Herman *et al,* 1993). Placenta is usually thought of as a non-contractile or passive organ (Herman *et al,* 1993). However, Farley and co-workers demonstrated that human placental anchoring villi have contractile properties (Farley *et al,* 2004). This finding supports the hypothesis that placenta has the ability to regulate its own intravillous volume. Farley also reported that NO may be involved in the regulation of the contractile properties, as GTN and SNP (NO donors) in her study, induced dose dependent relaxation of the villi. Furthermore, the fetal membranes contain all three isoforms of the enzyme necessary to synthesize NO (Marinoni *et al,* 1997; Dennes *et al,* 1997). The inducible isoform, iNOS, is specifically expressed during labor, both at term and preterm (Marinoni *et al,* 2000). Moreover, the fetal

membranes release NO metabolites (Ticconi *et al,* 1996). They are probably a major source of the increased levels of NO metabolites found in amniotic fluid at labor (Marinoni *et al*, 2000). In addition, NO donors can strongly stimulate PGE_2 release by fetal membranes (Marinoni *et al,* 2000). It is reasonable to presume that NO release from the fetal membranes also exerts a relaxing effect on placental villi and therefore may play a role in the detachment of placenta. It seems likely that NO induced relaxation of anchoring villi is a prerequisite for the retroplacentar myometrium to contract and the placenta to detach. Additionally, Ticconi and co-workers (2004) showed that oxytocin modulates NO generation by human fetal membranes at term, which could explain the promising results of combining oxytocin and nitroglycerin. Oxytocin stimulates the release of NO, and by adding both substances when retained placenta the effect of combined treatment is likely to be significantly improved by joint action. According to this hypothesis it could be NO that plays a more important role than oxytocin in the mechanism of placental detachment.

GENERAL DISCUSSION

In this thesis some aspects on the role of NO in cervical ripening, labor induction and placental detachment have been evaluated. It seems obvious that NO has an important role in all these processes, but many of its mechanisms of action are still unknown. The present thesis comprises clinical and experimental trials where two different NO donors, IMN and nitroglycerin, have been applied for the purpose of cervical ripening, labor induction and management of retained placenta. Before a medical agent can be accepted for clinical use three important questions have to be answered: 1) efficacy, 2) safety and 3) side effects of the agent. In this thesis attempts have been made to examine NO donors in respect to these questions.

1) Efficacy: The results indicated that NO promotes cervical ripening since vaginal treatment with IMN resulted in increased cervical distensibility. This effect could be mediated by a stimulation of PG synthesis since vaginal administration of IMN induced increased expression of COX-2. In accordance with a cervical ripening effect of IMN it was also observed that vaginal treatment with IMN initiates onset of labor in postterm pregnancies. Nitroglycerin in sequential combination with oxytocin was found to be extraordinarily effective in the management of retained placenta. It seems likely that nitroglycerin plays a key role in this context.

2) Safety: Safety aspects are extremely important in obstetric managements, as the clinical handling concerns two individuals - the fetus and mother. In three of the studies the safety of NO regarding the mother has been examined, while in two studies fetal safety was evaluated. As NO donors are known to be vasodilatating agents, effects on maternal circulation must be considered. In the present thesis treatment with NO donors showed effects on maternal hemodynamics since both IMN and nitroglycerin caused a significant decrease in blood pressure as well as an increase in pulse rate. However, these side effects were in no case of clinical importance since none of the women needed treatment due to hemodynamic instability. On the other hand, in the placenta study intravenous infusion was routinely used for prophylactic reasons to compensate for hypotension and blood loss. Due to the small number of women it is too early to draw definite conclusions as to hemodynamic adverse effects. On the other hand, nitroglycerin did not cause more bleeding compared to placebo. On the contrary, blood loss was significantly lower in women treated with nitroglycerin

compared to women treated with placebo probably due to the efficacy of the treatment. The extra time interval otherwise needed for removal of placenta under anaesthesia could thereby be avoided. Taken together, so far no serious maternal or fetal side effects following treatment with NO donors have been registered. However, interpretations have to be made with caution. In the present thesis, various parameters regarding fetal safety were examined. Prior to labor, examinations on umbilical artery resistance and pulsatility index, CTG and fetal heart rate were registered. Following delivery, umbilical artery pH, Apgar score and need of NICU care were registered. Compared to the placebo group no difference was observed concerning fetal side effects.

3). Side effects: It was obvious that NO donors commonly caused headache, nausea and palpitations. However, according to the VAS scales the side effects were of mild or moderate intensity. Nevertheless, the side effects are not negligible as nearly 90% of the women in the outpatient labor induction study reported headache. Therefore, it was unexpected that the majority of the women not only reported satisfaction with the treatment but also expressed that they could recommend the treatment to other women. It is probably wise not to draw too strong conclusions based on these reports since many of the women were tired of their postterm pregnancies and wished labor to be induced. Although side effects of NO donors were common, the overall impression remains that they seemed to be well tolerated.

Finally, in this thesis two aspects of importance need to be emphasized: The possibility of outpatient treatment with an NO donor for cervical ripening and labor induction, and the management of retained placenta by using a sequential adminstration of oxytocin and sublingual nitroglycerin. The study indicates that IMN administered vaginally for cervical ripening and labor induction is effective, safe and well tolerated. Hospital costs are increasing and there is a demanding need to limit these costs. Treatment for cervical ripening and labor induction are normally managed in an inpatient setting as PGs, commonly used for this purpose, may be associated with uterine hyperstimulation. Consequently, fetal monitoring is mandatory. Since NO donors do not cause uterine hypertonus the possibility to induce cervical ripening and labor in an outpatient setting arises. Besides, the labor induction rate seems to be increasing in many countries. If future studies confirm that NO donors are safe and effective the outpatient procedure could prove to be an alternative strategy. Such an approach would reduce hospital costs substantially and could also possibly contribute to higher maternal satisfaction with the labor induction procedure. The second aspect to

emphasize is the possible effect of nitroglycerin on management of retained placenta. Future studies are needed to verify that the sequential combination of a uterotonic agent, oxytocin or PG, and a relaxing agent, e.g. nitroglycerin, is an efficient treatment regimen for detachment of retained placenta. Since both nitroglycerin and PG tablets are inexpensive and easily stored, the combined treatment may be worldwide applicable. Excessive blood loss due to retained placenta can be avoided and many lives, especially in developing countries, can thereby be saved.

ACKNOWLEDGEMENTS – TACK

Erling Ekerhovd, min huvudhandledare, för alla år du inspirerat mig med dina imponerande kunskaper och ditt genuina intresse för forskning. Tack även för givande och alltigenom positivt samarbete!

Anders Norström, min handledare, för att du väckt mitt intresse för den mikroskopiska världen och för att du alltid med stor generositet delat med dig av dina kunskaper.

Björn Andersch, min handledare, för ditt trogna stöd, din vänskap och din unika förmåga att avrunda stundom allvarliga samtal med befriande skratt.

Gunnar Silfverstolpe, som från början lockade mig till Kvinnokliniken, Sahlgrenska Universitetssjukhuset. Utan denna glädjande och överraskande invit hade min avhandling aldrig blivit av.

Per-Olof Janson, för stor empati och uppmuntran i svåra stunder.

Ian Milsom, för din otroliga förmåga att mentalt lyfta även en vacklande doktorand.

Henrik Hagberg, för flera positiva och inspirerande samtal både inom vetenskapliga och icke-vetenskapliga områden.

Lars-Åke Mattsson, för visat engagemang i mina arbeten, och för att du förmedlat nya goda kontakter och samarbeten över klinikgränserna.

Margareta Wennergren, för alla uppmuntrande ord, för ditt föredömliga ledarskap, och för din förmåga att lösa alla problem på ett fantastiskt sätt.

Gunilla Sundell, för långvarig vänskap, och för att alltid ha varit en förebild för mig i min yrkesroll.

Sven Nielsen, för oväntat engagemang i min forskning, samt för detaljerad granskning av mina arbeten.

Febe Blohm, för alla praktiska råd, ditt fina stöd och din snabba respons på alla spörsmål.

Cecilia Kärrberg och Nina Radulovic, mina doktorandvänner, som jag delat många tankar med i både starka och svaga stunder.

Håkan Lilja och Hans Bokström, för delat intresse för mina forskningsområden och för att ni gav mig utrymme att genomföra mina projekt.

Thore Solum, för att jag fick komma till Kvinnokliniken, Borås för en tids tjänstgöring och för din positiva attityd till min forskning.

Lena Granström och Monica Eriksson-Orrskog, för er entusiasm och vilja att hjälpa till med överburenhetsstudien. Ni bidrog stort till att arbetet kunde slutföras inom rimlig tid.

Alla barnmorskor på förlossningen för er positiva attityd och vilja att samarbeta.

All operationspersonal som så villigt hjälpt till vid vävnadsprovtagning.

Alla kollegor, som på olika sätt uppmuntrat mig i mitt arbete.

Anja Andersson, för trogen hjälp med allt praktiskt och för total pålitlighet.

Personalen på Göteborgs Kvinnoklinik, för att ni alltid generöst gett mig den tid jag behövt för min forskning.

Min familj, Dan, Daniel, Andreas och Josefine, för att ni så tålmodigt uthärdat mina perioder av själslig frånvaro. Ni vet precis vad ni betyder för mig!

Mina föräldrar Aino och Karl-Erik, min bror Anders, postumt till min syster Helena, för att ni aldrig ifrågasatt mina beslut utan alltid visat tillgivenhet och tilltro.

Annika, Greta, Susanne och Åse, mina vänner, för äkta oskattbar vänskap.

Alla kvinnor, som deltagit i min forskning och som gett mig så mycket glädje i mina möten med er!

Ekonomiska bidrag för denna avhandling har erhållits från Stiftelsen Hjalmar Svensson, Göteborgs Läkaresällskap samt Regionala FOU-medel.

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I

Vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening at term: A randomized controlled study

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OBJECTIVE: Our aim was to examine the effect of the nitric oxide donor isosorbide mononitrate on the uterine cervix at term and to evaluate possible adverse effects of this treatment.

STUDY DESIGN: Term pregnant women were randomly selected to receive either 40 mg vaginally administered isosorbide mononitrate or placebo 4 hours before elective cesarean section. Cervical status, maternal blood pressure, maternal pulse rate, fetal heart rate, umbilical arterial Doppler indices, and various side effects were examined.

RESULTS: Isosorbide mononitrate induced a significant increase in cervical distensibility. It also caused a significant change in maternal blood pressure and maternal pulse rate. In addition, the frequency of maternal headache and palpitations was significantly higher in the isosorbide mononitrate group versus the placebo group. However, the intensity of these symptoms was moderate.

CONCLUSION: Vaginal administration of 40 mg of isosorbide mononitrate induces cervical ripening at term. Although the majority of women experienced side effects, no serious clinical maternal or fetal adverse effects, resulting in specific medication or emergency cesarean section, were diagnosed. (Am J Obstet Gynecol 2003;189:1692-7.)

Key words: Cervical ripening, isosorbide mononitrate, nitric oxide, side effects, term pregnancy

Cervical ripening, clinically diagnosed by softening, effacement, and dilatation of the uterine cervix, is commonly stimulated pharmacologically by the use of vaginally administered prostaglandins before induction of labor. However, prostaglandins may cause adverse maternal and fetal effects, mainly because of their stimulatory action on uterine contractions. It has been estimated that about 5% of women have uterine hypertonus after administration of prostaglandins for the induction of cervical ripening.[1] Uterine hypertonus often results in abdominal pain and anxiety, but it may also cause circulatory complications that could be fatal to the unborn child.

An ideal agent for cervical priming would induce adequate cervical ripening without adverse maternal and fetal effects. During the last few years it has become clear that intravaginal administration of nitric oxide donors, such as isosorbide mononitrate and glyceryl trinitrate, induces cervical ripening in first trimester pregnant women before surgical termination of pregnancy.[2,3] In addition, pretreatment with the nitric oxide donor isosorbide mononitrate has proved to be associated with fewer side effects than the prostaglandin analog gemeprost.[4] On the basis of these studies, it has been suggested that nitric oxide donors may produce cervical ripening for the induction of labor, without causing serious adverse effects.[5,6]

Before studies can be performed to determine the efficacy of nitric oxide donors for cervical ripening prior to induction of labor, it is important to examine the clinical effect of local treatment with nitric oxide donors on the uterine cervix as well as to evaluate possible adverse maternal and fetal effects of this medical treatment at term. In the current study we examined the effects of 40 mg of isosorbide mononitrate, administered intravaginally, in term pregnant women before elective cesarean section. The clinical effect of this treatment on the uterine cervix was measured by the use of a cervical tonometer. Maternal blood pressure, maternal pulse rate, various subjective maternal side effects, as well as arterial umbilical blood flow, fetal heart rate, and Apgar score

From the Department of Obstetrics and Gynecology, Sahlgrenska University Hospital.
Supported by grants from the Foundation of Handlanden Hjalmar Svensson and the Göteborg Medical Society.
Received for publication February 20, 2003; revised June 5, 2003; accepted June 21, 2003.
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0002-9378/2003 $30.00 + 0
doi:10.1016/S0002-9378(03)00865-2

Table I. Characteristics and clinical data of women participating in the study

	Isosorbide mononitrate (n = 30)	*Placebo (n = 30)*	*P value*
Age (y)	29.2 ± 5.1 29 (20-39)	28.7 ± 4.7 30 (20-37)	NS
Gestational age (wks)	38.0 ± 0.5 38 (37.0-38.6)	38.3 ± 0.4 38.3 (37.5-38.9)	NS
Pretreatment Bishop score	3.3 ± 1.1 3 (2-5)	3.4 ± 1.3 3 (1-5)	NS
Treatment period until cervical dilation (min)	253 ± 11.6 255 (240-270)	254 ± 12.4 255 (240-270)	NS
Cervical length			
At 0 min	36.3 ± 3.3 36 (30-42)	35.2 ± 3.6 35 (30-41)	NS
At 210 min	36.1 ± 3.1 36 (30-42)	35.1 ± 2.7 35 (30-41)	NS
Cumulative force to dilate the cervix to 10 mm (N)	2.7 ± 1.2 2.5 (1-5)	6.4 ± 2.1 6 (3-10)	<.0001
Umbilical pH value (arterial)	7.34 ± 0.03 7.34 (7.28-7.38)	7.33 ± 0.02 7.34 (7.29-7.38)	NS
Intraoperative blood loss (mL)	346.7 ± 140.7 300 (200-600)	360.0 ± 122.1 400 (200-600)	NS

Data are shown as mean ± SD, median and range (minimum-maximum). *NS,* Not significant.

were registered. Moreover, the intraoperative blood loss at subsequent operation was measured.

Material and methods

This double-blind, randomized, controlled study was carried out at Sahlgrenska University Hospital, Göteborg, Sweden. Before the initiation of the study, approval was granted by the human ethics committee of Göteborg University.

A total number of 60 white Swedish women scheduled for elective cesarean delivery caused by breech presentation or psychologic reasons were included in the study (Table I). Inclusion criteria were nulliparity, gestational age of at least 37 weeks, uncomplicated singleton pregnancy, normal cardiotocography, normal arterial umbilical Doppler indices, and immature cervical status (Bishop score <6 and cervical length ≥30 mm, measured by transvaginal ultrasonography). Exclusion criteria included pregnancy-associated diseases, cardiorespiratory disease, history of headache, alcohol abuse, cigarette smoking, intolerance to isosorbide mononitrate, and serious systematic diseases, defined as daily use of medication.

All women included in the study were 2 to 7 days before planned cesarean delivery fully informed of the nature and scope of the study, as well as the potential risks. At admittance about 6 hours before cesarean section, cardiotocography was performed to ensure that fetal heart activity was normal. After cardiotocography, a clinical examination of the uterine cervix was performed to confirm a persistent immature cervical status. Assessment of the cervix included consistency, length, dilatation, position, and station of the fetal presenting part as first described by Bishop and later modified by Calder et al.[7] Cervical length was also measured by transvaginal ultrasonography. In addition, the umbilical artery resistance index and the pulsatility index were determined and maternal pulse rate and blood pressure were recorded digitally. If inclusion criteria were fulfilled, a signed informed consent was obtained from each participant before recruitment.

The women were randomized to either of two groups: (1) 40 mg of isosorbide mononitrate (two 20-mg tablets) (Ismo, Boehringer Mannheim, Mannheim, Germany) 4 hours before the cesarean section or (2) two placebo tablets of similar design as the isosorbide mononitrate tablets 4 hours before the cesarean section. Randomization was performed by means of numbered, sealed envelopes prepared with random-number tables. Both the isosorbide mononitrate and the placebo tablets were administered into the posterior vaginal fornix by a research nurse. Neither the participating women nor the research nurse were aware of the agent administered. Maternal pulse rate, maternal blood pressure, umbilical artery resistance index, and pulsatility index were recorded at baseline and at 180 minutes. Fetal well-being was evaluated intermittently by cardiotocographic registration, fetal heart rate being specifically recorded at baseline and 210 minutes after intravaginal administration. For measurement of cervical length, transvaginal ultrasonography of the uterine cervix was conducted at baseline and at 210 minutes. Ultrasonography was performed by one and the same investigator, who was unaware of the type of intravaginal agent used. Before being transported to the operating theater, the women were asked to complete a symptom questionnaire. This questionnaire concerned the following symptoms: headache, hot flushes, palpitations, dizziness, abdominal pain,

pelvic pain, and nausea. If any of the mentioned symptoms were present, the women were also asked to describe the intensity of the symptoms based on a visual analog scale. For statistical assessment of the intensity of the reported symptoms, each scale was later graded from 0 (no symptom) to 10 (symptom of maximal intensity).

At the operating theater, spinal anesthesia was given and the women were placed in the lithotomy position. The peak force (in newtons) required to dilate the cervix was measured with a cervical tonometer[8] connected to cervical dilators with diameter from 5 to 10 mm, as previously described.[2] Measurement of cervical distensibility was conducted by one and the same investigator, who was not informed about the intravaginal agent administered. Cesarean section was then performed. Oxytocin (Syntocinon, Novartis, Täby, Sweden), 10 U administered intravenously, was given routinely after delivery of the placenta. Intraoperative blood loss was assessed at the end of each operation. All newborn infants were examined immediately after birth and monitored closely during the early postnatal adaptation period. Apgar score was documented 1, 5, and 10 minutes after birth. At the end of the study, the treatment allocation for each study number was revealed and the data were analyzed.

Statistical analysis. A sample size of 60 women in two groups, estimated by the Altman nomogram,[9] was calculated to yield 80% power at the 5% significance level of detecting a standardized difference of 0.75 N in the cumulative force required to dilate the cervix. Mean ± SD, median, and range were calculated for descriptive purposes. For comparison between groups, the Mann-Whitney nonparametric *U* test was used for continuous variables and the Fisher exact test for dichotomous variables. All tests were two tailed and conducted at a 5% significance level.

Results

No serious maternal complications or signs of fetal distress resulting in specific medication or emergency cesarean delivery were diagnosed during the study. All 60 women participating in the study had uncomplicated planned cesarean delivery.

Isosorbide mononitrate had a clear effect on the distensibility of the uterine cervix when measured 4 hours after initiation of treatment (Table 1). The force necessary to dilate the cervix was significantly lower in this group versus the placebo group ($P < .0001$). However, no change in cervical length could be measured by transvaginal ultrasonography approximately 210 minutes after administration of the nitric oxide donor.

There were no significant differences in mean age, gestational age, pretreatment Bishop score, and treatment period between the two groups (Table I). Maternal systolic blood pressure, maternal diastolic blood pressure, and maternal pulse were similar in the two groups before intravaginal administration of either isosorbide mononitrate 40 mg or placebo tablets (Table II). After a treatment period of 180 minutes, both the mean maternal systolic ($P < .001$) and diastolic ($P < .0001$) blood pressures in the isosorbide mononitrate group were significantly lower than in the placebo group. The mean maternal pulse rate in the isosorbide mononitrate group 180 minutes after intravaginal administration was 84.0 beats/min. In the placebo group, the mean maternal pulse rate at this time point was 76.5 beats/min. Thus, a significant difference of 7.5 beats/min in mean maternal pulse rate was measured ($P < .01$).

The most frequent symptomatic side effect in the isosorbide mononitrate group was headache, experienced by 24 of the 30 women (Table III). In the placebo group, 9 of the 30 women reported headache immediately before the start of cesarean delivery ($P < .001$). In addition, a significant increase ($P < .01$) in the frequency of palpitations was registered in women treated with isosorbide mononitrate at this time point. There was no statistically significant difference in reported symptoms regarding hot flushes, dizziness, abdominal pain, pelvic pain, and nausea between the two groups. Generally, the experienced side effects were not regarded as serious by the women themselves, according to the results of the questionnaire. None of the women required any kind of medical treatment because of the reported side effects.

Neither the umbilical artery resistance index nor the pulsatility index were found to be influenced by 40 mg of isosorbide mononitrate 180 minutes after intravaginal administration (Table II). All cardiotocographies intermittently performed during the preoperative observational period were normal and without any signs of fetal distress, fetal heart rate being within the normal range (110-150 beats/min) in all patients examined. No significant difference in fetal heart rate was registered between the isosorbide mononitrate group and the placebo group 210 minutes after intravaginal administration (Table IV). Umbilical arterial pH and Apgar score did not differ between the two groups. None of the newborn infants needed specific neonatal surveillance.

Uterine tonus, after delivery of the placenta and routine intravenous injection of 10 U of oxytocin, was found to be normal, except in four cases. Two of these cases were in the isosorbide mononitrate group. In each case, an additional dose of 10 U of oxytocin was injected, resulting in adequate tonus of the uterine body. No significant difference in intraoperative blood loss was found between the isosorbide mononitrate group and the placebo group.

Comment

The current study demonstrates that intravaginal administration of 40 mg of isosorbide mononitrate for

Table II. Mean maternal blood pressure, heart rate, UA PI, and resistance index at 0 and 180 minutes

	Isosorbide mononitrate (n = 30)	*Placebo (n = 30)*	*P value*
Maternal systolic blood pressure (mm Hg)			
At 0 min	112.0 ± 6.9	110.7 ± 7.5	NS
	110 (95-125)	110 (100-130)	
At 180 min	105.5 ± 5.5	116.0 ± 6.5	<.001
	105 (90-120)	115 (100-130)	
Maternal diastolic blood pressure (mm Hg)			
At 0 min	74.5 ± 4.4	73.0 ± 4.8	NS
	75 (70-80)	70 (65-80)	
At 180 min	64.3 ± 6.0	74.0 ± 4.6	<.0001
	65 (55-75)	75 (65-85)	
Maternal pulse rate (beats/min)			
At 0 min	73.2 ± 8.3	71.7 ± 7.4	NS
	72 (55-85)	72 (60-85)	
At 180 min	84.0 ± 8.4	76.5 ± 8.3	<.01
	85 (65-95)	75 (60-90)	
Pulsatility index			
At 0 min	0.82 ± 0.06	0.81 ± 0.07	NS
	0.82 (0.68-0.94)	0.80 (0.70-0.96)	
At 180 min	0.79 ± 0.06	0.80 ± 0.06	NS
	0.80 (0.66-0.92)	0.80 (0.66-0.92)	
Resistance index			
At 0 min	0.58 ± 0.05	0.57 ± 0.05	NS
	0.58 (0.48-0.67)	0.57 (0.48-0.66)	
At 180 min	0.57 ± 0.05	0.57 ± 0.06	
	0.56 (0.49-0.68)	0.56 (0.50-0.68)	

Data are shown as mean ± SD, median and range (minimum-maximum). *NS,* Not significant.

Table III. Number of patients with side effects in the period after administration of either isosorbide mononitrate (40 mg) or placebo until cesarean section and the intensity of these registered side effects according to a visual analog scale (0-10)

	Side effects			*Intensity*		
	Isosorbide mononitrate (n = 30)	*Placebo (n = 30)*	*P value*	*Isosorbide mononitrate (n = 30)*	*Placebo (n = 30)*	*P value*
Headache	24	9	<.001	2.97 ± 1.94 3 (0-7)	1.00 ± 1.68 1 (0-5)	<.001
Hot flushes	10	6	NS	1.27 ± 1.96 0 (0-6)	0.53 ± 1.22 0 (0-5)	NS
Palpitations	16	6	<.02	1.83 ± 1.93 2 (0-6)	0.63 ± 1.33 0 (0-4)	<.01
Dizziness	6	5	NS	0.73 ± 1.57 0 (0-5)	0.43 ± 1.07 0 (0-4)	NS
Abdominal pain	4	4	NS	0.47 ± 1.28 0 (0-5)	0.53 ± 1.41 0 (0-5)	NS
Pelvic pain	3	4	NS	0.33 ± 1.03 0 (0-4)	0.33 ± 0.96 0 (0-4)	NS
Nausea	1	1	NS	0.10 ± 0.55 0 (0-3)	0.10 ± 0.55 0 (0-3)	NS

Data are shown as mean ± SD, median and range (minimum-maximum). *NS,* Not significant.

a period of 4 hours makes the uterine cervix more distensible in term pregnant women. The study also shows that maternal blood pressure and maternal pulse rate are significantly changed 180 minutes after administration of isosorbide mononitrate. However, none of these changes resulted in specific medical treatment. Cardiotocography, umbilical Doppler indices, as well as fetal heart rate, were always normal during the observation period until elective cesarean section was performed. Most important, neither the term pregnant women nor the newborn infants showed any signs of serious adverse effects caused by the intravaginal treatment.

The finding that nitric oxide donors, such as isosorbide mononitrate and glyceryl trinitrate, induce

Table IV. Fetal heart rate at 0 and 210 minutes and Apgar score at 1, 5, and 10 minutes

	Isosorbide mononitrate (n = 30)	*Placebo (n = 30)*	*P value*
Fetal heart rate			
At 0 min	135.5 ± 9.5	135.0 ± 10.4	NS
	135 (115-150)	135 (115-150)	
At 210 min	135.3 ± 8.2	135.8 ± 7.6	NS
	135 (120-150)	135 (115-145)	
Apgar score			
At 1 min	9.0 ± 0.6	9.1 ± 0.6	NS
	9 (7-10)	9 (8-10)	
At 5 min	9.8 ± 0.4	9.9 ± 0.3	NS
	10 (9-10)	10 (9-10)	
At 10 min	10.0 ± 0.0	10.0 ± 0.0	NS
	10 (10-10)	10 (10-10)	

Data are shown as mean ± SD, median and range (minimum-maximum). *NS,* Not significant.

ripening of the uterine cervix has previously been demonstrated in first-trimester pregnant women.[2] Recently, it was shown that intravaginal administration of either 40 mg of isosorbide mononitrate or 0.5 mg of glyceryl trinitrate had ripening effects similar to that of 3 mg of prostaglandin E_2 on the induction of labor in term pregnant women.[10] In that study, little change in cervical Bishop score was found after administration of either of the two nitric oxide donors 4 hours after start of treatment. In the current study, no change in cervical length was observed by transvaginal ultrasonography 210 minutes after intravaginal application of isosorbide mononitrate. However, a significant increase in cervical distensibility was measured by dilatation of the cervical canal to a diameter of 10 mm by the use dilators connected to a cervical tonometer. A cervical response characterized by an increased distensibility and no change in cervical length seems to be typical for nitric oxide donors. This effect may partly be due to the fact that nitric oxide does not induce uterine contractions. A similar effect on the cervix has previously been demonstrated after local treatment with the nitric oxide donor sodium nitroprusside in guinea pigs during advanced pregnancy.[11]

The competence of the cervix during pregnancy appears to rely on an intact organization of the collagen framework, which constitutes about 80% of the cervical tissue.[12] Degradation and disorganization of collagen fibers at term seem to reduce cervical tensile strength and may be a prerequisite for obvious clinical changes, estimated by Bishop score, to occur. Among factors regulating cervical ripening (ie, mechanical factors, estrogens, cytokines, and other inflammatory agents), prostaglandins are regarded to have a crucial role. Nitric oxide appears to be involved in this process. We have previously shown that vaginally applied isosorbide mononitrate increases the expression of cyclo-oxygenase-2 in the cervix of first-trimester primigravid women.[3] We therefore suggest that the increased distensibility of the cervix, as observed in response to the nitric oxide donor in this study, may be due to stimulation of local prostaglandin synthesis. The short treatment period (4 hours) and absence of uterine contractions may explain the fact that no change in cervical length was observed. Further, we have previously demonstrated in vitro that nitric oxide relaxes cervical smooth muscle in late pregnancy.[6] Therefore, it cannot be excluded that the observed increased cervical distensibility to some extent also may be due to an effect on the smooth muscle component.

Maternal and fetal hemodynamics after intravaginal administration of isosorbide mononitrate in term pregnant women have recently been described in detail.[13] In a randomized controlled trial Nicoll et al recorded maternal blood pressure, maternal pulse rate, and fetal heart rate at baseline and then every 30 minutes until 360 minutes. In addition, umbilical artery resistance index and pulsatility index were measured. They found that in women who had been given isosorbide mononitrate a significant decrease in both the maternal systolic and diastolic blood pressures occurred during the study period versus the control group. No changes in umbilical artery Doppler indices were registered. The results of the current study are in agreement with these previous findings. Although significant changes in maternal hemodynamics were recorded after administration of isosorbide mononitrate, these effects did not prove to be of clinical importance. As in the current study, none of the participating women required treatment for hypotension or tachycardia.

The main symptoms after intravaginal administration of isosorbide mononitrate reported in the current study were headache and palpitations. It is well known that these most common side effects are due to the vasodilatory action of nitric oxide. In this study, a visual analog scale was used to assess the frequency and intensity of side effects. Although headache and palpitations were reported by the vast majority of women in the isosorbide mononitrate group, only one described any of the symptoms as having an intensity of 7 on a visual analog scale between 0 and 10.

Thus, almost all the reported symptoms were of moderate intensity. However, the fact that none of the participating women had headache of high intensity or needed medication, may be due to the relatively short treatment period until cesarean delivery was performed. Because all the participating women were informed about possible adverse effects before giving their informed consent, the relatively high number of women who had headache in the placebo group may be due to the information given before recruitment to the study.

Another aspect of intravaginal administration of isosorbide mononitrate is the possible relaxing effect on the uterine body. Several studies in vitro have shown that nitric oxide has a relaxing effect of the myometrium.[14-16] Glyceryl trinitrate has also been used as a tocolytic agent for the treatment of preterm labor.[17,18] A relaxing effect on the uterine body after intravaginal treatment with isosorbide mononitrate, causing uterine hypotonus and thus increased blood loss, would preclude nitric oxide donors for the induction of cervical ripening. As demonstrated in the current study, intravaginal administration of 40 mg of isosorbide mononitrate did not inhibit uterine contraction after cesarean delivery as evaluated by intraoperative and postoperative uterine tonus and blood loss. None of the women had an intraoperative blood loss of more than 600 mL and blood transfusions were never necessary.

The number of women in this study is too small to make any conclusive statements regarding the safety of vaginally administered isosorbide mononitrate. Headache and palpitations, experienced by the majority of treated women, may not be considered as a minor side effect. Likewise, the reduction of systolic and diastolic blood pressures might be regarded as an important adverse reaction. Nevertheless, the current study points to the fact that intravaginal isosorbide mononitrate treatment is well tolerated by the women as well as the newborn infants. We therefore conclude that isosorbide mononitrate promotes cervical ripening at term by increasing the distensibility of the tissue without causing serious clinical maternal or fetal side effects. The use of nitric oxide donors for the induction of cervical ripening at term may thus prove to be a major therapeutic advance.

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II

OBSTETRICS

Outpatient vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening and labor induction postterm: a randomized controlled study

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OBJECTIVE: Our aim was to examine the efficacy, safety, and acceptability of isosorbide mononitrate for cervical ripening and labor induction in women in an outpatient setting.

STUDY DESIGN: Two hundred pregnant women of at least 42 weeks' gestation with an unripe cervix were randomly selected to receive vaginally either 40 mg isosorbide mononitrate or placebo tablets.

RESULTS: Twenty-two women treated with isosorbide mononitrate went into labor within 24 hours compared to 8 women in the placebo group ($P < .05$). In women who did not go into labor, cervical status was similar in the 2 groups the next day. Headache was a common side effect. No maternal or fetal side effects of clinical importance were registered.

CONCLUSION: Outpatient cervical ripening and labor induction with isosorbide mononitrate seems to be an effective, safe, and well tolerated procedure. The definitive clinical efficacy and safety needs to be evaluated in larger series of patients.

Key words: cervical ripening, induction of labor, isosorbide mononitrate, nitric oxide

Cite this article as: Bullarbo M, Orrskog ME, Andersch B, et al. Outpatient vaginal administration of the nitric oxide donor isosorbide mononitrate for cervical ripening and labor induction postterm: a randomized controlled study. Am J Obstet Gynecol 2007;196:50.e1-50.e5.

Postterm pregnancy, defined as a pregnancy with a gestational length of 294 days or more, occurs in 5% to 10% of all births.[1] Postterm pregnancies are associated with increased rates of multiple maternal and fetal complications.[2,3] Elective labor induction is therefore often carried out to reduce the risk of adverse outcome, as an alternative to expectant management with serial fetal monitoring and selective labor induction. The success of labor induction is related to cervical ripening, and various prostaglandin regimens are commonly employed to ripen the cervix. However, prostaglandin may cause adverse fetal and maternal effects, mainly due to their stimulatory action on uterine contractions. It has been estimated that about 5% of women have uterine hypertonus after administration of prostaglandin.[4] For this reason, cervical ripening is carried out on an inpatient basis so that the fetus can be monitored during the ripening process.

The ideal agent for cervical ripening would induce adequate cervical ripening without causing uterine contractions. Since the lack of contractions obviates the need for fetal monitoring, such an agent could be given on an outpatient basis. The advantages of outpatient cervical ripening and labor induction include patient convenience, reduced workload on labor and delivery units, and reduced hospitalization costs.

Nitric oxide, a free radical gas with a short half life, is thought to be a fundamental mediator of cervical ripening.[5] Nitric oxide (NO) donors, such as isosorbide mononitrate, nitroglycerin, and sodium nitroprusside may prove to be agents that can be administered in an outpatient setting since they induce cervical ripening without causing uterine contractions or other adverse effects of clinical importance during the ripening process.[6-11] In addition, several studies have indicated that a substantial number of women given isosorbide mononitrate for cervical ripening at term go into labor within the next 24 hours.[7,12] The present study was conducted in order to examine the possible effect of isosorbide mononitrate on cervical ripening and initiation of labor in women postterm and to study the safety of this treatment in an outpatient setting. Possible maternal and fetal side effects as well as maternal satisfaction with the outpatient induction procedure were also assessed.

Materials and Methods

This double-blind, randomized, controlled study was conducted at Sahlgrenska University Hospital, Gothenburg, Sweden, and Borås Hospital, Borås, Sweden, between November 2002 and April 2005. Before initiation of the study, ap-

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Received Apr. 7, 2006; revised June 5, 2006; accepted Aug. 15, 2006.

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0002-9378/$32.00

doi: 10.1016/j.ajog.2006.08.034

proval was granted by the human ethics committee of Gothenburg University. All women included were fully informed of the nature and scope of the study, as well as potential side effects. Written informed consent was obtained from each woman before recruitment.

Inclusion criteria were: (1) uncomplicated singleton pregnancy, (2) cephalic presentation, (3) gestational age at least 42 weeks, (4) Bishop score ≤ 5, (5) normal amniotic fluid index measuring > 5 cm, (6) reactive fetal heart pattern, and (7) intact membranes. Gestational age was assessed by a routine ultrasonographic examination at 17-19 weeks of pregnancy. Examination of the cervix included an assessment of consistency, length, dilatation, position, and station of the fetal head as described by Bishop and later modified by Calder and coworkers.[13] Measurement of the closed cervical length was performed by transvaginal ultrasonography.[14] Exclusion criteria included regular uterine contractions, cardiorespiratory disease, history of headache, alcohol abuse, intolerance to isosorbide mononitrate, and serious disease, defined as daily use of medication.

A total number of 200 women fulfilled the inclusion criteria and agreed to participate in the study. The women were randomized to either of 2 groups: (1) 40 mg of isosorbide mononitrate (2 20-mg tablets) (Ismo, Roche, Basel, Switzerland) or (2) 2 placebo tablets of similar design as isosorbide mononitrate. Randomization was performed by means of numbered, sealed envelopes prepared with random-number tablets. Both the isosorbide mononitrate and the placebo tablets were inserted into the posterior vaginal fornix by an obstetrician. Neither the participating women nor the obstetrician were aware of whether isosorbide mononitrate or placebo tablets were administered.

After administration of the tablets before returning home, each woman was scheduled for an appointment the next day. The women were also asked to complete a symptom questionnaire regarding possible maternal side effects during the priming interval. This questionnaire concerned the following symptoms: headache, hot flushes, palpitations, dizziness, nausea, and abdominal pain. For assessment of the intensity of the symptoms a visual analogue scale graded from 0 (no symptom) to 10 (symptom of maximal intensity) was used. If onset of labor occurred before the next day, the women were to contact the labor and delivery unit according to routine praxis. If regular contractions had not been established within the next day another cardiotocography and assessment of the cervix were performed. Induction of labor was then carried out according to the local induction-of-labor protocol. Women with a Bishop score ≥ 6 underwent amniotomy, while 1 mg of prostaglandin E_2 (Minprostin, Pfizer, Täby, Sweden) was administered vaginally for further cervical ripening if Bishop score was < 6 or if amniotomy was not feasible. The study investigators were not involved with the women's inpatient cervical ripening or intrapartum labor management.

Primary outcome measure was to evaluate the efficacy of 40 mg of isosorbide mononitrate in promoting onset of labor following cervical ripening for up to 24 hours in an outpatient setting. Secondary outcome measures were assessment of maternal side effects, mode of delivery, blood loss, and neonatal outcome in the 2 groups of women. Neonatal outcome was determined by Apgar score, pH in the umbilical artery, and need of neonatal intensive care. Finally, when discharged from hospital each woman was asked to return a questionnaire regarding her opinion about the outpatient procedure and if she would recommend it to other women.

Statistical Analysis

According to audits carried out in our units approximately 7 out of 100 postterm women would go into labor spontaneously within 24 hours. For evaluation of the efficacy of isosorbide mononitrate for induction of labor a power of 80% at the 5% significance level was reached if 22 out of 100 women in this group went into labor within 24 hours compared to 7 women in the placebo group. Thus, a total number of 200 women (100 women in each group) were included in the study. Fisher exact test was used for assessment of the efficacy of isosorbide mononitrate for induction of labor. Changes in Bishop score and cervical length were analyzed using 2 sample t test, while Mann-Whitney U-test was used for assessment of maternal side effects. All categorical outcomes in the 2 groups were compared using Fisher exact test. Logistic regression was used to assess the effect of isosorbide mononitrate, parity, baseline cervical length, and baseline Bishop score on spontaneous labor outcome. All significance tests were two-tailed. A $P < .05$ was considered to be statistically significant.

Results

There were no significant differences between the 2 groups of women with respect to maternal age, parity, and gestational age (Table 1). All participating women completed the study.

Twenty-two of the women (22%) treated with isosorbide mononitrate and 8 of the women (8%) in the placebo group went into labor before the return appointment the following day ($P < .05$; Table 2). Mean time from vaginal administration of tablets to the first painful contractions was 14.0 hours in the isosorbide mononitrate group and 15.3 hours in the placebo group, respectively. The 95% confidence interval (CI) for the difference between the proportions of women who went into labor was 0.04-0.24. Regression analysis with spontaneous labor as dependent variable and case-control status, parity, baseline cervical length, and baseline Bishop score as independent variables, showed that case-control status and baseline Bishop score remained significant ($P = .003$ and $P = .001$, respectively). The odds ratio for going into labor was 4.0 (95% CI 1.6-9.8) for the treatment group compared to the placebo group, and 2.0 (95% CI 1.3-2.9) for each unit increase in baseline Bishop score.

Two women who went into labor following treatment with isosorbide mononitrate underwent emergency cesarean section due to fetal distress. The other women who went into labor within 24 hours had uncomplicated vaginal deliv-

TABLE 1
Basic data of women participating in the study

	Isosorbide mononitrate (n = 100)	Placebo (n = 100)	*P* value
Age (y)	31.2 ± 4.4 (21-41)	30.6 ± 5.3 (19-43)	NS
Parity (n)			
Nulliparous	72	74	NS
Multiparous	28	26	
Gestational age (wk)	42.2 ± 0.3 (42.0-43.0)	42.1 ± 0.2 (42.0-43.0)	NS

Data are shown as mean ± SD and range (minimum-maximum). *NS*, not significant.

eries, except for 1 woman in the placebo group who had a cesarean due to failure of progress.

In women who did not go into labor, Bishop score as well as cervical length were found to be similar in the 2 groups at the return appointment (Table 2). Sixty-four women initially treated with isosorbide mononitrate and 74 women in the placebo group had a Bishop score < 6. These women were given 1 mg of prostaglandin E_2 vaginally for further cervical ripening. In 14 women in the isosorbide mononitrate group and in 18 women in the placebo group Bishop score was ≥ 6 and amniotomy was carried out for induction of labor.

Neonatal outcome was similar in the 2 groups (Table 3).

The most common side effect in women treated with isosorbide mononitrate was headache, experienced by 88 women (88%). The intensity of headache was reported to be moderate in most cases with a median of 4 (range 0-9) on the visual analogue scale. In the placebo group 8 women (8%) reported headache of mild or moderate intensity. Nausea of mild intensity was reported by 19 women (19%) in the isosorbide mononitrate group and by 5 women (5%) in the placebo group. All other reported side effects were infrequent.

Ninety-four women (94%) in the isosorbide mononitrate group and 99 women (99%) in the placebo group returned the questionnaire regarding how they had experienced the outpatient procedure. The vast majority of women in

TABLE 2
Clinical data of women who went into labor and women who did not go into labor

Total number of women	Isosorbide mononitrate (n = 100)	Placebo (n = 100)	*P* value
Women who went into labor	(n = 22)	(n = 8)	.01
Baseline Bishop score	2.5 ± 1.0 (1-4)	3.1 ± 1.0 (1-4)	NS
Baseline cervical length (mm)	28.9 ± 8.6 (15-51)	28.2 ± 8.4 (17-42)	NS
Parity			
Nulliparous	12	6	NS
Multiparous	10	2	
Interval between administration of agent and first painful contraction (h)	14.0 ± 5.0 (4-23)	15.3 ± 4.2 (10-22)	NS
Women who did not go into labor	(n = 78)	(n = 92)	
Bishop score			
Baseline	1.8 ± 1.2 (0-5)	2.0 ± 1.1 (0-5)	NS
Change at return appointment	1.7 ± 1.4 (0-5)	1.3 ± 1.5 (1-5)	NS
Cervical length (mm)			
Baseline	31.2 ± 9.4 (10-55)	31.1 ± 8.2 (9-47)	NS
Change at return appointment	−6.0 ± 8.8 (5-31)	−5.2 ± 10.2 (10-40)	NS
Parity			
Nulliparous	60	68	NS
Multiparous	18	24	

Data are shown as mean ± SD and range (minimum-maximum). *NS*, not significant.

TABLE 3
Data on delivery and neonatal outcome

	Isosorbide mononitrate (n = 100)	Placebo (n = 100)	*P* value
Mode of delivery			
Vaginal delivery	86	83	NS
Cesarean section	14	17	NS
Blood loss (mL)	635 ± 616 (150-3700)	566 ± 449 (100-3500)	NS
Blood loss >1000 mL (n)	14	12	NS
Birthweight (g)	3867 ± 476 (2505-5590)	3955 ± 484 (2990-5865)	NS
Apgar score < 7 at 5 minutes (n)	2	1	NS
Apgar score at 5 minutes	9.5 ± 1.1 (2-10)	9.6 ± 0.9 (6-10)	NS
Umbilical artery pH <7.05	4	8	NS
Admission to NICU	13*	9†	NS

Data are shown as mean ± SD and range. *NS*, not significant; *NICU*, neonatal intensive care unit.

* Cause: infection (4), general seizures (2), heart disease (2), meconium aspiration (3), high bilirubin levels (1), hypoglycaemia (1).

† Cause: infection (4), heart disease (1), meconium aspiration (1), nutritional problems (2), hypoglycemia (1).

both groups were either positive or very positive to the treatment. Eighty-nine of the women (94.7%) in the isosorbide mononitrate group and 93 of the women (93.9%) in the placebo group reported that they would recommend the procedure.

Comment

This study shows that 40 mg of isosorbide mononitrate seems to have an effect on cervical ripening and induction of labor postterm. The results also indicate that outpatient isosorbide mononitrate induced cervical ripening is likely to be a safe procedure, since neither fetal nor maternal side effects of clinical importance were registered.

Significantly more women treated with isosorbide mononitrate went into labor within 24 hours compared to women in the placebo group. The result is in line with previous reports where 27% to 39% of term pregnant women went into active labor or had a favorable Bishop score following cervical ripening with either isosorbide mononitrate or nitroglycerin for 24 hours.[7,12,15] Thus, a linkage between the degree of cervical ripening or cervical level of nitric oxide and time point for onset of contractions may exist. In fact, in 1 study commencement of uterine contractions was found to be associated with increased levels of cervical nitric oxide metabolites.[16] It has also been demonstrated that isosorbide mononitrate induces cervical production of cyclo-oxygenase 2, an enzyme that is involved in prostaglandin synthesis.[17] Prostaglandins are known to have a stimulating effect on uterine contractions. In addition, vaginally administered isosorbide mononitrate increases cervical tissue levels of cyclic guanosine monophosphate (cGMP). This second messenger of nitric oxide is thought to promote relaxation of cervical smooth muscle, a pathway which may be involved in cervical ripening.[18,19]

Forty milligrams of vaginally administered isosorbide mononitrate have previously been demonstrated to stimulate cervical ripening after a treatment interval of 3-4 hours and to induce rearrangement of the cervical ultrastructure in the first trimester similar to the changes seen during spontaneous cervical ripening at term.[10,17,20] In the present study, although women treated with isosorbide mononitrate had a higher mean Bishop score than women given placebo tablets at the appointment the next day, the difference in Bishop score was not significant. However, only women who had not gone into labor within 24 hours could be examined. Thus, 22 of the 100 women (22%) treated with isosorbide mononitrate could not be assessed. Since almost all women who went into labor had uncomplicated vaginal deliveries, it is reasonable to conclude that cervical ripening in these women was adequate. In previous reports, a gradual increase in Bishop score has been described following vaginal administration of isosorbide mononitrate at term when examined at intervals of 3 hours until amniotomy or onset of labor.[7,12]

Previously it has been demonstrated that cervical nitric oxide production is very low in women postterm. Thus, it has been suggested that reduced cervical nitric oxide release may contribute to prolonged pregnancy.[21] Vaginal administration of a nitric oxide donor as performed in the present study, could therefore result in sufficient cervical levels of nitric oxide to promote efficacious cervical ripening and induction of labor.

In the present study, the effect of isosorbide mononitrate was assessed during a treatment interval of only 24 hours. Women who went into active labor did not experience painful or regular contractions before 14-23 hours after administration of isosorbide mononitrate. A further increase in the effect of isosorbide mononitrate would most likely have occurred if vaginal administration of the nitric oxide donor had been repeated. A strategy with repeated administration of the agent is normally carried out when prostaglandin is employed for cervical ripening and labor induction. Isosorbide mononitrate is

known to be a slow-releasing nitric oxide donor. When administered into the vagina in the late third trimester, peak serum levels of isosorbide mononitrate were still not achieved at 6 hours.[22] Therefore, a treatment interval before another dose of isosorbide mononitrate is administered should clearly be more than 6 hours.

The investigation confirms previous reports that vaginally administered isosorbide mononitrate does not induce uterine hyperstimulation. No signs of abnormal fetal heart rate until the active phase of labor were registered. This finding is in agreement with a recent report, where no abnormal fetal heart tracings were identified after isosorbide mononitrate therapy in 199 women at term in an inpatient setting.[11] Thus, the risk of fetal compromise during the ripening process seems to be minimal. In addition, no difference in mode of delivery, blood loss, umbilical pH, Apgar score, or need for neonatal intensive care was found between women treated with isosorbide mononitrate and women who were given placebo tablets.

Maternal preference for outpatient cervical ripening and labor induction in uncomplicated pregnancies, thereby potentially improving patient convenience and reducing hospital costs, has previously been described.[23] The questionnaire clearly demonstrated that maternal side effects caused by isosorbide mononitrate were acceptable, although headache of mild to moderate degree was common. Maternal satisfaction and acceptability were found to be high, and the vast majority of women reported that they would recommend the outpatient procedure to other women.

However, although this was a double-blind randomized study, the high frequency of headache in women treated with isosorbide mononitrate may have influenced their comments. Since the women were informed about possible side effects of the nitric oxide donor before being included in the study they may have guessed whether they had received isosorbide mononitrate or placebo tablets. This may have prompted them to report higher satisfaction rates with the novel regimen.

Taken together, this study indicates that vaginally administered isosorbide mononitrate for cervical ripening and induction of labor seems to be safe, effective, and well tolerated. Further studies are necessary to identify the ideal formulation, dose, and frequency of administration of nitric oxide donors, as well as to confirm the safety of the procedure when repeatedly administered. ■

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European Journal of Obstetrics & Gynecology and Reproductive Biology 130 (2007) 160–164
ejog European Journal of Obstetrics & Gynecology and Reproductive Biology
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Isosorbide mononitrate induces increased cervical expression of cyclooxygenase-2, but not of cyclooxygenase-1, at term

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Received 6 October 2005; received in revised form 7 December 2005; accepted 10 January 2006

Abstract

Objective: Prostaglandin and nitric oxide (NO) are both known to be involved in cervical ripening at term. The aim of the study was to investigate if NO has an effect on cervical expression of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), the two main isoenzymes involved in prostaglandin synthesis, and to localize these enzymes within the cervix.
Study design: Women with an unripe cervix scheduled for elective caesarean section at term were randomly selected to receive vaginally either the NO donor isosorbide mononitrate (IMN) or placebo 4 h before surgery. At the operating theatre, cervical tissue specimens were obtained for immunoblotting and immunohistochemistry.
Results: Increased expression of COX-2 was found in specimens exposed to IMN compared to specimens obtained from women in the placebo group. There was no difference in the expression of COX-1. Immunohistochemistry revealed similar localization of the two enzymes in treated and untreated women.
Conclusions: Vaginal administration of IMN induces increased cervical expression of COX-2, but not of COX-1. This pathway may be of importance in the process of cervical ripening at term.

Keywords: Cervical ripening; Cyclooxygenase; Nitric oxide; Prostaglandin

1. Introduction

The biochemical events during cervical ripening resemble an inflammatory reaction, involving influx of leukocytes, an increase in extracellular water content, and rearrangement of collagen fibres [1–3]. Prostaglandins have been considered to be central mediators of cervical ripening [4]. Prostaglandin (PG) synthesis is catalysed by cyclooxygenase (COX). The two main isoenzymes of cyclooxygenase, COX-1 and -2, are differently regulated. COX-1 is constitutively expressed in numerous tissue types and its expression is, in general, not regulated. In contrast, COX-2 is typically undetectable in most tissues under normal physiological condition but can be expressed at high levels following stimulation.

An endogenous nitric oxide (NO) system is present in the human uterine cervix [5,6]. In addition, increased expression of NO synthases, enzymes that catalyse NO production, has been found in cervical biopsies at term [5,7,8]. Nitric oxide donors, such as isosorbide mononitrate (IMN) and nitroglycerin, have been documented to induce cervical ripening in pregnant women [9,10]. Nitric oxide has a multifunctional role in inflammation and various proinflammatory effects of NO have been described, including an increase in vascular permeability, cytotoxity and tissue damage, changes in glycosaminoglycan synthesis and apoptosis. Apoptosis is known to play an important role in cervical ripening [11]. Thus, several lines of evidence indicate that NO is a mediator of cervical ripening. Furthermore, an interaction between NO and PG synthesis has been suggested to be present in the cervix at term [12]. Such a linkage between two proinflammatory substances could be of importance in promoting cervical ripening.

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0301-2115/$ – see front matter
doi:10.1016/j.ejogrb.2006.01.021

The aim of the present study was to investigate if NO has an effect on cervical expression of COX-1 and -2 at term and to localize the two enzymes within the cervix.

2. Materials and methods

2.1. Patients and tissue sampling

The study was approved by the ethics committee of Gothenburg University. Twenty-four healthy women, scheduled for elective caesarean section were, after informed consent, included in the study. Inclusion criteria were gestational length > 37 weeks, no former vaginal delivery, uncomplicated singleton pregnancy and unripe cervical status (Bishop score < 6). All women included were 1–4 days before caesarean section informed of the nature and scope of the study, as well as the potential side effects of IMN. Cervical status was assessed in the morning the day of caesarean section. If inclusion criteria were met, the women were randomised to either of two groups: (1) 40 mg IMN (two 20-mg tablets) (ISMO®, Boehringer Mannheim, Mannheim, Germany) 4 h before the caesarean section or (2) two placebo tablets of similar design as IMN 4 h before caesarean section. Randomisation was performed by means of numbered, sealed envelopes prepared with random-number tablets. Twelve women were allocated to each group. The tablets were administered into the posterior vaginal fornix by an obstetrician. Neither the participating women nor the doctor were aware of the agent administered. At the operating theatre, spinal anaesthesia was given and the women were placed in the lithotomy position. Cervical tissue specimens were obtained transvaginally from the anterior lip of the cervix by use of a 14 gauge Tru-Cut® biopsy needle (Allegiance Healthcare Corporation, McGore Park, IL, USA). Three cylindrical biopsies of approximately 1.5×15 mm size (wet weight 25–40 mg) were obtained from each woman. The tissue specimens were immediately transferred to liquid nitrogen and frozen at -70 °C until analysis. Caesarean section was then performed.

2.2. Immunoblotting

The immunoblotting procedure for assessment of COX-1 and -2 expression has previously been described in detail [6]. Briefly, a monoclonal antibody raised against purified ovine COX-1 (Cayman Chemical Company, Ann Arbor, MI, USA), diluted 1:1000, was used for measurements of COX-1 expression. For determination of COX-2 expression, a monoclonal antibody raised against a synthetic peptide from the human COX-2 580–599 amino acids sequence (Cayman Chemical Company), diluted 1:1000, was used. As positive controls commercially available standards, human COX-1 electrophoresis standard and human COX-2 electrophoresis standard (Cayman Chemical Company) were used.

2.3. Immunohistochemistry

Tissue specimens for immunohistochemistry were embedded in OCT (Sakura Finetek, Zoeterwoude, The Netherlands). Sections, 6 μm thick, were rinsed in phosphate-buffered saline (PBS), treated with 0.3% hydrogen peroxide in methanol for 30 min, and then exposed to normal horse serum (Vector Laboratories Inc., Burlingame, CA, USA) for 20 min at room temperature. They were then incubated with the primary monoclonal antibody against either COX-1 or COX-2 (Cayman Chemical Company) diluted 1:100 in 1% bovine serum albumin/PBS at 4 °C overnight. The sections were rinsed in 0.05% Triton-X (Sigma Chemical Company, St. Louis, MO, USA) in PBS and then incubated with biotinylated horse anti-mouse immunoglobulin G (Vector Laboratories) as secondary antibody for 30 min followed by the addition of avidin–biotin peroxidase complex (Vector Laboratories) for 60 min. To visualize immunoreactivity the specimens were exposed to 0.05% 3′,3′-diaminobenzidine-tetrahydrochloride (Sigma Chemical Company) in PBS containing 0.03% hydrogen peroxide for 7 min. Two sections were prepared from each of the women in the IMN and the placebo group, respectively. Negative controls were treated with PBS without any monoclonal antibody. Sections of epithelial ovarian carcinoma were used as positive controls [13].

2.4. Data analysis

Statistical analysis for immunoblotting was performed using the Mann–Whitney *U*-test. The results were expressed as median and range. $P < 0.05$ was considered to be statistically significant.

For immunohistochemical analysis, the slides were examined by two independent observers using light microscopy. Localization of positive staining was carried out, but no attempt to quantify staining by objective methods was performed.

3. Results

In all women included in the study the treatment interval following administration of either IMN or placebo tablets until caesarean section was uneventful. No side effects caused by the NO donor or the placebo tablets were registered.

No statistical difference in the expression of COX-1 was found in women treated with IMN (median 5.2; range 0.7–19.5) compared to women in the placebo group (median 5.0; range 3.3–5.6; Fig. 1). The expression of COX-2 was significantly higher ($P < 0.001$) in women treated with IMN (median 16.8; range 8.1–31.7) than in women who had been given placebo tablets (median 1.5; 0.4–5.8; Fig. 2).

Positive immunostaining for both COX-1 and -2 was found in smooth muscle of the vessel wall, in bundles of stromal smooth muscle, and in glandular and squamous

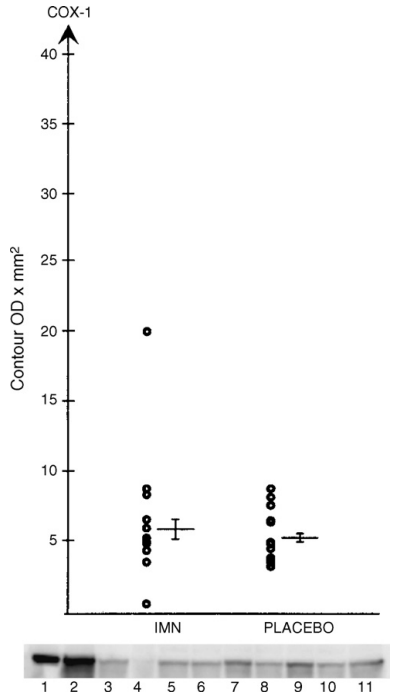

Fig. 1. Cervical expression of COX-1 in 12 women treated with IMN and in 12 women given placebo tablets (mean ± S.E.M.) and representative immunoblots of COX-1. Lane 1: human COX-1 electrophoresis standard. Lanes 2–6: IMN. Lanes 7–11: placebo.

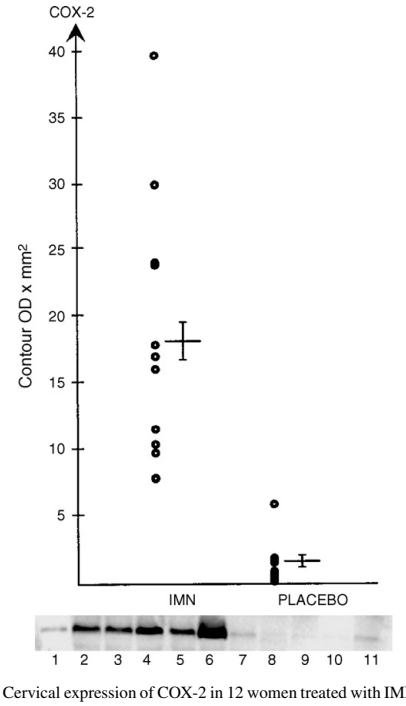

Fig. 2. Cervical expression of COX-2 in 12 women treated with IMN and in 12 women given placebo tablets (mean ± S.E.M.) and representative immunoblots of COX-2. Lane 1: human COX-2 electrophoresis standard. Lanes 2–6: IMN. Lanes 7–11: placebo.

epithelium (Fig. 3). In addition, immunopositive cells, probably representing inflammatory cells, were observed as isolated cells or as clusters of cells. No difference in distribution of COX-1 positive cells or staining intensity of these cells was observed between the two groups. Although the number of immunopositive cells per visual area was too low to make any statistical calculation there was an overall impression by the two independent observers that the staining for COX-2, especially in the "inflammatory" stromal cells, was stronger in women treated with IMN as compared with women given placebo tablets. In sections where the squamous epithelium happened to be included, staining intensity was found to be stronger for COX-1 than for COX-2.

4. Discussion

Cervical ripening has been described as a complex biochemical cascade in which hormones, cytokines, leucocytes, prostaglandins, nitric oxide, and various other substances are involved [12,14]. Prostaglandins and NO are thought to play a fundamental role in cervical ripening, but the mechanisms regulating these biochemical mediators have not been clarified.

Previously, it has been shown that intravaginal administration of 40 mg IMN for 4 h induces cervical ripening at term [10]. The present study demonstrates that the cervical expression of COX-2, but not of COX-1, is increased following intravaginal treatment with the NO donor for 4 h, as assessed by immunoblotting. This finding indicates that an interaction between NO and prostaglandin synthesis is present in the uterine cervix at term. Several studies have demonstrated that NO can induce the expression of COX-2, thereby increasing the release of endogenous prostaglandin [15–19]. Nitric oxide exerts its effects either via cyclic guanosine monophosphate (cGMP) as a second messenger or via cGMP-independent pathways. In cervical tissue specimens obtained from women before surgical termination of pregnancy in the first trimester, levels of cGMP as well as COX-2 have been found to be increased following intravaginal administration of IMN [20].

When immunoblotting is carried out to assess COX expression it is important to bear in mind that the binding to COX-antibodies comprises both active and inactive enzymes. Consequently, the semiquantitative estimation of the enzymes does not directly relate to enzyme activity. This means that we can only presume that the increase in COX-2 expression was due to new enzyme synthesis.

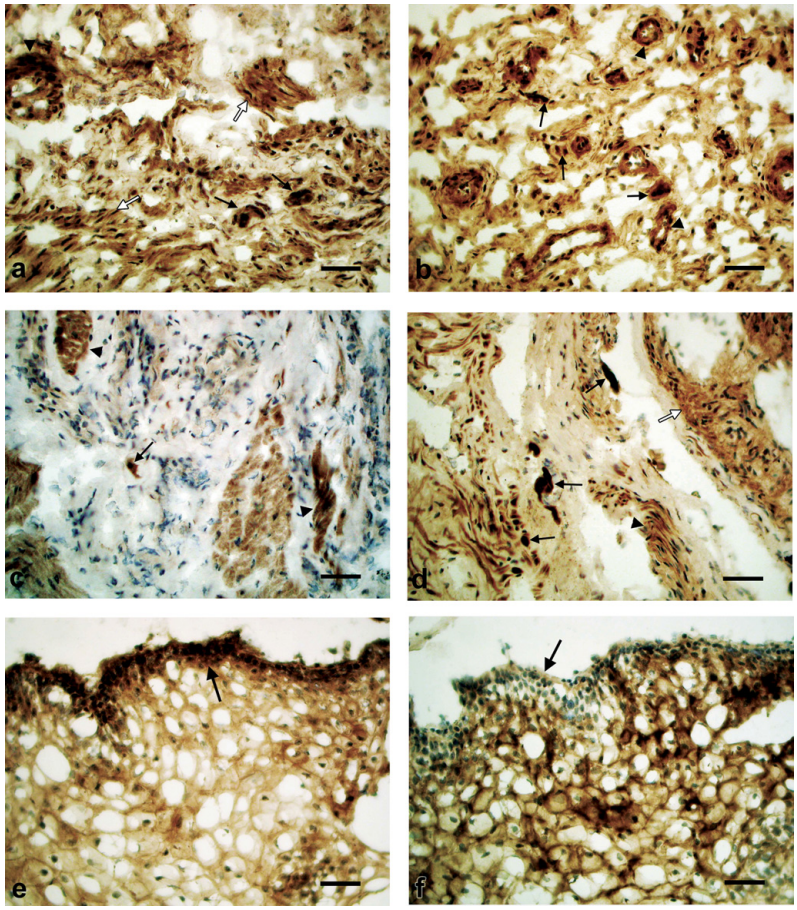

Fig. 3. Immunohistochemical staining for COX-1 and -2 in cervical specimens obtained from women treated with IMN and women given placebo tablets. Scale bars = 50 μm. (a) Placebo. Positive staining for COX-1 seen in smooth muscle of blood vessels (arrowhead), stromal smooth muscle (open arrow), and in stromal interstitial cells (arrow). (b) IMN treatment. Cervical tissue where positive staining for COX-1 is seen in smooth muscle of blood vessels (arrowhead) and in clusters of interstitial cells in the perivascular space (arrow). (c) Placebo. Positive staining for COX-2 is seen in smooth muscle (arrowhead) and in an isolated stromal cell (arrow). (d) IMN treatment. Positive staining for COX-2 is seen in smooth muscle of blood vessels (open arrow), stromal smooth muscle (arrowhead) and in perivascular and stromal cells (arrow). (e) IMN treatment. Positive staining for COX-1 is seen in epithelial cells (arrow). (f) IMN treatment. Faint positive staining for COX-2 is seen in epithelial cells (arrow).

In support of the present results, a recent report demonstrated that COX-2 mRNA, but not COX-1 mRNA, was increased at term, as assessed by RT-PCR technique [21].

Immunohistochemistry did not reveal any difference in cell types that turned out to be COX immunopositive between the two groups of women. Nevertheless, it was an overall impression that the staining for COX-2, especially in interstitial cells, was more intense in women treated with IMN as compared to women in the placebo group.

The distribution of COX positive cells found in the present study is similar to the results demonstrated in a recently published study comparing COX-1 and -2 distribution in cervical tissue specimens obtained from preterm and term pregnant women [21]. In the latter study, the expression of both COX-1 and -2 was increased at term. It therefore seems likely that both COX-1 and -2 are increasingly expressed towards term, but that NO preferentially has a stimulating effect on COX-2.

In the present as well as the previously cited study [21], positive immunostaining for COX-1 as well as COX-2 was found in smooth muscle. In an earlier study, positive staining for inducible NO synthase was described in cervical smooth muscle cells at term [6]. While connective tissue constitutes the main component of the human uterine cervix, smooth muscle cells constitute only 10–15% of the human uterine cervix [22]. The role of cervical smooth muscle has only to a

certain extent been examined and may have been underestimated. It has been shown that prostanoids, especially prostaglandin E_2, inhibit contractions of the cervical muscle in vitro and that the sensitivity to prostaglandin E_2 is increased at term [23]. In addition, relaxing effects of cervical smooth tissue following administration of NO donors have been demonstrated in vitro in tissue specimens obtained from pregnant women [24,25].

Several studies have concluded that prostaglandins can stimulate the release of NO [19]. In a recent study, it was reported that misoprostol, a prostaglandin E_1 analogue, stimulates cervical NO release in pregnant women and that the sensitivity of NO synthesis to misoprostol was enhanced at term [26]. On the other hand, it has also been shown that NO stimulates prostaglandin E_2 release from cervical tissue explants and that cultured cervical tissue after in vivo treatment with IMN increases prostaglandin $F_{2\alpha}$ release significantly [27,28]. Thus, a chain reaction where the initial NO stimulation caused by prostaglandin is followed by an endogenous release of prostaglandin triggered by NO has been suggested [26]. The interaction between NO and prostaglandin synthesis indicates a possible joint action of the two substances which may be of importance for optimal cervical ripening at term.

Acknowledgements

This study was supported by grants from Handlanden Hjalmar Svensson and the Gothenburg Medical Society.

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International Journal of Gynecology and Obstetrics (2005) **91**, 228–232

International Journal of
GYNECOLOGY
& OBSTETRICS

www.elsevier.com/locate/ijgo

CLINICAL ARTICLE

Sublingual nitroglycerin for management of retained placenta

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Received 16 June 2005; received in revised form 23 August 2005; accepted 31 August 2005

KEYWORDS
Nitroglycerin;
Oxytocin;
Retained placenta

Abstract

Objective: The aim of the study was to investigate the effect of sublingual nitroglycerin for management of retained placenta and to assess possible adverse effects of the treatment. *Method:* Twenty-four women were randomly selected to receive either 1 mg nitroglycerin or placebo tablets sublingually if intravenous oxytocin and controlled umbilical cord traction had failed to expel the placenta. Success rate for delivery of placenta, blood pressure, pulse rate, blood loss, and various side effects were examined. *Result:* All 12 women in the nitroglycerin group had successful delivery of placenta, while removal of placenta was successful in only one of the 12 women in the placebo group. No adverse effects of clinical importance were registered. *Conclusion:* Sublingual nitroglycerin for treatment of retained placenta seems to be effective without causing serious adverse effects. However, the definite clinical value needs to be evaluated in larger series of patients.

1. Introduction

Retained placenta occurs in 2.0–3.3% of all vaginal deliveries [1,2]. For management of retained placenta administration of oxycontin to promote uterine contractions combined with controlled umbilical cord traction is usually performed. Oxytocin is either administered intravenously or into the umbilical vein. Since injections of oxytocin into the umbilical vein in most cases do not reach the placental bed, the use of an infant nasogastric tube to thread down the umbilical vein has been recommended [3]. According to a recent Cochrane review, umbilical vein injection of diluted oxytocin via a nasogastric tube appears to be effective for management of the retained placenta [4]. In one study, a success rate of 93.3% in delivering retained placentas was reported using this method [5].

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0020-7292/$ - see front matter
doi:10.1016/j.ijgo.2005.08.020

When this procedure fails, operative manual removal under anesthesia is necessary. General anesthesia is often used since it provides both good analgesia as well as adequate cervico-uterine relaxation necessary for manual removal of the placenta. However, general anesthesia is associated with an increased risk of complications, such as aspiration pneumonitis, hemodynamic instability and delayed emergence [6].

Several reports have indicated that intravenously administered nitroglycerin is effective for successful delivery of retained placenta [7,8]. Nitroglycerin is a well-known nitric oxide donor. In vitro, nitric oxide donors, including nitroglycerin, induce immediate relaxation of the term pregnant myometrium as well as the uterine cervix [9–12]. In vivo, rapid uterine relaxation of short duration is obtained following intravenous injection of 100–200 μg nitroglycerin [13].

Since both oxytocin and nitroglycerin have shown to be beneficial for management of retained placenta, we decided to use both agents. In addition, in order to simplify the medical procedure, intravenous oxytocin was combined with sublingually administered nitroglycerin tablets.

2. Materials and methods

This trial was designed as a prospective, double-blind, randomized controlled study. Inclusion criteria were uncomplicated singleton pregnancy with spontaneous vertex delivery of a healthy child. Exclusion criteria were gestation less than 37 weeks, postpartum hemorrhage requiring immediate intervention, uterine malformation, intolerance to nitroglycerin, maternal age less than 18 years, suspected placental accretism, and serious maternal disease, defined as daily use of medication.

During the recruitment period, all women who underwent vaginal delivery had standard active management of the third stage of labor according to written hospital protocols, including intravenous or intramuscular administration of 5 IU oxytocin (Syntocinon®, Novartis, Täby, Sweden) within minutes after delivery of the neonate. Maternal effort was encouraged to expel the placenta. If this was insufficient, controlled umbilical cord traction was used. This procedure involved applying steady, gentle traction to the cord with a Kelly clamp while providing counter tracking against the uterine fundus. If the placenta still remained undelivered 30 min after delivery of the neonate an additional dose of 10 IU oxytocin was given intravenously to induce more efficient uterine contractions and thus promote placental separation. It has previously been indicated that a third stage of more than 30 min should be regarded as a prolonged third stage, because complications arise when this interval is exceeded [1]. Five minutes after administration of oxytocin another controlled umbilical cord traction for 5 min was carried out. Women were considered eligible for recruitment to the study if the placenta remained undelivered 40 min after completion of the second stage of labor, despite additional administration of 10 IU oxytocin followed by controlled umbilical cord traction.

Once the diagnosis of retained placenta had been made, a rapidly running intravenous infusion was initiated and all women were hemodynamically monitored. A total number of 24 women were recruited for the study. The women were randomized to either of the two groups: (1) 1 mg of nitroglycerin tablets (two 0.5 mg tablets) (Nitromex®, Alpharma AB, Stockholm, Sweden) or (2) two placebo tablets of similar design as the nitroglycerin tablets. Randomization was carried out by means of numbered opaque, sealed envelopes, prepared with random-number tables and was performed by a research nurse. The tablets were administered sublingually approximately 50 min after child delivery. Neither the participating women nor the obstetrician on duty were aware of the agent administered. Five minutes after administration of either nitroglycerin or placebo tablets, controlled cord traction was again performed for a maximum duration of 5 min. No extra anesthesia was given during this procedure, except inhalation of nitrous gas, if wanted by the women. If placenta was delivered by means of controlled umbilical cord traction the procedure was regarded as successful. Additional intravenous oxytocin, usually 5 IU, was given once the placenta had been delivered.

If the placenta remained undelivered, operative manual removal was conducted under either regional (epidural or spinal) or general anesthesia. To reduce postoperative hemorrhage, 5 IU of oxytocin was routinely administered intravenously at the end of the operative manual removal. Maternal blood pressure and pulse rate were measured immediately prior to the sublingual administration of either nitroglycerin or placebo tablets. These measurements were repeated 15 min later for assessment of possible hemodynamic effects caused by the nitroglycerin tablets. Blood loss during the third stage of delivery was registered in each case. In addition, all the women completed

Table 1 Characteristics and clinical data of women participating in the study

	Oxytocin + nitroglycerin ($n = 12$)	Oxytocin + placebo ($n = 12$)	*P*-value
Maternal age (years)	33.17 ± 4.78 (26, 39)	31.67 ± 3.94 (27, 40)	NS
Gestational age (weeks)	281.83 ± 8.15 (265, 292)	283.50 ± 9.39 (264, 294)	NS
Nulliparous (*n*)	8	8	
Multiparous (*n*)	4	4	
Epidural anesthesia (*n*)	6	7	
Successful delivery of placenta (*n*)	12	1	<0.0001
Failed delivery of placenta (*n*)	0	11	<0.0001
Total blood loss (mL)	400 ± 108.71 (250, 650)	662.50 ± 144.80 (400, 900)[a]	<0.0001

Data are shown as mean ± SD, range.
[a] Intraoperative blood loss at the operating theater included.

a questionnaire about possible side effects of nitroglycerin.

2.1. Statistical analysis

A sample size of 24 women (12 in each group) was calculated to yield a power of 90% at the 5% significance level when assuming a success rate of 80% in the treated group compared to a 20% success rate in the placebo group. Mean ± SD and range were calculated for descriptive purposes. For statistical comparison the Student's *t*-test was used for continuous variables and the Chi squared test was used for dichotomous variables.

3. Results

No significant difference in mean maternal age, gestational age or parity was found between women treated with oxytocin + nitroglycerin versus women treated with oxytocin + placebo (Table 1). All 12 women in the nitroglycerin group had successful delivery of placenta within 5 min of controlled cord traction. Of the 12 women given placebo tablets only one woman had successful delivery of placenta by means of controlled cord traction. In the other eleven women in this group operative manual removal of placenta under regional or general anesthesia was necessary.

In women treated with nitroglycerin, a significant decrease in systolic and diastolic blood pressures was measured 15 min after administration of the drug as compared to immediately before administration of nitroglycerin (Table 2). In the placebo group a significant decrease in systolic blood pressure was registered. While a slight increase in pulse rate 15 min after administration of nitroglycerin was registered, a significant decrease in pulse rate was measured in the placebo group (Table 3).

Mean blood loss was found to be significantly higher in the placebo group versus the nitroglycerin group (Table 1). None of the women treated with nitroglycerin had a blood loss of more than 650 mL. Mean blood loss in the two groups was similar when measured before operative manual removal of the placenta was carried out, in cases where controlled umbilical cord traction had failed. Thus, operative manual removal of the placenta under regional or general anesthesia resulted in an additional mean blood loss of 275 ± 101.1 mL (range 50—400 mL). However, the blood loss did not cause any clinical

Table 2 Hemodynamic measurements in women immediately before administration of either nitroglycerin tablets or placebo tablets and 15 min after administration of tablets

	Pre-nitroglycerin ($n = 12$)	Post-nitroglycerin ($n = 12$)	*P*-value
Systolic BP (mm Hg)	119.17 ± 7.33 (110, 130)	112.92 ± 7.82 (100, 125)	0.003
Diastolic BP (mm Hg)	76.25 ± 5.69 (70, 85)	71.25 ± 4.33 (65, 80)	0.001
Pulse (bpm)	76.67 ± 5.35 (70, 86)	78.67 ± 4.21 (74, 86)	NS
	Pre-placebo ($n = 12$)	**Post-placebo** ($n = 12$)	*P*-value
Systolic BP (mm Hg)	117.08 ± 7.22 (105, 130)	114.58 ± 6.20 (105, 120)	0.026
Diastolic BP (mm Hg)	72.92 ± 7.53 (60, 85)	71.67 ± 6.51 (60, 80)	NS
Pulse rate (bpm)	75.50 ± 5.73 (66, 86)	71.50 ± 3.73 (62, 76)	0.004

Data are shown as mean ± SD, range. NS, nonsignificant.

Table 3 Comparison between differences in hemodynamic parameters in women receiving either nitroglycerin or placebo tablets (measurements obtained 15 min after administration of tablets minus measurements obtained prior to administration of tablets)

	Nitroglycerin ($n=12$)	Placebo ($n=12$)	*P*-value
Difference in systolic BP (mm Hg)	−6.25 ± 5.69 (−15, 5)	−2.50 ± 3.37 (−10, 0)	NS
Difference in diastolic BP (mm Hg)	−5.00 ± 3.69 (−10, 0)	−1.25 ± 3.11 (−5, 5)	0.013
Difference in pulse rate (bpm)	2.00 ± 3.52 (−4, 6)	−4.00 ± 3.81 (−10, 2)	0.001

Data are shown as mean ± SD, range. NS, nonsignificant.

complications in any of the women and no blood transfusions were given.

The most frequent symptomatic side effect of nitroglycerin was headache, experienced by four women. Two of these women described the headache as mild, while the two other women had headache of moderate intensity. In the placebo group, one woman reported headache of moderate intensity within 10 min following administration of placebo tablets. In all women, the headache disappeared spontaneously within 2 h. One woman in the nitroglycerin group and one woman in the placebo group reported hot flushes. No other symptomatic side effects were reported.

4. Discussion

The present study indicates that sublingual nitroglycerin is both effective and safe in cases of prolonged third stage labour. The finding that intravenously administered nitroglycerin promotes placental separation has previously been described in several reports [8,13—16]. When nitroglycerin is injected intravenously uterine relaxation usually occurs within 45—60 s and the relaxation normally lasts no longer than 2 min [13]. In contrast, sublingual nitroglycerin reaches its maximum plasma concentration within 5 min with a half-life of approximately 3 min [17]. Although not examined in detail in the present study, a slight increase in uterine bleeding indicating uterine relaxation and placental separation was observed in some of the women about 3 min after administration of nitroglycerin tablets. This observation is in agreement with the fact that vasorelaxation induced by sublingual nitroglycerin for treatment of angina pectoris normally occurs within 2—3 min following sublingual administration. Furthermore, a dose of 1 mg nitroglycerin, as used in the present study, is a standard dose for treatment of angina pectoris.

The potential risk that a dose of 1 mg nitroglycerin would induce hypotension of clinical importance seems to be ruled out. In the present study, systolic as well as diastolic blood pressures were significantly decreased 15 min after administration of nitroglycerin. In the same group of women a slight, but nonsignificant increase in pulse rate was measured. The rapidly running intravenous infusion may to a certain extent have compensated for a further decrease in blood pressure.

Another important clinical aspect of sublingual nitroglycerin for management of retained placenta is the possibility that it could induce prolonged uterine relaxation, thereby causing increased intrapartum blood loss. In the present study, no prolonged uterine relaxation following administration of sublingual nitroglycerin was registered. Following injection of oxytocin immediately after placental delivery recovery of uterine tone occurred in all women without any delay. A rapid reversal of uterine relaxation has also been described to take place when intravenous nitroglycerin is administered during obstetric emergencies [8,13,16]. Thus, the risk of nitroglycerin induced uterine hemorrhage seems to be low. However, nitroglycerin should never be administered in cases of hypovolemia or postpartum hemorrhage. When nitroglycerin is given for management of retained placenta a rapidly running intravenous infusion is mandatory and ephedrine should always be available in case of a hypotensive reaction.

Little is known about the pathophysiology of retained placenta. Dynamic ultrasonographic imaging of the third stage of labor has demonstrated that retro-placental myometrial contractions are necessary for placental separation and that lack of retro-placental contractions results in retained placenta [18]. Herman et al. suggested that placental separation is caused by shearing forces between a passive placenta and active placenta-site uterine muscle, which diminish placental site surface area and thus tear septa in the spongiosa. They hypothesized that dynamic interrelationships between the decidua in the placenta-site and corresponding uterine smooth muscle control placental separation. Recently it was demonstrated that placental anchoring villi are able to contract and relax and that this ability is under the control

of nitric oxide [19]. This mechanism may be of importance for the regulation of intraplacental volume, but it could also play a role in placental separation. In another recently published study it was demonstrated that oxytocin exerts an overall stimulating effect on nitric oxide release by fetal membranes at term and that this stimulating effect is more marked after labour than before labour [20]. An interaction between oxytocin and nitric oxide, as demonstrated in the latter study, could play a role in promoting placental separation.

The results of the present trial are extremely promising, but in line with previous reports where intravenous nitroglycerin has been used for management of retained placenta. So far, twenty-nine women with retained placenta have been treated according to the presented treatment regimen. In only one of these cases was operative manual removal under anesthesia necessary. Sequential administration of oxytocin and nitroglycerin for management of retained placenta should therefore be considered in women who are hemodynamically stable and with no signs of postpartum hemorrhage. However, larger studies are necessary to identify the exact role of nitroglycerin for the management of retained placenta and to establish standard treatment protocols.

Acknowledgment

This study was supported by grants from the Foundation of Handlanden Hjalmar Svensson and the Göteborg Medical Society.

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