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Clinical application of nitric oxide donors and blockers

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Cervical ripening is associated with complex changes in the extracellular matrix components of the uterine cervix (McInnes *et al.*, 1980; Calder, 1981; Stys, 1986; Uldbjerg *et al.*, 1992). The major macromolecular components of the extracellular matrix are collagen, proteoaminoglycans, elastin and various glycoproteins such as fibronectins (Uldbjerg *et al.*, 1983). Changes in cervical characteristics in pregnancy have been attributed to collagen content and metabolism (Uldbjerg *et al.*, 1983; Petersen *et al.*, 1991; Leppert *et al.*, 1992), proteoaminoglycans (decorin and byglycan) (Uldbjerg *et al.*, 1983) and matrix metalloproteinases. Indeed, ripening is associated with a decrease in total collagen content; increase in collagen solubility and increased matrix metalloproteinase activity. Extracellular matrix turnover in the cervix is high; thus, mechanical properties of the cervix can change quickly.

Cervical ripening has been likened to an inflammatory response (Liggins, 1981). During ripening there is an influx of inflammatory cells (macrophages, neutrophils, mast cells, eosinophils, etc.) into the cervical stroma. Proinflammatory cytokines (interleukin-1 and interleukin-8) and other mediators, such as prostaglandins (PG), are thought to play a key role on extracellular matrix metabolism and cervical ripening (Ito *et al.*, 1987, 1988; 1990; Chwalisz *et al.*, 1994). There is strong evidence that sex steroid hormones play a regulatory role in this process as well (Hegele-Hartung *et al.*, 1989; Chwalisz *et al.*, 1991; Chwalisz, 1993). Indeed, oestrogen stimulates collagen degradation *in vitro* while progesterone blocks the oestrogen-induced collagenolysis (Rajabi *et al.*, 1991). Moreover, the administration of progesterone receptor antagonist induces cervical ripening in all species studied to date, including humans (Chwalisz *et al.*, 1987; Chwalisz and Garfield, 1994).

For years, PGs have been considered to be the central mediators of cervical ripening by inducing collagenolytic activity and synthesis of PG-S_1 proteoglycans (Uldbjerg *et al.*, 1992; Kelly, 1994). Within hours of administration, PGE_2 produces clinical and histological cervical changes resembling physiological ripening which normally developed over several weeks of gestation. However, the observation that neither indomethacin (Chwalisz, 1994) nor the specific cyclooxygenase (COX)-II inhibitor flosulide (Shi *et al.*, 1996) inhibit the physiological and antiprogestin-induced cervical ripening has called into question the central role attributed to prostaglandins in cervical ripening.

Nitric oxide accumulates at the site of inflammation (Evans *et al.*, 1995). Proinflammatory cytokines enhance the expression of the inducible form of NO synthase (iNOS) (Chwalisz *et al.*, 1996). At high concentrations NO can act as an inflammatory mediator (Chwalisz *et al.*, 1996). Recently, the expression of iNOS by human myocytes was shown to increase during pregnancy and to decrease significantly around the time of labour and delivery, both at term and preterm (Bansal *et al.*, 1997). However, no decrease in NOS enzyme activity (total enzymatic activity) was found in myometrium, placenta and membranes obtained from women undergoing Caesarian section in women in labour when compared to that from tissues of women not in labour (Thomson *et al.*, 1997b). In contrast, iNOS expression and NO production are increased in the cervix of rats in term and preterm labour (Buhimschi *et al.*, 1996). Treatment of guinea pigs with L-NAME blocks cervical ripening (Chwalisz *et al.*, 1996). Taken together, these observations support a role for NO in cervical ripening.

Chwalisz *et al.* (1997) suggest in their report that NO participates in cervical ripening during pregnancy and that pharmacologic manipulation of the NO bioavailability may have considerable clinical applications. Chwalisz *et al.* (1997) report that direct application of an NO donor (sodium nitroprusside) to the cervix can induce the biomechanical and anatomical changes characteristic of cervical ripening in guinea pigs. These observations are consistent with those recently reported by Thomson *et al.* (1997a,b) in humans. These investigators conducted a randomized clinical trial comparing the efficacy of two NO donors with no treatment on cervical ripening prior to surgical termination of pregnancy in the first trimester. NO donors reduced the cumulative force required to dilate the cervix to 8 mm and thus were more effective than no treatment (Thomson *et al.*, 1997a).

Prostaglandins were introduced to induce cervical ripening before labour, and hopefully to reduce the Caesarean section rate attributable to cervical factors. Unfortunately, a clear and unequivocal reduction in the rate of Caesarean section after induction of cervical ripening with prostaglandins has not been demonstrated (Keirse, 1992). Moreover, the effects of

prostaglandins, even when used to ripen the cervix, are not confined to the cervix. Prostaglandins may cause uterine hyperstimulation with potentially adverse effects in patients with a previous uterine scar or at risk for fetal distress (Egarter *et al.*, 1990). NO donors are an attractive alternative for cervical ripening because they can induce this effect without stimulating myometrial contractility (Chwalisz *et al.*, 1996). Nonetheless, in the randomized clinical trial of Thomson *et al.* (1997a), a PGE_1, analogue (gemeprost) was more effective than the NO donors isosorbide mononitrate and glyceryl trinitrate. Further studies are needed to determine if other NO donors are better in achieving cervical ripening as well as the optimal formulation and dose for such an agent. While prostaglandins may be suitable for first trimester termination of pregnancy, NO donors may be safer closer to term. Therefore, further comparison of the safety and efficacy of these two distinct pharmacological approaches (prostaglandins *versus* NO donors) to the induction of cervical ripening is desirable.

It is noteworthy that the reported clinical effects of NO donors on the uterus may depend upon the route of administration. Systemic administration of NO donors during pregnancy inhibits uterine contractility. This effect has been used clinically for manual removal of a retained placenta (De Simone *et al.*, 1990), breech extraction (Greenspoon and Kovacic, 1991) and pharmacological inhibition of uterine contractility in preterm labour (Lees *et al.*, 1994; Rowlands *et al.*, 1996) Indeed, Rowlands *et al.* (1996) have recently reported that treatment of spontaneous preterm labour with 50 mg patches of nitroglycerine (a NO donor) resulted in uterine quiescence as well as closure and lengthening of the uterine cervix (Rowlands *et al.*, 1996). This is in contrast to the local application of these compounds to the cervix which result in the induction of cervical ripening (Chwalisz *et al.*, 1997; Thomson *et al.*, 1997b) These apparent paradoxical effects may be explained by the tissue concentrations required to achieve a biological effect on the cervix and myometrium. Pharmacokinetic studies will be required to assess the rate of absorption of locally administered NO donors and the effects of these compounds on maternal and fetal cardiovascular systems.

The use of agents capable of blocking local NO bioavailability may also have potential clinical application in the treatment of premature cervical ripening. This area is largely unexplored at this time. Today, there are no pharmacological methods to reverse or prevent premature cervical ripening. The effectiveness of cerclage, the standard clinical therapy for cervical dilatation in the mid-trimester remains controversial (Novy *et al.*, 1990; MRC/RCOG, 1993). Systemic administration of L-NAME, a non-specific inhibitor of NOS, to pregnant guinea pigs has been associated with induction of uterine contractility but delayed cervical ripening (Buhimschi *et al.*, 1996). The possibility that local treatment with NO inhibitors may have effects limited to the uterine cervix is intriguing. Inasmuch as iNOS appears to be more important that endogenous NO synthase eNOS in cervical ripening, selective iNOS inhibitors may be more suitable for this indication.

Premature cervical shortening and/or cervical funnelling detected with ultrasound is a major risk factor for preterm delivery (Romero *et al.*, 1993; Gomez *et al.*, 1994; Jams *et al.*, 1996). No treatment exists for this condition. The recognition that NO plays an important role in the regulation of cervical ripening opens new avenues of investigation into the management of premature ripening of the cervix (cervical incompetence and preterm labour), induction of labour at term with an unripe cervix and other clinical conditions in which evacuation of the uterus is required.

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Nitric oxide—another factor in cervical ripening

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It is just 50 years since David Danforth (1947) ushered in a new era of enlightenment concerning the birth process. Before 1947, most of the attention directed towards understanding human parturition had been focused on the contractility of the myometrium. In the view of anatomists, physiologists and obstetricians, the cervix was to the uterus what the anus was to the rectum – namely a muscular sphincter whose function was to remain closed until it was necessary for the contents to escape. Although William Hunter had clearly stated in his classic atlas 'The anatomy of the human gravid uterus' (1774) '*ad cervitem uteri, fibrae musculosae, in fasciculos collectae, nullae conspiciuntur*' (at the cervix no distinct bundles of muscle fibres are seen), misconceptions only began to be dispelled with Danforth's highly controversial paper 'The fibrous nature of the human cervix' (Danforth, 1947). His claim that the cervix was principally a connective tissue structure was initially rejected and even ridiculed but his demonstration that normal and abnormal cervical function in pregnancy and labour can only be understood in terms of connective tissue biology gradually gained acceptance. This heralded five decades of increasing enlightenment.

A proper understanding of pregnancy maintenance on the one hand and parturition on the other, demands consideration of the highly complex, independent control mechanisms of myometrial contractility in the corpus and connective tissue behaviour (especially concerning collagen) in the cervix, and the relationship between them. Just as the past century has seen a bewildering array of substances contributing to the control of the myometrium, the past half century has seen emerging a similar welter of factors which may prevent or promote cervical ripening. To such agents as placental steroids (Henneman, 1973; Gordon and Calder, 1977), prostaglandins (Calder and Embrey, 1973), relaxin (Hisaw and Hisaw, 1964), and chemokines (Barclay *et al.*, 1993), and agents which modify their effects, can now be added nitric oxide (NO). Chwalisz *et al.* (1997) have shed new insights on the control of the cervix during pregnancy. Their experimental data from guinea pig studies and the theoretical discussion they advance argue very strongly for a crucial role for NO in this process. Debate will doubtless follow concerning the relative importance of different factors. It seems beyond dispute however that no single factor is responsible or even pre-eminent in cervical ripening and there is ample scope for further study of this intriguing process.

Different investigators have chosen to focus on either the collagen or the ground substance which envelopes it. A fuller understanding will inevitably require clarification of the intimate relationship between these different components, as well as the dynamic roles of the resident cellular elements and those of visiting leukocytes. The biochemical events which interplay within the milieu of the cervix should provide a fertile area of exploration extending well into the 21st century. Animal models such as those employed in these most recent basic studies will require to be mirrored by observations in human pregnancy.

From a clinical perspective, the need to induce labour and to search for more effective, safe and acceptable techniques of replicating normal parturition continue to offer opportunities to elucidate the biology of the cervix. Currently the gold standard for inducing cervical ripening in human pregnancy is the local application of prostaglandins (PG), notably PGE_2 (Calder and Greer, 1992). While this represents a considerable therapeutic advance it is still far from perfect. There are two obvious deficiencies. The first is the difficulty of divorcing cervical ripening from provocation of myometrial contractions.