Prostaglandin endoperoxide H synthase inhibitors and other tocolytics in preterm labour

Bryan F. Mitchell, David M. Olson

Abstract

Preterm delivery (≤37 weeks of gestation) is the major obstetrical complication in developed countries, yet attempts to delay labour and prolong pregnancy have largely been unsuccessful. One of the many reasons it is so difficult to prevent preterm birth is that the nature of preterm labour changes as a function of gestational age, maternal lifestyle factors or infection, to list a few of the reasons. The inhibitors of prostaglandin endoperoxide H synthase (PGHS), known as the Non-steroidal Antiinflammatory Drugs, have been viewed with interest as tocolytics with promising effectiveness under most conditions of preterm labour. Three isoforms of PGHS exist; the first two, PGHS-1 and -2, have been studied for their catalytic activity, X-ray crystallographic structure, and physiological roles in the adult and the foetus. Mixed inhibitors and isoform-specific inhibitors of PGHS have been developed, and their roles in delaying preterm labour are examined and compared to other tocolytics.

1. Introduction

Preterm delivery (≤37 weeks of gestation) is the major obstetrical complication in developed countries [1]. It occurs in 5–10% of births and is associated with >75% of perinatal death and long-term infant disability. Accordingly, a great deal of basic science and clinical research has focused on the regulation of uterine contractions and prevention of preterm labour. Development and evaluation of drugs to stop uterine contractions—called tocolytic agents—have been the subjects of hundreds of research papers. Despite these efforts, there is little indication that they have had any favourable impact on the clinical problem of preterm birth. For example, in Canada, the incidence of preterm delivery in 1997 was 7.1% [2]. This represents a significant increase over the previous two decades. Three main factors explained the increasing incidence: better ascertainment due to better pregnancy dating through use of ultrasound; increasing frequency of iatrogenic delivery prior to 37 weeks gestation for maternal or foetal indications; increased incidence of multiple pregnancies mainly due to more frequent use of assisted reproductive technologies. The Canadian Perinatal Health Report 2000 [3] issued by Health and Wellness Canada continues to emphasize that, “Preterm birth accounts for 75–85% of all perinatal mortality in Canada and is an important determinant of neonatal and infant morbidity, including neurodevelopmental handicap, chronic respiratory problems, infections and ophthalmologic problems.” It is often thought that delaying preterm birth for those pregnancies less than 30 weeks gestational age, which represents 1% of births [3], is most important and that if the pregnancy can achieve 30 weeks, then the greatest concern is past. However, recent evidence showed that children at age 10, who were born between 30 and 34 weeks, had significantly diminished language development [4]. This demonstrates that prematurity at any gestational age can carry...
risks. Therefore, the health, emotional and societal expense of preterm birth is substantial, costing in the billions of dollars each year [1]. The prevention of preterm birth is considered the most important perinatal challenge facing industrialized countries [1,5].

In this chapter, we will discuss the use of PGHS inhibitors as potential treatment to prevent preterm birth. We also shall briefly discuss the use of alternate therapies that have been tested in randomized controlled trials. Though the detailed experimental data describing the role of prostaglandins (PGs) in parturition are provided in other chapters of this volume, we will begin with a brief overview of the physiology of uterine contractility as necessary to understand the potential mechanisms of action of the putative tocolytic agents.

2. Uterine contractions

This subject will be addressed in greater detail in another chapter in this volume. But it is necessary to briefly review it here because therapies to delay preterm birth are based upon what is known about the biochemistry and physiology of birth. There are five separate physiological events of parturition: membrane rupture, cervical dilation, myometrial contractility, placental separation, and uterine involution [6]. Thus, although the uterus is the effector organ of labour, it is recognized that the overall control of parturition is not limited to changes in uterine contractility alone. Since uterine contractility is the most studied of the five physiological events of parturition, we will focus on it as the endpoint of our discussion and a model for the other physiological processes.

Throughout most of pregnancy, the uterus is in a resting phase and is poorly responsive to many contractile stimulants such as oxytocin. This phase is characterized by the long-lasting, low amplitude contractions referred to as “contractures” in sheep and “Braxton-Hicks” contractions in women. Near the time of parturition, the uterus leaves this “quiescence” phase and enters the phase of “activation” [7]. During this transition, the uterus attains increased concentrations of receptors to contractile stimulants and increases its content of gap junctions that provide rapid electrical coupling between muscle cells, thus enabling the strong, coordinated contractions characteristic of active labour. With the appearance of appropriate stimulants, including PGs, oxytocin and likely many others, the uterus enters the “stimulation” phase with strong and regular contractions that result in cervical effacement and dilation and descent of the foetus through the birth canal eventuating in birth.

As in other smooth muscle, the contractile force of the myometrium is dependent on phosphorylation of myosin and subsequent shortening of the actin-myosin filaments of the uterine muscle. This reaction is regulated by the enzyme, myosin light chain kinase (MLCK). There are three principal intracellular signaling pathways that may regulate MLCK and hence are important targets of current tocolytic drugs (Fig. 1). The receptors for PGF2α and for oxytocin are G-protein coupled to membrane phospholipase C. Stimulation of these pathways lead to increased intracellular calcium that activates MLCK resulting in uterine contractions. In contrast, another subset of G-protein coupled receptors, of which the β-adrenergic agonist receptor is a representative example, are linked to adenyl cyclase. Stimulation of these receptors will increase intracellular cAMP that in turn lowers calcium and inactivates MLCK, thus inhibiting uterine contractions. In an analogous manner, stimulation of guanylyl cyclase and generation of cGMP by nitric oxide donors may have the same effect though there is much less data to support the physiologic relevance of this pathway in the uterus.

Before describing tocolytic approaches to the arrest of preterm labour, we will first describe briefly the synthesis of PGs, their relationship to labour at term and preterm, and how the nature of preterm labour changes during gestation. This information will establish important perspectives upon which to review the data on tocolytics.

3. Prostaglandin synthesis

PGs are 20-carbon chain fatty acids that function as local hormones and are produced by all cells of the body. Biologically active eicosanoids (PGs, leukotrienes, lipoxins, and other 20-carbon fatty acids) are formed from the polyunsaturated fatty acid, arachidonic acid (5,8,11,14-cis eicosatetraenoic acid). Arachidonic acid is a common constituent of phospholipids in all
membranes within a cell, cholesteryl esters and triglycerides. The liberation of arachidonic acid from phospholipids is the initial step in the synthesis of PGs. This is accomplished directly by the catalytic action of members of the phospholipase A2 (PLA2) family of enzymes, or indirectly by the action of phospholipase C (PLC).

The second step in PG synthesis is the oxygenation and reduction of arachidonic acid to form an unstable intermediate endoperoxide. This step is catalyzed by PG endoperoxide H synthase (PGHS) or cyclooxygenase. Two isoforms of PGHS have been identified and well studied, PGHS-1 and -2. They are both homodimeric, haeme-containing, glycosylated proteins which catalyze two enzyme reactions, a cyclooxygenase reaction and a peroxidase reaction, forming, first, PGG2, which then undergoes a two electron reduction to PGH2. The mature processed forms of PGHS-1 and -2 have a great deal of homology in their 576 and 587 amino acids, respectively, [8] between 60% and 65% identity between isoforms within a species and 85–90% sequence identity for similar isoforms between species. The main exceptions are six residues in from the C-terminal region of PGHS-2, an extra 18 amino acids are inserted. The last four may facilitate binding to the nuclear and endoplasmic reticulum membranes or the entire insertion may mark the isofrom for rapid proteolysis [9,10]. PGHS-2 also lacks 17 amino acids that are present in the N-terminal region of PGHS-1. However, the amino acid residues thought to be important for catalysis are conserved [11], and the two isoforms have about the same affinity (Km) and capacity (Vmax) to convert arachidonic acid to PGH2 [12].

The X-ray crystallographic forms of PGHS-1 and -2 are nearly superimposable. But it is the substitution of valine in PGHS-2 for isoleucine in PGHS-1 at positions 434 and 523 (the residues in PGHS-2 are given the equivalent number as their counterparts in PGHS-1) that permits the design of inhibitors that are specific for PGHS-2 or -1. The smaller size of the Val 523 exposes a side-pocket off the main substrate channel in PGHS-2, which increases the volume of the PGHS-2 active site, a fact that is exploited by specific inhibitors of PGHS-2. The longer side chain of isoleucine in PGHS-1 prevents access to this side pocket and thereby considerably lowers specificity of PGHS-1 inhibitors or non-selective non-steroidal antiinflammatory drugs (NSAIDs) [13–16].

The single best characterized distinction between PGHS-1 and PGHS-2 is their differential regulation of expression. PGHS-1 can be detected in most tissues although not within all cells of a tissue and is therefore considered to be “constitutive”, or constantly expressed [17]. On the other hand, PGHS-2 is found at variable levels in tissues and is expressed only in response to cytokines, growth factors, or tumour promoters [18], hence it is known as the “inducible” enzyme. PGHS-2 is more highly concentrated on the nuclear envelope than PGHS-1 [19], and oxygenates lower concentrations of arachidonic acid (<1 mM) more efficiently than PGHS-1 [20], suggesting that arachidonate can be streamed within a cell for preferential oxygenation by PGHS-2.

A third distinct isofrom of PGHS has been discovered, PGHS-3 or COX-3 [21]. Along with a partial COX-1 protein, it is derived from PGHS-1 but retains intron 1 in its mRNA. It is found in the cerebral cortex of dogs followed by heart and in lesser amounts in other tissues including placenta and foetal tissues. COX-3 is inhibited more readily by acetaminophen than is PGHS-1, but aspirin and indomethacin are among its most potent inhibitors. Indeed, its presence was predicted by the properties of acetaminophen, an NSAID with potent antipyretic and analgesic actions, but relatively little antiinflammatory function, which was not characteristic of other NSAIDs that are better inhibitors of PGHS-1 or -2 [22,23]. At this time there is no evidence whether COX-3 synthesizes PGs for the physiological processes of birth.

The third enzymatic step of PG synthesis is the conversion of PGH2 to one of the biologically active PGs (D2, E2, F2a, I2, or TXA2). It is presumed that most individual cell types contain primarily one isomerase, reductase, or synthase that converts the endoperoxide to one PG characteristic of that cell. Although researchers have examined this level of synthesis over the past 2 years, there is little evidence to suggest that this step is either rate limiting or regulated in terms of PG synthesis in the human foetal membranes. We believe there may be some regulated isomerase or reductase activity in the decidua, but further research is necessary in this regard.

The PGs are now recognized as the “triggers” of labour [24] because the myometrium contracts in response to exogenous PGs, in vivo and in vitro [25–28]. PG synthetic enzymes (including PGHS-2 mRNA in humans as we first showed) and levels in uterine tissues and fluids increase before or at the time of labour [29–37], and inhibitors of PG synthesis decrease uterine contractility, in vitro [38–40], and delay birth and prolong pregnancy, in vivo [24,41–44].

4. The changing nature of preterm labour

From a mechanistic point of view, we know very little about human preterm birth compared to what is known about term birth. It is clear, however, that preterm birth is different from term birth, and that preterm birth at 24 weeks is different from preterm birth at 34 weeks. One example of the differences between term and preterm birth is the abundance or activity of PG synthetic or metabolic enzymes and concentrations of maternal plasma and foetal amnion PGs. For instance, the concentrations of tissue PGE2 and PGF2α in amnion
and placenta are significantly lower at preterm birth than at term birth [45]. This is due to the specific activity of PGHS, which we showed is considerably lower at preterm labour than at term labour in human foetal membranes. The specific activity in the human amnion rises from \(6 \pm 2\) to \(28 \pm 7\) pg PG\(E_2/\mu g\) protein/min in women delivered by cesarean section preterm but not in labour to those following spontaneous preterm labour [46]. This contrasts to the term birth levels, which are much higher, rising from \(18 \pm 4\) to \(39 \pm 6\) pg PG\(E_2/\mu g\) protein/min in women delivered by elective cesarean section at term to those delivering spontaneously at term. The situation in the chorion is similar except that the preterm levels rise proportionally less, from approximately \(9 \pm 2\) to \(22 \pm 3\) and from \(17 \pm 3\) to \(32 \pm 2\) (all increases are \(P<0.05\)) at term labour [33–35]. These data are confirmed by Sadowsky et al. [40] where they showed that although human amnion PGHS-2 protein mass and PG\(E_2\) concentrations rise with labour at preterm or term, the levels at preterm labour are equal to or lower than in non-labouring term tissues. Hence the PG synthetic capacity of membranes is considerably lower at preterm birth than at term birth when there are no signs of infection.

These foetal membrane PGs may interact with receptors in the foetal membranes [47,48] or diffuse or be transported to decidua or myometrium. However, high levels of chorionic prostaglandin 15-hydroxy dehydrogenase (PGDH), the primary enzyme that catalyzes PGs into inactive metabolites, prevents intact PGs from the foetal or maternal compartments from crossing over to the other side [49–51]. In light of information (below) regarding high decidual PG synthetic capacity throughout gestation, this metabolic barrier may be acting opposite to conventional thought, that is it may prevent maternal PGs from interacting with foetal membrane PG receptors which might lead to activation of mediators, such as matrix metalloproteinases (MMPs) [52], that promote membrane rupture. In pathological (infected) preterm birth or in about 15% of idiopathic preterm births, the specific activity of PGDH in chorion decreases, potentially allowing greater diffusion or transport of PGs across the chorion to facilitate myometrial contraction [53,54]. This capacity for PGs to traverse the chorion intact under these conditions still remains to be shown directly.

The PGHS synthetic capacity in decidua throughout gestation is different than in foetal membranes. Neither PGHS-1 nor -2 mRNA abundance, enzyme activity or protein concentrations change in decidua during gestation or with labour onset in women [29,40] or in baboons [55]. The specific activity of (total) PGHS is very high (111 \(\pm 3\) pg PG\(E_2/\mu g\) protein/min) in decidua, about 3- to 4-fold greater than in foetal membranes. Some controversy exists whether PG concentrations or output from the decidua increase with term labour onset as some studies showed no changes [40,56] while others suggest that there is an increase in PG output from decidual cells with labour at term [57–60] which may reflect changes in the specific PGE and F synthases. One report indicates there is no increase in PG output in decidua with preterm labour [40]. The human myometrium does demonstrate an increase in PGHS-2 mRNA with term labour onset [61], but another report [40] indicates that no increase in PG\(E_2\) output occurs with preterm or term labour. However, the myometrium produces mostly PG\(I_2\), which was not examined, but more likely has an effect upon vascular tone than uterine contractility [24].

Prostaglandin synthesis changes in tissues obviously lead to changes in PG concentrations in fluids, which provide more valuable information about PGs in relation to the changing nature of preterm and term birth. Evidence exists to suggest that amniotic fluid PG concentrations may be relatively low at some preterm births, an observation that reflects the low synthetic capacity of amnion at preterm birth without infection. Romero and Mitchell and others [62] showed that amniotic fluid levels of PG\(E_2\) did not rise at preterm labour compared to not-in-labour matched controls (the mean values were actually lower), but PG\(F_2\alpha\) concentrations did increase from 252 \(\pm 53\) to 731 \(\pm 363\) pg/ml, although this rise was not statistically significant. Only with infection-associated preterm labour (which occurs in 30–40% of all preterm births) was there a significant increase in amniotic fluid PG levels. During normal birth maternal plasma levels of the PG\(F_2\alpha\) metabolite rise with cervical dilatation, increasing considerably from late pregnancy not in labour (59 \(\pm 8\) pg/ml) to 143 \(\pm 32\) pg/ml in early labour and 283 \(\pm 55\) pg/ml in late labour. In contrast, the plasma levels were 63 \(\pm 17\) pg/ml in preterm labour without infection, which were not different from term not-in-labour values [63,64].

Another key association with preterm birth that changes as gestation advances is the infection rate. For instance, 70% of spontaneous preterm birth \(<30\) weeks gestational age is associated with intrauterine infection, whereas after 30 weeks a decreasing rate from 40% to 30% (at term) of spontaneous birth is associated with infection [65]. Pregnancies and births associated with infection are characterized by increased levels of cytokines (interleukin (IL)-1\(\beta\), IL-6, IL-8, and tumour necrosis factor-\(\alpha\)) in the foetal membranes [66], amniotic fluid [67–70], lower genital tract [71–73], and in the lower segment of the uterus [74]. Interestingly, normal pregnancies in the third trimester and term deliveries without infection are also associated with increased levels of IL-8 in myometrium [75] and increased levels of IL-1\(\beta\) and IL-8 in the amnion and chorio-decidua [76]. Furthermore, the synthetic capacities of PGs are stimulated by inflammatory agents [77–79] which leads to increases in intrauterine fluid and tissue concentrations.
5. History of tocolytic agents

There are several theoretical approaches that could be taken to prevent preterm delivery. Ideally, the process of uterine activation could be stopped before the clinical onset of labour. Unfortunately, there is no good method of detecting uterine activation nor is there any known method of preventing or arresting it in women, though progesterone appears to be very effective in several animal models including sheep, rats and mice. At present, tocolytic strategy is directed towards halting or reversing the stimulation phase once labour is clinically apparent. Perhaps the lateness of this intervention in the process of parturition explains the relatively poor results obtained. As an introduction to the use of PGHS inhibitors to arrest preterm labour, we will first consider briefly the history of evaluation of tocolytic agents to establish important perspectives in which to review the data and then review the experience using other tocolytics.

As noted, essentially all human trials of tocolytic agents have evaluated drugs targeting the stimulation phase of labour. Interestingly, though the steroid progesterone has been demonstrated to prevent uterine activation and prevent parturition on several animal species, it has not been examined in pregnant women. This may be due to early data that concluded, on the basis of maternal serum progesterone concentrations, that progesterone was not important in the mechanism of human parturition. It may also be secondary to the difficulty in synthesizing a suitable preparation of the lipid soluble steroid suitable for pharmacologic preparation.

The earliest tocolytic trials of the late 1960s and 1970s evaluated the efficacy and safety of ethanol to inhibit preterm labour [84]. This drug was thought to impair hypothalamic secretion of oxytocin and thus inhibit the progress of labour, though the scientific support of this mechanism was sparse. The trials showed that intravenous ethanol treatment could delay parturition by several days with a very high incidence of unwanted maternal side effects associated with acute alcohol intoxication. However, a retrospective analysis of ethanol-treated women revealed that, despite the prolongation of pregnancy, the incidence of respiratory distress syndrome and neonatal death was twice as high as in the control groups [85]. Ethanol is no longer used as a tocolytic agent.

The next class of tocolytic agents that were evaluated was the \( \beta \)-adrenergic agonists. These drugs included isoxuprine, ritodrine, salbutamol, terbutaline and hexaprenaline. Literally dozens of randomized control trials (RCT) and many metaanalyses of these trials have been performed. There is consensus that these drugs will prolong pregnancy for at least 72h but there is no evidence of improved perinatal outcome [86]. Unwanted maternal and foetal side effects, including significant cardiovascular and metabolic complications, are very frequent. There are several reports of maternal death secondary to treatment with \( \beta \)-agonists, particularly...
when used in combination with glucocorticoid therapy to accelerate foetal pulmonary maturation. These deaths have usually been associated with pulmonary edema from excessive fluid retention and myocardial arrhythmia, often associated with hypokalemia. In Canada, the manufacturer voluntarily withdrew the only drug of this class that was approved for use as a tocolytic agent (ritodrine), hence its inclusion in the history section of this article. There is some continued use of these drugs in other countries.

This history of tocolytic therapy brings into focus three major issues. The first issue is that most tocolytic drugs have no specificity for uterine smooth muscle. If they are administered in sufficient quantity to relax uterine smooth muscle, it is highly likely that they also will relax vascular and/or cardiac smooth muscle to produce prohibitive side effects, even serious maternal complications. Most of these drugs freely cross the placenta and may result in equally deleterious effects in the foetus. The first lesson learned from the history of tocolytics is that these drugs can have significant negative maternal and foetal consequences that are important to factor into consideration of the therapeutic index for these drugs.

The second important issue concerns the interestingly high success rate of placebo treatments that occur in these trials. On average, the success rate in prolonging pregnancy for 48 h ranges from 40% to 80%. This can be interpreted in two different ways. First, it may be that the diagnosis of labour was incorrect. The clinical diagnosis of labour usually requires both evidence of uterine activity (contractions that are palpable and occurring with a specified frequency for a specified duration of time) as well as evidence that the contractions are effective (causing cervical effacement or dilation or associated with rupture of the membranes). Still, it is a very subjective diagnosis. Despite active current research in this area, there is no test or combination of tests to accurately determine the presence of real labour as opposed to “false labour” that will not eventuate in parturition even if untreated. Therefore, the surprisingly high efficacy of placebo treatment may be explained by a false diagnosis of labour. This makes it essential to compare a proposed tocolytic agent to a placebo before concluding that it has efficacy. In contrast, an alternate interpretation of these data is that the placebo treatment does have a real effect and that the majority of any success achieved by tocolytic drugs is by a mechanism that involves a significant, physiological placebo effect.

The third, and perhaps most important issue that the early tocolytic trials bring out is the definition of an appropriate outcome measure. Essentially all trials have utilized prolongation of pregnancy by 24 or 48 h or 7 days. Others have measured birthweight or proportions of babies achieving a specified gestational age. But the older literature clearly indicated that these end-points may be attained but the eventual outcome of the baby may not be improved or actually may be worse! Clearly, the only appropriate outcome measure is a decrease in foetal-infant mortality and morbidity, an endpoint that few trials have had the objective or ability to assess. There is no doubt that a healthy baby at 30 weeks gestation is preferable to a sick baby at 30 weeks plus 2 days. If we fail to address appropriate outcome measures, we will repeat the historical mistakes of causing more harm than we do good with any drugs used in pregnant women.

A final observation from previous tocolytic trials relates to the proportion of women that might be appropriate for therapy to stop labour. In some instances, treatment with tocolytic agents would be futile (advanced labour with cervical dilation >4 cm) or even harmful (presence of infection or other evidence of maternal or foetal compromise). Indeed, experience has shown that only a minority of women in preterm labour will actually be candidates for attempted tocolysis (Table 1). Until we have more effective methods of early diagnosis or prevention of preterm labour, even the best tocolytic intervention may provide only disappointingly small returns.

6. PGHS inhibitors as tocolytic agents

6.1. The basis of PGHS inhibition

The use of non-steroidal antiinflammatory drugs (NSAIDs) extends back thousands of years when plant extracts containing salicylic acid were employed in the treatment of pain, inflammation and fever [87–89]. The pharmaceutical industry has long been involved in the production of NSAIDs; aspirin, which is derived from the acetylation of salicylic acid, was first introduced in 1897. NSAIDs are the most commonly prescribed drugs in the world today [89]. However, it was not until 1971 that John Vane discovered that aspirin and other NSAIDs inhibit PG synthesis [87].

These drugs owe their popularity to the fact they are first choice analgesics and antiinflammatory agents for headache and chronic inflammatory diseases including
The advent of acetylsalicylic acid (aspirin) and indomethacin, for instance, is only about 10-fold more selective for PGHS-2, while the mixed action inhibitors, indomethacin and aspirin, are slightly more specific selective for PGHS-2, while the mixed action inhibitors, indomethacin and aspirin, are slightly more specific.

The next generation of NSAIDs was the result of animal studies in which drugs were developed to combat inflammatory processes but minimize the gastric disorders. This led to the development of meloxicam, nimesulide, and NS-398. These have been marketed for approximately 25 years, but it was not until the identification of PGHS-2 that it was realized these compounds were specific inhibitors of PGHS-2 and -1, respectively. PGHS-2 inhibitors interact with the enzyme, and their kinetics appear to be time-dependent and pseudoirreversible. Conversely, when specific PGHS-2 inhibitors interact with PGHS-1, their kinetics are rapid, competitive, and irreversible. The practical importance of these kinetics becomes evident during in vivo inhibition of PGHS-1 and -2. When PGHS-2 inhibitors are present in blood or tissues at sufficiently low levels that are below the half-maximal inhibitory concentrations (IC50) of PGHS-1, the enzymatic activity of PGHS-1 will barely be altered, while PGHS-2 is inactivated.

This concept was tested and confirmed by Warner et al. [95] who compared most of the available inhibitors (Table 2). They developed a new assay, known as the William Harvey Modified Whole Blood Assay, which tests inhibitors for PGHS-1 and -2 against TXB2 formed from platelets (PGHS-1 test) and PGE2 formed from interleukin-1β-treated A549 cells in the presence of whole human blood (PGHS-2 test). Further, as most inhibitors of PGHS-1 or -2 frequently do not have parallel concentration-response curves, and it is more meaningful to test compounds at concentrations where inhibitors inactivate the enzymatic activity, they compared the IC50 values of the inhibitors. Their results show that some of the new and old PGHS-2 inhibitors are 20 to >50-fold more selective for PGHS-2 than PGHS-1 (rofecoxib and NS398), but not all are. Celecoxib, for instance, is only about 10-fold more selective for PGHS-2, while the mixed action inhibitors, indomethacin and aspirin, are slightly more specific (≤5-fold) for PGHS-1. Because NSAIDs are somewhat fat soluble and their distribution, metabolism and excretion may vary in individuals due to a number of factors, we suggest that in all clinical trials using NSAIDs for tocolysis, whole blood be withdrawn from treated mothers and tested for PGHS-1 inhibition (platelet production of TXB2) and PGHS-2 inhibition (endogenous leukocyte or cultured A549 cell production).

### Table 2

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 COX-1 (µM)</th>
<th>IC50 COX-2 (µM)</th>
<th>Ratio IC50 COX-2/COX-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>8.0</td>
<td>30</td>
<td>3.8</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.46</td>
<td>2.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Meloxicam</td>
<td>22</td>
<td>2.0</td>
<td>0.091</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>41</td>
<td>7.0</td>
<td>0.17</td>
</tr>
<tr>
<td>NS398</td>
<td>65</td>
<td>1.0</td>
<td>0.015</td>
</tr>
<tr>
<td>Celecoxib (Celebrex)</td>
<td>28</td>
<td>3.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Rofecoxib (Vioxx)</td>
<td>&gt; 100</td>
<td>5.0</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Modified from Warner et al. [95]. Comparisons were made with the concentration of NSAID that achieved 80% inhibition of enzyme activity (IC50).

rheumatoid disorders. Unfortunately, the first series of compounds produced in this class (e.g. aspirin, indomethacin) also led to severe gastric disorders, including ulceration, hemorrhage, and gastric damage, especially in chronic users. These problems are so bad, that about 1% of chronic users develop ulcers or other complications leading to 100,000 hospitalizations annually in the USA [90] and 2000–2500 deaths each year in the UK [91,92]. The primary isoform in the gastro-intestinal system is PGHS-1, which produces PGs (e.g. prostacyclin, PGI2) that have a cytoprotective role through the maintenance of mucosal blood flow and in promoting stem cell survival and proliferation of the epithelial cells in the crypts of Lieberkuhn [93]. The original NSAIDs inhibited both PGHS-1 and -2 (Table 2).
of PGE₂) to ascertain the degree of selective [96] PGHS inhibition obtained. To date, none of the tocolytic trials using PGHS inhibitors have made these tests, which could lead to alterations in the dosages used and might have implications for maternal or foetal health, especially since it may only be necessary to inhibit PGHS-2 of maternal origin to delay preterm labour. Indeed, it may only be necessary to lower PG levels marginally to achieve tocolysis sufficient for patient transfer to a tertiary centre or to administer glucocorticoids.

6.2. Maternal-foetal distribution of PGHS inhibitors

The best study examining the distribution and pharmacodynamics of indomethacin in the mother and foetus during and following chronic administration to the mother is from the University of British Columbia using sheep as the experimental animal (Fig. 3) [97]. Indomethacin was infused at the rate of 7.5 µg/kg/min for 3 days, and it was observed that only 5.2 ± 1.1% crossed the placenta to the foetus. This rate was low and due to the polarity of the compound and the permeability characteristics of the sheep’s epitheliochorial placenta. However, the inhibitor lingered in the foetus for much longer than it did in the mother following cessation of infusion for the same reason—it crossed back over the placenta to the mother slowly, especially as maternal clearance had to precede it, so it could diffuse down its concentration gradient. The concentrations in the amniotic fluid were about 10% those in the foetal plasma; foetal renal clearance was extremely low (3.8 ± 1.1 µl/min/kg); most (70%) of the glucuronidated indomethacin was found in the foetal urine, suggesting foetal rather than maternal metabolism; and none was detected in the foetal tracheal fluid leaving the placenta as the only means of total body clearance of the drug. In previous studies the foetal to maternal concentration ratio of indomethacin in sheep was 0.28 [98] or 0.04 [99]. The human, with a hemochorial placenta, has a much higher foetal to maternal concentration ratio of 1.0 [100]. This is consistent with the higher permeability of hydrophobic agents in this type of placenta [101], and while it should lead to more rapid clearance of the drug from the foetus, it also implies a more rapid build-up of the inhibitor. When a PGHS inhibitor can cross the placenta as easily as it seems possible in humans, or accumulates for a considerable time as indomethacin does in the sheep foetus, it becomes important to understand more about the effects of the drug on the physiology of the foetus.

6.3. PGs, PGHS, and PGHS inhibitors in foetal development and physiology

PGs play roles in the organogenesis and physiology of the foetus and newborn. Their actions on organogenesis
include effects on cell proliferation in several organs [102,103], both the mediation and stimulation of growth factor actions [104–107], the regulation of angiogenesis [108,109], the progression of pulmonary alveolarization [110] and the control of renal nephrogenesis [111–114]. Their effects on cell and systemic physiology include the transport of free fatty acids from pulmonary fibroblasts to epithelial cells [115], regulation of lung liquid secretion [116,117], the secretion of lung surfactant [118,119], the enhancement of intestinal enzyme activities [120], central effects on febrile, respiratory and cardiovascular responses [121] and the regulation of vascular tone in the lungs [122], kidney [123,124], brain cerebral microvasculature [125], the small intestine, and the duc tus arteriosus [126–128]. They stimulate ACTH release from the foetal pituitary and cortisol release from the adrenal gland [129] and they control urine formation [124,130].

Evidence suggests a developmental regulation of PGHS activity. The conversion of arachidonic acid to PGE2 in foetal lung tissue increases with developmental age up to the adult in sheep, suggesting an increase in the activity of PGHS [131–133]. The data from rat small intestine are similar, in that conversion of arachidonic acid to PGs increased from one week old newborns to adults, and in the human, the PGE2 content of gastric secretions increases with age in newborns 35 weeks of gestational age and older. There are species differences as to which isoform of PGHS is the dominant and regulated form in the developing lung. In the foetal lamb, Brannon and Shaul and their colleagues published studies indicating that PGHS-1 mRNA increases significantly in pulmonary artery smooth muscle, endothelial, and airway epithelial cells [134,135], whereas Asano et al. [136] described PGHS-2 as the dominant and constitutive form in cultured human lung epithelial cells. We have reported that PGHS-1 mRNA levels in human foetal tissues obtained from approximately day 50 of gestation to normal term and into the first 9 days of the newborn period are unchanged in kidney and intestine and decrease in lung with increasing developmental age whereas PGHS-2 mRNA levels increase from the first to the third trimester in these tissues and further in the newborn period in the lung and kidney (Fig. 4) [137]. This pattern mimics that for PGHS-2 in the human foetal membranes [34]. Similar increases in PGHS-2 mRNA expression are noted in foetal lambs from day 105 to 135 in hippocampus and in the sensory-motor cortex, but not in the cerebellum, where expression was very low [138]. Interestingly, PGHS-2 mRNA is the predominant isoform expressed in the cortex and cerebral microvasculature of the newborn pig, but levels are at their peak in the newborn and decline in juvenile animals. It is very intriguing to note that the human data and much of the animal data, although scarce, suggest that the increase in PGHS gene expression is most dramatic in the third trimester and reaches a peak in the early newborn. In some tissues, expression will decrease in older newborns, and in others it may continue to increase to adulthood.

Many of the roles of PGs in foetal development and physiology have been identified by studies utilizing gene deletion (knockout) for PGHS-2 in mice, which indicate that renal nephric dysgenesis, cardiomyopathies, and peritonitis occur in the newborns that normally die shortly after birth [111,139]. It is suggested that loss of PGHS-2 results in a postnatal maturation arrest in the subcapsular nephrogenic zone of the kidney. There were no remarkable foetal or neonatal effects with PGHS-1 gene deletion [140].

The use of PGHS inhibitors is another experimental tool used to discern the function of PGs in foetal tissues.

![Fig. 4](image-url)
In newborns of mothers receiving indomethacin during pregnancy, the absence of foetal PGs leads to an increased risk for intraventricular hemorrhage, necrotizing enterocolitis, either closure of the ductus arteriosus, or a patent ductus if pulmonary vascular resistance is too high, loss of renal urine formation and a subsequent decrease in amniotic fluid volume and its consequences upon pulmonary development [123,141,142]. Both PGHS-1 and -2 are present in the ductus of the foetal sheep, but PGHS-2 is found primarily in the endothelial cells lining the lumen. Use of NS398 produces the largest contraction of the ductus, in vitro, more so than the PGHS-1 inhibitor, valeryl salicylate, or indomethacin. Similar results were also obtained in vivo [126,143]. Other studies show that in utero exposure to indomethacin causes vasoconstriction, hypoxia and ultimately death of the smooth muscle cells in the media of the foetal sheep ductus arteriosus, which may lead to patent ductus in the newborn [144].

Additionally, indomethacin infused to the foetal sheep causes a mild respiratory acidosis and hypoxemia and decreased blood flow in other vascular beds, including the cerebral hemispheres and other brain regions [145]. Another well established aspect of foetal indomethacin exposure and decreased PG production is a stimulation of foetal breathing movements [145–147]. Foetal exposure to indomethacin leads to major changes in organ blood flow patterns [148], increased susceptibility to hypoxic pulmonary vasoconstriction [149] and a direct inhibition of pulmonary alveolarization [126]. Administration of the PGHS-2 inhibitor, meloxicam, to foetal sheep in preterm labour depressed the hypothalamic-pituitary-adrenal axis as evidenced by the lowering of the plasma concentrations of ACTH and cortisol [43]. The acute administration of indomethacin did not decrease renal urine formation when administered to ewes and foetuses in one study [116], but it did decrease urine formation in another study when infused to the foetus over an 8-h period [150]. A PGHS-2 specific inhibitor, SC58236, administered to neonatal rats at the time of nephrogenesis, blocked nephron development [112]. Thus it is clear that PGs are essential for development and function in a number of foetal organs.

One of the newest considerations is the role of PGHS in the foetal response to stress. We examined the mRNA expression of renal PGHS-2 and the PGE2 receptors, EP2 and EP4, to placental restriction in pregnant ewes [151]. The reduction in the total mass of the placenta throughout pregnancy led to growth restricted foetuses and hypoxemia. It also increased the mRNA abundance for PGHS-2 and EP2 in the foetal kidney. An inverse correlation developed between pO2 and PGHS-2 mRNA, and it appeared that the capacity of the foetal kidney to synthesize and respond to PGs in these stressed conditions could direct the expression of renin as there was a direct correlation between both EP2 and EP4 mRNA and renin mRNA. The effect of PGHS inhibitors on the hypoxicem or growth-restricted foetus has not been explored.

6.4. Effects of PGHS inhibitors on adult physiology and health

As mentioned earlier, the chronic administration of first generation (mixed) PGHS inhibitors caused severe gastric disturbances that led to death in some cases. This motivated the design of second and third generation drugs to treat chronic inflammation but reduce the unwanted gastric effects. The new third generation PGHS-2 inhibitors, celecoxib and roficoxib, based upon the structure activity relationship of the X-ray crystallographic image of PGHS-2, have vastly improved the gastrointestinal safety and tolerability of treating chronic arthritis [152–154]. Further, they may be able to play effective roles in treating intestinal polyposis, colorectal carcinogenesis, and Alzheimer’s disease [155].

Questions are being raised, however, regarding possible hazards in the use of the new PGHS-2-specific inhibitors. Their inhibition of PGI2 synthesis may lead to increased prothrombic activity, and caution should be exercised in patients at risk of cardiovascular complications. The possibility of altered renal function and wound healing are also concerns [154–156]. Indeed, a controversial retrospective study published recently highlighted increased risks for developing a thrombotic cardiovascular event with rofecoxib versus naproxen (which has inhibitory activity for both PGHS-1 and -2) [157,158]. (For more about the debate, read the Letters to the Editor in JAMA 286:2808-2813, 2001 and the CMAJ 167:739-741, 2002.) Also, traditional NSAIDs cause renal sodium retention and reduced glomerular filtration rate leading potentially to peripheral edema, increased blood pressure, weight gain, and congestive heart failure. Several recent reviews of studies indicate that neither rofecoxib nor celecoxib have effects different than the older NSAIDs, implying they may lead to renal failure [159–163]. Clearly, close monitoring of maternal as well as foetal effects of specific PGHS-2 inhibitors is warranted during tests of their tocolytic potential.

6.5. Indomethacin as a tocolytic

Given the rapidly accumulating evidence that PGs are involved in the mechanism of parturition, it is not surprising that PGHS inhibitors were proposed early on as potentially useful tocolytic agents. Three RCTs have compared indomethacin, a non-specific PGHS inhibitor, to placebo [164–166]. If a meta analyses is done, there is a highly significant prolongation of pregnancy achieved with indomethacin (Table 3). On first glance, it appears...
that this drug would be a good choice for tocolytic therapy in women. It should be noted however, that there was no evidence in single trials or in the meta analyses of any benefit to the baby. Additionally, during the 1970s and 1980s, it was becoming clear that inhibition of prostaglandin synthesis could have serious negative consequences in the foetus. This is due to the importance of foetal PGs in development of the kidney, foetal hemostasis and foetal vascular regulation, including maintenance of vasodilation of the ductus arteriosus and regulation of splanchnic and cerebral blood flow. Thus, in the later trials, it was important to determine whether any of these potential adverse effects were occurring in the foetus of women treated with indomethacin.

In 1991, a RCT was reported comparing indomethacin to β-agonist therapy and specific foetal side effects were measured [167]. Though indomethacin treatment was equally efficacious to the β-agonists, it was accompanied by some disturbing foetal consequences. Three of the 22 indomethacin-treated foetuses had neonatal courses complicated by primary pulmonary hypertension, a possible consequence of foetal constriction of the ductus arteriosus. Two others had a persistent patent ductus arteriosus that required medical treatment for closure. No such incidents occurred in the β-agonist treated babies. Even more alarming was a retrospective report in 1993 comparing outcomes in babies treated with indomethacin at < 30 weeks gestation compared to babies matched for gestational age but treated with other tocolytic drugs [142]. The indomethacin-treated group had a significantly increased incidence of necrotizing enterocolitis, severe intraventricular hemorrhage and persistent patent ductus arteriosus requiring surgical ligation. They also had a higher serum creatinine level that would be compatible with a toxic effect of indomethacin on the foetal kidneys. The most recent placebo-controlled RCT for indomethacin, reported in 1999, was designed specifically to assess the safety as well as efficacy of the drug [166]. Unfortunately, enrolment was difficult and the study was terminated after achievement of only 10–15% of the calculated sample size. Even with these small numbers, increased flow velocity was measured in the ductus arteriosus indicating ductal constriction after indomethacin in 2 of 19 foetuses compared to none in the controls. Further, there was decreased urine output in the first 24 h in 6 if the 12 indomethacin-treated babies in whom this was measured. This was statistically greater than the 2 of 13 in the placebo-treated group. There were no differences in urine output at 48 h of age.

Several investigators have confirmed the effects of indomethacin on foetal renal function and ductal flow velocity. A decrease in amniotic fluid volume is commonly seen in women treated with indomethacin, probably reflecting a decrease in foetal urine output [168]. Indeed, indomethacin has been proposed as a treatment for polyhydramnios, though most clinicians feel the potentially toxic effects of the drug on the developing kidneys outweigh any potential benefit. The same consideration should be given to the use of indomethacin as a tocolytic agent. The effects of indomethacin on ductal constriction appear to be dependent on gestational age [141,169]. Increased flow velocity is more easily measured at later gestational ages but it is clear that it occurs even in foetuses between 24 and 30 weeks gestation. Although the changes in urine flow and ductal flow velocity appear to be transient and reverse after several hours or days, this does not guarantee that they are harmless. Recent intense research in the area of “foetal programming” has provided strong support for the concept that transient disturbances in foetal physiology may stimulate compensatory mechanisms that provide short-term benefit to the foetus but may persist to become disadvantageous in later life [170].

### 6.6. Isoform selective PGHS inhibitors

It is perhaps unfortunate that all the early studies with PGHS inhibitors in pregnancy were performed using indomethacin. This is a non-selective drug that inhibits both PGHS-1 and PGHS-2. It has a broad range of well-described side effects and it is interesting that its use was superceded by other NSAIDs in most clinical conditions except preterm labour. It is possible that other PGHS inhibitors may not be associated with the extent of foetal side effects noted above for indomethacin.

With the advent of PGHS-2-selective inhibitors, there was hope that these drugs would provide tocolytic efficacy with a less worrisome profile of foetal side effects. Indeed the first report of maintenance of pregnancy using a PGHS-2 inhibitor, nimesulide, in a single case study where no changes in the amniotic fluid index or ductal pulsatility index were evident, did promote this optimism [171]. This hope is tempered somewhat by the findings that the tissue distribution of PGHS isoforms is quite variable among species. For example, PGHS-2 inhibitors cause ductal constriction in

### Table 3

<table>
<thead>
<tr>
<th>Study</th>
<th>Indomethacin</th>
<th>Placebo</th>
<th>R.R. (95% CI)</th>
<th>Outcome measure: Delivery within 48 h.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niebel 165 (10)</td>
<td>3/15</td>
<td>10/15</td>
<td>0.30 (0.10, 0.88)</td>
<td>14/18</td>
</tr>
<tr>
<td>Zuckerman 164 (9)</td>
<td>1/18</td>
<td>14/18</td>
<td>0.07 (0.01, 0.48)</td>
<td></td>
</tr>
<tr>
<td>Panter 166 (11)</td>
<td>3/16</td>
<td>8/18</td>
<td>0.42 (0.13, 1.32)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7/49</td>
<td>32/51</td>
<td>0.43 (0.30, 0.63)</td>
<td></td>
</tr>
</tbody>
</table>

a Relative risk (Fisher’s Exact test) (95% CI).
the sheep foetus [143] but this was not observed in the pig foetus [172]. There is very little information regarding human foetal distribution of PGHS isoforms, although the data available [137] indicate a significant increase in PGHS-2 mRNA expression in the third trimester in lung, kidney and intestine. A recently reported RCT comparing celecoxib with indomethacin in 24 pregnant women at 24–34 weeks gestation confirmed the increased foetal ductus flow velocity and diminished amniotic fluid index with indomethacin but observed no such changes with celecoxib [173]. The tocolytic efficacy of the drugs was not reported, as the trial was not designed to assess this. However, an anecdotal report suggests that maternal consumption of PGHS-2 specific inhibitors can be associated with irreversible renal failure in the neonate [174], and two studies since then confirm that nimesulide treatment leads to foetal oligohydramnios [175,176].

To date, there are no large RCTs to evaluate the efficacy and safety of PGHS isoform-specific inhibitors. There remains hope that a member of this class of tocolytic drugs will provide a high therapeutic index that may reduce mortality and morbidity resulting from preterm birth.

7. Alternative tocolytic agents

Because of potential toxicity and debatable therapeutic efficacy, many clinicians elect not to use indomethacin or other PGHS inhibitors for treatment of preterm labour. The purpose of this section is to provide a very brief synopsis of the alternative classes of drugs that are being used as tocolytic agents.

7.1. β-adrenergic agonists

Perhaps the best clinical trials in this area of research have been performed to evaluate this group of drugs. The most recent Cochrane Library systematic review indicates evidence of prolongation of pregnancy for at least 72 h [86]. However, despite enrollment of hundreds of pregnant women in these clinical trials, there is no convincing evidence of improved perinatal outcome for the baby. Unfortunately, even though the drugs are very specific inhibitors for the β2 subclass of receptors, these receptors are also present on vascular smooth muscle. As noted previously, cardiovascular and metabolic side effects limit the usefulness of these drugs as tocolytic agents. Additionally, tachyphylaxis develops within several hours and the patients become resistant to the effects of the inhibitors. Though this class of drugs remains the “gold standard” for tocolytic agents with respect to evidence-based use, their clinical use appears to be diminishing steadily.

7.2. Magnesium sulfate

This interesting drug is perhaps the most commonly used tocolytic agent in North America. The putative mechanism of action for this divalent cation involves competition with Ca2+ for entry into the cell through calcium channels and for activation of MLCK by binding with calmodulin. Its common use is interesting because a meta analysis of the two randomized placebo control studies that have evaluated the drug found that it is no more efficacious than the placebo [177,178]. On the other hand however, several trials have found its efficacy to be comparable to β-agonists, indomethacin or calcium channel blockers [179]. Although high infusion rates can lead to serious neural, respiratory or cardiac toxicity due to excessive concentrations of magnesium, this is not a common problem with low infusion rates and the drug has a lower side effect profile than most other tocolytic agents. Though it is widely debated whether or not magnesium sulphate has any tocolytic efficacy, a population-based study from Northern California in 1995 suggested that it might have other beneficial effects [180]. In a study of more than 150,000 children born between 1983 and 1985, investigators found a 70–90% reduction in the rate of cerebral palsy in children exposed to prenatal magnesium sulphate. This was not found for other tocolytic agents and was not explained by concomitant administration of glucocorticoids. This finding undoubtedly added to the popularity of magnesium sulphate as a tocolytic drug of choice. However, further controversy followed.

A large RCT designed to determine prospectively the potential neuroprotective effects of magnesium (the MAGnet trial) was terminated after the first interim analysis revealed a marked increase in the death rate of children treated with magnesium sulphate [181]. The report of these results in 1997 documented that 9 of the 75 foetuses receiving magnesium had died in the first year of life compared to only 1 of 75 in the control group. This 12% overall pediatric mortality rate with magnesium sulphate clearly would prohibit its use. Additionally, in the largest placebo control trial of magnesium sulphate, there were 7 total pediatric deaths in the 76 magnesium-treated group compared to 1 of 80 placebo treated controls. This increased the sense of alarm. However, careful review of the causes of death in the 16 deaths from these two studies reveals no pattern that could biologically be explained by magnesium toxicity. Four babies had multiple congenital abnormalities. A set of twins died from twin–twin transfusion syndrome. Two babies were asphyxiated at birth and two others were born with birthweight < 900 g and died of complications of prematurity. One died from placental abruption several weeks after magnesium had been given. Interestingly, 5 infants from the MAGnet study died of respiratory arrest or sudden
7.3 Calcium channel blockers

Increased intracellular Ca\(^{2+}\) is essential for uterine contractions. Since this increase relies partly on influx of Ca\(^{2+}\) into the cell, there is good rationale for the use of calcium channel blockers (nifedipine and nicardipine) to inhibit uterine contractions. However, as with most tocolytic agents, they have no specificity for the uterus and side effects from relaxation of vascular smooth muscle are likely to restrict use of effective concentrations. There have been no studies comparing these drugs to a placebo and therefore, like magnesium sulphate, no good evidence of efficacy. As with magnesium sulphate, several trials have compared them to \(\beta\)-agonists and have found them to be comparable, usually with fewer side effects [184]. Though still experimental, their use as a tocolytic agent seems to be increasing, particularly as a replacement for \(\beta\)-agonists.

7.4 Nitric oxide donors

There is a theoretic role for nitric oxide in regulation of uterine activity. In rats, the inducible form of nitric oxide synthase is present in the endometrium and appears to decrease at the time of uterine activation [185]. A similar situation may be present in the pregnant human uterus [186]. Therefore, augmentation of nitric oxide production may provide a physiological method to induce myometrial relaxation. The current dogma is that this gaseous molecule stimulates guanylyl cyclase to produce cGMP that leads to inactivation of MLCK. However, it has recently been reported that nitric oxide may act in other, yet unknown mechanisms to induce smooth muscle relaxation [187,188].

The efficacy of nitric oxide donors (glyceryl trinitrate patches) has been evaluated in one pilot study where they were found to be no different from placebo patches [189]. In another study, nitric oxide (nitroglycerine tablets) was shown to have no greater uterine relaxation activity than a placebo, even at doses of nitroglycerine that caused a measurable decrease in blood pressure [190]. These data suggest that this class of tocolytic agents also will be severely restricted by non-specific cardiovascular side effects.

7.5 Oxytocin antagonists

Over the past two decades, a large number of analogues of the neuropeptide hormones oxytocin and vasopressin have been developed. Some of these analogues act as agonists and some as antagonists. Some are very selective for either the oxytocin or the vasopressin receptor but many will bind to both receptors [191]. Since there is considerable evidence that oxytocin plays a role in the initiation or propagation of human labour, there is rationale for using oxytocin antagonists to treat preterm labour. Unlike other tocolytics, there is hope that this class of drugs may have specific uterine effects since the major site of oxytocin receptors is in the uterus and myoepithelial cells of the breast. Though there has been suggestion that oxytocin may have some vasoactive properties, it has been shown that it has no vasoconstriction or vasorelaxation effects when directly applied to mesenteric or uterine vessels from non-pregnant or pregnant rats [192]. The only oxytocin antagonist that has been evaluated in a clinical trial (atosiban) has considerable cross-reactivity with the vasopressin receptor, which may give rise to undesirable side effects [193].

In a large RCT comparing atosiban to a variety of \(\beta\)-agonists, the oxytocin antagonist was found to be equally as efficacious as the \(\beta\)-agonists and much better tolerated by pregnant women [194]. A large, randomized placebo control trial also has been performed [195]. Unfortunately, the results are difficult to assess. The randomization was conducted in blocks to distribute registration equally within the various participating centers. Unfortunately, there was no intentional stratification for gestational age and, in the final analysis of the 491 women enrolled, there was a biased distribution...
according to gestational age with the atosiban group having significantly more women at lower gestational ages (<26 weeks). At >28 weeks gestation, atosiban was superior to placebo at prolonging gestation by >48 h. However, in common with essentially all other literature on tocolysis, there was no significant effect on neonatal outcome. Indeed, at <26 weeks gestation, there was a significantly increased infant mortality rate in the atosiban-treated foetuses. Almost certainly, this was due to the unbalanced inclusion of very preterm foetuses in the two groups. In the atosiban group, 10 babies died of the 27 that were <26 weeks gestation at admission compared to the 0 of 16 in the placebo group. In the atosiban-related deaths at this gestational age, 7 of the 10 were <24 weeks gestation with birthweight <650 g. Thus, the safety of atosiban is unclear at very low gestational ages. Future developments in this field hopefully will yield more specific antagonists with an improved therapeutic index.

8. Glucocorticoid therapy in preterm labour

The main clinical relevance of a 48-h endpoint for determining success with a tocolytic agent is derived from the fact that this time interval is thought to be optimal for effectiveness of glucocorticoids to accelerate maturation of the foetal lung. Prenatal administration of glucocorticoids is perhaps the most solid, evidence-based therapy for management of preterm labour [196]. However, the bulk of that evidence is now 15–30 years old. Recent research has raised concerns about potential negative effects of foetal glucocorticoids [197]. In animal models, and with some supporting evidence in humans, glucocorticoid therapy may stimulate maturation at the expense of permanent growth restriction [198]. Reduction in the numbers of fully functional cells could have obvious detrimental effects on many organ systems throughout development and in the adult. This has stimulated a second look at this form of treatment [199].

At the present time, most guideline-producing agencies continue to recommend a single course of therapy but are hesitant to recommend repeated doses. Clearly, this is an area where results from future research may significantly change recommended practices.

A great deal has changed in the management of the preterm newborn in the last two or three decades. The incidence and severity of respiratory distress syndrome has decreased markedly, due in large part to therapy with artificial surfactant. Though the original surfactant RCTs suggested that glucocorticoid therapy still offered some benefits to infants who were treated with surfactant, these trials were designed to evaluate the efficacy of the surfactant preparation [200,201]. The analyses regarding glucocorticoid therapy were post hoc retrospective reviews that would not constitute first class evidence. Furthermore, these data are now a decade old. It seems logical to suggest that with earlier and more aggressive post-natal therapy with artificial surfactant, the value of prenatal glucocorticoids may be relatively less. If so, this may well influence the assessment of costs and benefits when contemplating tocolytic therapy.

Even if we were to accept that glucocorticoids provided a favourable risk–benefit ratio for the foetus, it is an assumption that treatment would be facilitated by the use of tocolytic drugs, even those that have efficacy for delaying birth by 48 h. This assumption may well be false. Three RCTs evaluating efficacy of tocolytic drugs have evaluated glucocorticoid therapy as a secondary outcome measure. In all three (Table 4), the treatment was significantly better than the placebo control for prolonging pregnancy >48 h. However, in all three, the placebo-treated patients actually had a higher (though not statistically different) rate of completing glucocorticoid therapy.

9. Future considerations

The work reviewed has generated several considerations of the use of PGHS inhibitors, or other agents that delay preterm labour, that future research should address. Several suggestions for research on PGHS inhibitors or other aspects of the prostaglandin synthesis-receptor system are discussed in the following paragraphs.

The optimal period (or the wrong period) of gestation or foetal development to use PGHS inhibitors should be investigated. The acute affects on the foetus may be different at 24 weeks than they are at 34 weeks. For example, renal development or function or ductal function may have differing sensitivities to PGHS inhibitors during foetal development so that there are times during gestation when certain PGHS inhibitors are safer or carry less risk. In contrast, the foetal prostaglandin synthesis-receptor system responds to
stress as demonstrated during hypoxia in the foetal sheep kidney [151], therefore inhibiting it may not be in the best interest of the foetus at that time, even if the mother is in preterm labour. Knowing the degree of foetal stress and its responses to it may alter therapeutic treatment for prevention of preterm labour. But there are few data in these areas and more research is necessary to make informed decisions.

The long-term effects of foetal exposure to PGHS inhibitors have not been studied. There are no data on the effects of foetal renal exposure to PGHS inhibitors on adult blood pressure regulation or adult renal function even though it is recognized that PGHS-2 knockout foetuses have nephric dysgenesis and rarely survive beyond the newborn period [111, 139]. This knowledge increases the likelihood that disruption of nephrogenesis by PGHS inhibitors may lead to long-term consequences involving renal function or blood pressure regulation.

An assessment of how the risk of preterm labour is managed may focus attention on developing tocolytics for specific needs, for instance a “stabilization tocolytic” to arrest uterine contraction for transport of mother to a tertiary centre or for assessment of maternal and foetal health versus a “long-term tocolytic” for delay of labour and maintenance of pregnancy for more than 48 h.

Trials should be conducted testing the effectiveness of combination therapies such as those recently reported in sheep in preterm labour that received both nimesulde and atosiban to block PGHS-2 activity and the oxytocin receptor, respectively [41]. Such combinations may sufficiently suppress the mechanisms of preterm labour so that lower concentrations of PGHS inhibitors can be administered, thereby avoiding or diminishing some of their side effects.

Novel efforts to modify or block components of the prostaglandin synthesis-receptor system should be explored. Reviews and studies have pointed out the effects of n-3 fatty acid diets in extending the length of gestation [202, 203]. Animal diets high in dihomo-γ-linolenic acid (DHA), an n-3 fatty acid substrate for PGHS, lead to lower levels of the biologically active PGs of the 2 series. Faroese women with diets high in n-3 fatty acids have longer gestations and their babies have higher birthweights [204], and when pregnant women consumed a fish-oil supplement, gestation was extended by 4 days on average [205]. Women at risk of preterm birth may benefit from diets high in n-3 fatty acids, as a subsequent study showed that fish oil supplementation reduced the risk of a recurrent preterm delivery from 33% to 21% in a large European multicentre trial [206].

Recently, we reported that tyrphostins, inhibitors of tyrosine kinases, block LPS-induced preterm labour in mice [207]. We determined several years ago that tyrosine phosphorylation mechanisms were associated with enhanced expression of PGHS-2 mRNA in cultured human amnion cells [208], and that tyrphostins inhibited PGHS-2 mRNA expression [209]. Many signalling pathways employ tyrosine phosphorylation, including cytokine pathways, and tyrphostins are not specific for PGHS-2 signalling, so more investigation is required to learn how they specifically block preterm labour.

Changes in expression levels of the PGF2α receptor (FP) are associated with uterine activation and preterm birth in mice [80,81], thus research exploring the role and antagonism of the FP receptor in preterm labour should prove fruitful. Indeed, recently an FP receptor antagonist, THG113, has been developed by Theratechnologies in Montreal that effectively delays preterm labour in mice [210]. This peptide antagonist only slightly (1%) crosses the placenta, is rapidly metabolized, and has no known side effects in mice. In collaboration with Dr. Jon Hirst in Melbourne, we now have the first evidence in sheep that THG113 also decreases uterine electromyographic activity and delays preterm labour induced by RU486, the progesterone receptor antagonist. Antagonizing FP action in women may be suitable for short-term tocolysis, such as during transport or assessment as suggested above, for administration of glucocorticoids, or perhaps for longer-term delay of labour and maintenance of pregnancy.

10. Summary and conclusions

Preterm delivery remains the most important obstetrical problem of the developed world. A relatively small proportion of women in preterm labour are candidates for attempted tocolytic therapy. Though the data are few and the quality of the studies diverse, there is some evidence to suggest that PGHS inhibitors can prolong gestation. However, there is considerable concern regarding the safety of these drugs for the foetus. Though there is hope that more isoform-selective PGHS inhibitors will offer an improved therapeutic index, there currently are no data to evaluate them in women with preterm labour. A host of other tocolytic agents has been evaluated but none shown to have any beneficial effects of neonatal outcome. The concept of prolonging gestation for 48 h to permit administration of glucocorticoids to accelerate foetal pulmonary maturation urgently needs to be re-addressed in the context of current neonatal care.

There is agreement that a very important factor in neonatal outcome with preterm birth is that delivery occurs in a centre with immediately available expertise in neonatology. No studies have assessed the efficacy of tocolytic drugs to facilitate successful transport of women in suspected preterm labour from outlying areas to regional centres with expertise in neonatology.
An accurate and clinically useful method to diagnose labour remains elusive. It is not surprising then that we know so little about treatment of preterm labour. It is very possible that the many RCTs evaluating tocolytic therapy are demonstrating a strong placebo effect. At the present time, this placebo effect may represent the management plan with the best therapeutic index—a combination of good efficacy with a low rate of acceptable side effects.

Our hope for the future is that research into the regulation of parturition will open new avenues of prevention. Our current strategy of arresting or reversing the stimulation phase of labour may not yield any better results than we now have. The breakthrough may come with further understanding of and the ability to recognize and control the process of uterine activation.

New generations of PGHS inhibitors may play a central role in these developments. However, at present there are grave doubts about the value of any type of tocolytic therapy and perhaps the primary consideration should be, “First, do no harm”.

Acknowledgements

Work from the authors’ laboratories was supported by the Canadian Institutes of Health Research, the Alberta Heritage Foundation for Medical Research, and the Molly Towell Perinatal Research Foundation. The contributions of Mr. Dean Zaragoza and Ms. Sheila McManus are gratefully acknowledged.

References

[9] J.C. Otto, W.L. Smith, Photolabeling of prostaglandin endoperoxide H synthase-1 with 3-trifluoro-3-(m-[125I]iodophenyl)dia-


C. Quinioi, K. Peri, X. Hou, D. Abran, S. Chemtob, PHG113 is a selective PGF2α receptor antagonist which delays preterm labor, J. Soc. Gynecol. Invest. 8 (2001) 79A.