Emerging tocolytics: challenges in designing and testing drugs to delay preterm delivery and prolong pregnancy

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The global rate of preterm delivery (before 37 completed weeks of pregnancy) is increasing and there are no effective means available to prevent this rise. Prematurity is the principal cause of neonatal mortality and a major cause of pediatric morbidity and long-term disability. Current strategies to prolong pregnancy are based on inhibiting the mechanisms that effect uterine smooth muscle (myometrium) contractions in women who are in preterm labor. Most drugs in this group were developed for other purposes. Newer strategies are designed to maintain a state of uterine quiescence and pregnancy, preventing the myometrium from initiating contractions and entering preterm labor. Again, it may be possible to use existing drugs for pregnancy maintenance. Several financial and practical barriers exist for developing completely new drugs to delay labor. Designing clinical trials to test tocolytics is complicated, as the health of two patients must be considered and the nature of preterm birth and its outcomes are different at early preterm labor (< 28 weeks) and late preterm labor (34 – 36 weeks).

Keywords: atosiban, morbidity, myometrium, neonatal, NSAIDs, pregnancy, prematurity, preterm birth, preterm labor, progesterone, tocolytics, uterus


1. Background

Prematurity is the principal cause of neonatal mortality [1] and a major cause of pediatric morbidity and long-term disability [2-4]; it is associated with at least 50% of all pediatric neurodevelopmental disorders [5]. Infants born preterm (< 37 completed weeks of gestation) are at increased risk for a range of adverse neonatal outcomes, including retinopathy of prematurity [6], respiratory distress syndrome and bronchopulmonary dysplasia [7], severe brain injury [8], necrotizing enterocolitis [9], and neonatal sepsis [10]. Long-term sequelae include risk for motor and sensory impairment (including cerebral palsy, visual impairment, and hearing impairment) [11,12], learning problems and neurocognitive impairment (including lower IQ and lower academic achievement) [13-18], and behavioral problems (e.g., attention deficit hyperactivity disorder, ADHD) [19-24]. Although the risk for mortality and morbidity is higher among early preterm births (< 32 weeks’ gestation), late preterm births (34 to 36 weeks’ gestation) [25] are more common and rates are increasing [26], thus creating a serious public health problem. Recent evidence is demonstrating that late preterm birth infants are also at risk for subtle developmental delays [27].

In spite of approximately 40 years of study and pharmaceutical testing to develop drugs that have the potential to arrest uterine myometrial contractility,
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Figure 1. Survival distributions for gestational age in weeks (wk) (p < 0.0001) of all infants who were admitted to neonatal intensive care units (n = 145 at 26 weeks, 90 at 25 weeks, 54 at 24 weeks, and 13 at ≤ 23 weeks).


56 delay labor and prolong pregnancy, the global rate of preterm delivery is increasing. In Alberta, Canada, the preterm birth rate increased from 7.2% in 1997 to 9.1% in 2004, and is currently higher than that of any other province in Canada (range 7.4 – 9.1%) [28]. In the United States, the rate is also increasing: it is now 12.1% on average, and much higher in certain locales or among African-American populations [29]. The same is true in Western Europe and Asia, although the rates are much lower than in North America.

Advancements in neonatal medicine, technologies and treatment over the past decade have led to improvements in the health and survival of infants born preterm, although the incidence of acute complications and chronic medical conditions in the surviving newborns has not decreased, partially because babies are surviving at younger gestational ages than previously [17,30,31]. The trend for increased survival and increased incidence has resulted in rapidly rising healthcare expenditures [32,33]. In addition, the emotional and social costs to parents, families and communities as a consequence of preterm birth are immeasurable. Families and society incur increased costs for long-term care and remediation of developmental delay or disability, and vision or hearing impairments. Parental absence from the workforce adds more financial and emotional stress. Further increased costs arise from the need for special education programs and increased utilization of health and social services as a result of chronic disabilities associated with preterm birth. In the United States, a conservative estimate of the yearly costs related to prematurity was $26 billion in 2005, and would undoubtedly be much more today [34]. This is incentive enough to develop effective therapies to reduce the rate of preterm deliveries.

All major international organizations, including the Institute of Medicine [34], World Health Organization, and several important health research funding bodies, including the March of Dimes [35], the National Institutes of Health, the Canadian Institutes of Health Research and others, agree that preterm birth is an important health problem that must be better addressed. However, as discussed below, preterm birth is a complex syndrome of several different etiologies, making the identification of specific drug targets more difficult. Successful approaches to address the issue of preterm birth will require improved prediction of who is at risk; improved preventative measures; for those who are in active labor, better tocolytics (drugs that arrest labor); and innovative interventions for preterm infants and their parents to improve their health and behavioral outcomes. This review will focus on drugs used for tocolysis.

2. Medical need

The clear medical need in relation to preterm birth is to prolong pregnancy as long as possible and medically advisable for optimal fetal and maternal health. Every week that pregnancy is prolonged beyond the point of viability (around 22 – 23 weeks gestational age) is another week of maturation for the fetus, with vastly improved survival (Figure 1) and decreased risk of short- and long-term morbidity. These drugs are intended to decrease or stop contractions of uterine smooth muscle, or myometrium. Tocolysis is the inhibition of labor or the delaying or halting of delivery. It derives from Greek 'tokos' (birth) and 'lysis' (dissolution) – meaning, in this sense, the dissolution of labor.

3. Scientific rationale

3.1 Basis for tocolytic therapeutic action

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 [36,37]. Thus, although the myometrium is the primary effector organ of labor, it is recognized that the overall control of parturition is not limited to changes in myometrial contractility alone although it is the target of most tocolytics.

Throughout most of pregnancy, the uterine myometrium is in a resting phase and is unresponsive to many contractile
Figure 2. Phases of pregnancy. Progesterone, prostacyclin (PGI₂), prostaglandin E₂ (PGE₂), relaxin, parathyroid hormone-related peptide (PTHrP), and nitric oxide (NO) are associated with pregnancy maintenance; estrogen and the uterine activation proteins, connxin-43 (CX-43), PGH synthase (PGHS-2, Cox-2), PGF₂α receptor (FP), and the oxytocin receptor (OTR), are associated with preparation for labor; PGs and oxytocin then stimulate the activated uterus to expel the baby, followed by uterine involution. Adapted from [36] with permission from McGraw-Hill Companies.

stimulants such as oxytocin. This phase is characterized by 138
the long-lasting, low amplitude contractions referred to as 139
‘contractures’ in sheep and ‘Braxton-Hicks’ contractions in women. Near the time of parturition, the uterus leaves this ‘quiescence’ phase (Phase 0) (Figure 2) and enters the phase of ‘activation’ (Phase I). During this transition, the uterus attains increased concentrations of receptors to contractile 145
stimulants and increases its content of gap junctions (of which the primary protein component is connxin-43, CX-43) that provide rapid electrical coupling between muscle cells, thus enabling the strong, coordinated contractions that are characteristic of active labor. With the appearance of appropriate stimulants, including prostaglandins (PGs), oxytocin and likely others, the uterus enters the ‘stimulation’ phase (Phase II) with strong and regular contractions that accompany cervical effacement and dilation and the descent of the fetus through the birth canal, eventuating in birth. Following delivery of the fetus and placenta, the uterus involutes, reducing to its pre or non-pregnant size and returning to its normal menstrual cycling (Phase III) [37].

As in other smooth muscle, the contractile force of the myometrium is dependent on phosphorylation of regulatory light chains and subsequent shortening of the actin-myosin filaments of the uterine muscle. This reaction is regulated by the enzyme myosin light chain kinase (MLCK). Three principal intracellular signaling pathways that regulate MLCK and hence are important targets of current tocolytics are illustrated in Figure 3. The receptors for PGF₂α and for oxytocin are G protein-coupled to membrane phospholipase C. Stimulation of these pathways leads 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 adenylyl cyclase. Stimulation of these receptors will increase intracellular cAMP, which in turn lowers calcium and inactivates MLCK, thus inhibiting uterine contractions. In an analogous manner, stimulation of guanylate cyclase and generation of cGMP by nitric oxide may have the same effect (though there are fewer data to support the physiologic relevance of this pathway in the uterus) [38]. Some of these actions occur in the decidua, making it another tissue target for tocolytics.

3.2 Tocolytic strategies
All current tocolytics are designed to stop preterm uterine contractions after they have started, and they do so by attacking one of the above-mentioned mechanisms responsible for causing uterine contractions. Better strategies would be to prevent the uterus from initiating preterm contractions by preventing uterine activation. Current research is targeting new drugs (not termed tocolytics) at this level, and it is hoped that clinical trials will be initiated shortly to test drugs that prevent uterine activation. The rationale, therefore, to delay preterm delivery is to target nonsymptomatic women before they enter preterm labor or to submit symptomatic women (those with preterm contractions) to tocolytic therapy. This review will review these strategies, put them into perspective relative to gestational age, discuss primary and secondary outcomes, and suggest criteria for future clinical trials.

4. Competitive environment
Tocolytics for preterm labor are developed in a relatively low-competition market, with very few drug companies investing in their research and development. This is in contrast to other pathologies such as cancer, high blood pressure or diabetes, which attract the interest of several companies, resulting in numerous drugs flooding the market. The reasons are numerous why preterm labor is at a disadvantage for attracting interest from major
pharmaceutical companies and the investment of resources into new drug development.

4.1 Financial disincentives
First among these deterrents is the large financial investment to develop a new tocolytic, like any other new therapeutic, when the size of the preterm labor market is considered. This market is limited to only pregnant women, and further reduced to those at risk for preterm labor (6 – 18%); finally, only about 1 in 10 women in preterm labor would fit the eligibility criteria for administration of a tocolytic. In the US, the population of women eligible for tocolytics is estimated at < 100,000 a year [39]. This small market would probably net annual sales of < $500 million [40]. Another disincentive is that women need only take tocolytics for a short duration, from a few hours to several days. This minimizes the profitability of a new tocolytic compared to a medication that a patient takes for a chronic disease or for a recurring seasonal malady, or one that affects large numbers of both genders and all ages.

4.2 Biochemical complications
The fetus has unique biochemical requirements for normal growth, development or specialized physiological functions that may differ from that of the maternal host. Consequently, the therapeutic potential and impact of the drug must be weighed against its potential toxic effects on the fetus. An example is indometacin, a NSAID, which can arrest uterine contractions by inhibiting prostaglandin synthesis, and is the most commonly used tocolytic in Canada. Like all NSAIDs, some indometacin crosses the placenta to the fetus, where prostaglandins have unique roles. Here it can decrease ductus arteriosus patency, alter normal urine production thereby decreasing levels of amniotic fluid, perhaps blunt nephrogenesis, and perhaps change blood flow to the brain, lung and intestine, and attenuate cell proliferation and surfactant release [41]. Hence the benefit of stopping preterm uterine contractions and extending pregnancy for the fetus must be weighed against the cost of altering fetal physiology during the period of indometacin administration.

4.3 Risk of adverse events in preterm labor
The association of a drug with an adverse fetal or newborn health event raises the potential for litigation even when the therapeutic benefit of a drug is high and the likelihood of fetal and maternal harm is very remote. No drug will be able to prevent every preterm delivery. A fetus whose mother is entering preterm labor is considered to be a healthy baby. But if an adverse event should happen when a tocolytic is being administered, even when the event is unrelated to the drug (such as fetal hypoxia due to placental abruption) or is a consequence of extreme prematurity, the potential for assessing blame to the manufacturer of the drug and the practitioner involved remains. Given the complex etiology of preterm labor, the risk is high that some babies whose mothers receive a tocolytic will have bad outcomes.

4.4 Concerns about warning labels
Related to this is the reluctance of pharmaceutical companies to test potentially effective drugs under development for other applications for their therapeutic potential in treating preterm labor. We encountered this in the early 1990s when...
Merck was developing Vioxx (rofecoxib) for the chronic inflammatory pain market [97]. We had discovered that expression of COX-2 (inducible COX-2 or prostaglandin endoperoxide H synthase-2 (PGHS-2), an enzyme involved in prostaglandin synthesis) in human intrauterine tissues, was responsible for the increased uterine synthesis of prostaglandins for labor at term and preterm, and wanted to test the efficacy of their COX-2 inhibitors in animal models of preterm birth. They refused to give us test amounts of such drugs – fearing, as it was explained to us, that an adverse outcome, even in animals, might delay the launch of rofecoxib. Indeed, after it was on the market, the warning label on each vial indicated it was contraindicated for pregnant women, although there was no evidence to support this claim. Subsequent trials in England testing the effectiveness of rofecoxib in women at risk of preterm delivery required the investigators to purchase the drug from their hospital pharmacies [42]. Unfortunately, rofecoxib was not effective in reducing the preterm delivery rate; but at least we now know that information, thanks to the persistence of these investigators.

### 4.5 Difficulty in defining primary outcome

Another deterrent to the development of new tocolytics is defining the primary outcome of the clinical trial. Prolonging gestation by 48 h or 7 days, or prolonging the time until the recurrence of labor, have each been used as a primary outcome in clinic trials of tocolytics as a measure of effectiveness [43-45]. Prolonging mean gestational length in Phase III trials is now viewed by the US FDA as a surrogate measure for improved neonatal outcomes, given the many months or years and expense required for long-term follow-up to assess subtle developmental delays or adverse health risks [46]. However, the FDA would probably still want to review infant outcome data before approval of any drug treatment for preterm labor. It is uncertain whether their interest would be a safety or efficacy end point. For most clinicians, though, the cessation of painful uterine contractions is the clinical end point. Most would not send a woman home with painful contractions and would keep her in hospital, even if she were unlikely to deliver. In practice, delaying delivery for 24 h, 48 h or 7 days has not demonstrated improved newborn outcomes [47] as revealed with β-mimetics, atosiban and indometacin. With the widespread use of artificial surfactant, the need to administer glucocorticoids to mothers to stimulate newborn surfactant production becomes less urgent. Therefore, delay of delivery for up to 48 h may be less important, except as the time that is required to transfer a pregnant woman to a tertiary unit. In the future, the length of delay of delivery should be tied more closely to improve neonatal health status rather than an arbitrary time point.

### 4.6 Trial design

The final complicating factor is that the design of clinical trials needs to consider the gestational age of women they are targeting and the likelihood that the pregnant women enrolled in the trial will deliver early. This derives because measures of morbidity change with gestational age as they reflect the developmental stage of the preterm newborn (Figure 1).

## 5. Potential development issues

As mentioned above, one of the major development issues is to design an appropriate trial to clinically test new tocolytics. Unfortunately, there are no templates of a successful design that are well suited for both early (<28 weeks gestation) and late (34 – 36 weeks gestation) preterm labor. We offer some suggestions that trials should consider in their design. This is not, however, an exhaustive list of criteria.

### 5.1 Eligibility criteria

The inclusion criteria for these trials are gestational age between 24 and 34 weeks gestation (as confirmed by ultrasound before 20 weeks gestation and/or by reliable menstrual dates), singleton pregnancies. The women will be stratified according to gestational age (<28 weeks, 28-32 weeks, >32 weeks). For tocolytic studies, preterm labor will be defined as 1) regular uterine contractions present at ≥ 30 sec duration at a rate of ≥ 4 per 30 min confirmed by external tocography 2) cervical dilation of 0-3 cm (nulliparas women) or 1-3 cm (primiparous or multiparous women) 3) cervical effacement ≥ 50%. The exclusion criteria are suspected chorioamnionitis (maternal fever ≥37.5°C, elevated white blood cell count, elevated C-reactive protein, or fetal tachycardia), premature rupture of membranes or any condition that mandates delivery, such as clinical chorioamnionitis, severe pre-eclampsia, non-reassuring fetal state, placental abruption and major fetal anomalies.

### 5.2 For symptomatic women (those in preterm labor or Phase II)

It is very difficult to perform a placebo-controlled, double-blind randomized study for preterm delivery, and especially for early preterm deliveries (Figure 2) First, many women in this category are not eligible to be enrolled in a trial. This is because about a third are medically induced; others have ruptured membranes or there are concerns about infection, pre-eclampsia or fetal anomalies. Additionally, women in preterm labor may be reluctant to enroll in a placebo-controlled trial, as they would want to receive the treatment for the potential benefit of their infant. Indeed, it is not always possible to prevent the obstetricians from biasing the study, as it is very difficult to completely disguise the treatment from the placebo, and they would prefer giving the treatment to their patients in preterm labor. Hence it is more pragmatic to compare the treatment with another known tocolytic. Unfortunately, there is no ‘gold standard’ comparison tocolytic. The choices for this are usually ritodrine (a β-mimetic) first and then magnesium sulfate or a calcium channel blocker.
Their use instead of a placebo would make a true randomized double-blind trial more feasible. In addition, a back-up or second-line (i.e., ‘rescue’) therapy is recommended in case the treatment or comparative tocolytic is not effective in a certain timeframe (e.g., 2 – 24 h). The choice for back-up treatment is ritodrine alone and then with magnesium sulfate. In the case of fetal distress, Caesarean section would be the last resort. Back-up therapies, however, complicate the interpretation of the results. Last, since nearly every drug has some side effects, it is necessary to demonstrate that the therapeutic value of the drug is greater than its complications. This becomes the ethical imperative for each drug used.

5.3 For asymptomatic women (those not in preterm labor)

This situation is different than for symptomatic women, as the treatments will be designed to keep pregnant women in Phase 0 (Figure 2), not allowing them to progress into Phase I or II. As such, they will not yet be in preterm labor; trials will therefore require more patients, because 90% of subjects will deliver at term (controls) and only 10% will deliver preterm (cases). Therefore, in order to increase the number of women at high risk for early delivery, certain inclusion criteria – such as at least one previous preterm delivery or a second trimester miscarriage, a short cervix by ultrasound, or a positive fetal fibronectin test – are helpful. Since no women are in preterm labor at the time of enrolment, it may be possible to design randomized, placebo-controlled, double-blinded studies.

5.4 Early preterm versus late preterm studies

As mentioned previously, the primary and secondary outcomes will be more difficult to determine on late preterm pregnancies (34 – 36 weeks) than on early preterm pregnancies. There is a smaller window of time to extend the average length of pregnancy (primary outcome) and the older babies are healthier babies; hence their deficits (secondary outcomes) are more subtle, and may not be evident until later in life. This means that a longer follow-up period may be required. On the other hand, there are many more patients in the late preterm group, so it is easier to increase the power of the trial. The early preterm group comprises about 10% of total preterms, and there is more variation in their outcomes. For instance, the newborn mortality at 24 weeks gestation is 50 – 60%, but this decreases to about 10 – 20% at < 28 weeks, yet both are grouped together as early preterms. Also, some 70% of early preterm deliveries are associated with an infectious process [48], which precludes them being included in a clinical trial, as it is better for such a baby to be delivered into a neonatal intensive care unit than to exist in a hostile environment.

5.5 Primary and secondary outcomes

The primary outcome of current tocolytic clinical trials has often been an increase in the average length of pregnancy compared with placebo or alternative treatment. Prolonging gestation is ineffective if neonatal outcomes are not improved, and can be deemed harmful if the mother or fetus is put at risk. Using pregnancy maintenance as a surrogate for improved neonatal outcomes is therefore dangerous and often misleading. Future trials of potential tocolytics will face the challenge of defining a measure of neonatal morbidity and mortality to use as the primary outcomes indicating tocolytic safety and efficacy. These measures should be stratified by gestational age at birth (< 28, 28 – 32, > 32 weeks) to reflect the changing health issues of neonates born at advancing gestational age. Secondary outcomes may include additional measures of neonatal health issues such as oxygen requirement, respiratory distress, necrotizing enterocolitis, paraventricular leucomalacia, or retinopathy of prematurity. Other secondary outcome measures could include a health economics assessment of treated versus untreated babies. The most meaningful studies will report both primary and secondary outcomes or combine them into a composite measure of neonatal health.

6. Existing treatment and therapeutic class review

The existing strategy to delay delivery is to administer a drug that suppresses uterine contractions during Phase II (Figure 2), as tocolytics are only administered to patients who are in active preterm labor. The problem in the field is that there are no approved specific treatments in North America, and the only approved product in Europe is atosiban, an oxytocin receptor antagonist. This is due in part to a paucity of evidence on tocolytics that precludes developing evidence-based guidelines for their use and therefore leads to regional differences in their use.

6.1 Ethanol

The earliest tocolytic trials of the late 1960s and 1970s evaluated the efficacy and safety of ethanol to inhibit preterm labor [49]. This drug was thought to impair hypothalamic secretion of oxytocin and thus inhibit the progress of labor, 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 [50]. Ethanol is no longer used as a tocolytic agent.

6.2 β-Adrenergic agonists

The next class of tocolytic agents that was evaluated is the β-adrenergic agonists. These drugs include isoxsuprine, ritodrine, salbutamol, terbutaline and hexaprenaline, and have been used extensively in the past 20 years [51]. β-Mimetics activate adenyl cyclase to form cyclic adenosine 3’,5’ monophosphate...
(cAMP). Increased levels of cAMP then decrease the activity of MLCK (Figure 3).

Ritodrine is currently the most commonly used tocolytic agent in the world, although there are large regional variances in its use. In Canada, for instance, it is not approved for use. Literally dozens of randomized control trials (RCT) and many meta-analyses of these trials have been performed [52]. There is consensus that these drugs will prolong pregnancy for at least 48 h, compared to placebo, but there is no evidence of improved perinatal outcome. The complications associated with the use of β-agonists are the high frequency of unwanted maternal and fetal side effects due to stimulation of β-adrenergic receptors. Common maternal side effects include palpitations, tremor, nausea, headaches and chest pain [53,54]. Pulmonary edema may occur. More importantly, there are several reports of maternal death secondary to treatment with β-agonists, particularly when used in combination with glucocorticoid therapy to accelerate fetal pulmonary maturation. In Canada, the manufacturer voluntarily withdrew ritodrine in 2000, although in the United States, Japan, China and elsewhere it is still the preferred tocolytic [55].

6.3 Magnesium sulphate

This interesting drug is the most commonly used tocolytic agent in the United States [55]. The putative mechanism of action for this divalent cation involves competition with Ca⁺⁺ for entry into the cell through calcium channels and for activation of MLCK by binding with calmodulin. However, in a recent Cochrane review, magnesium sulfate was found to be ineffective at delaying birth [56]. 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. Because it is no more effective than placebo, magnesium sulfate is not recommended for use as a tocolytic [56].

6.4 Oxytocin antagonists

Over the past two decades, a large number of analogs of the neuropeptide hormones oxytocin and vasopressin have been developed. Some of these analogs 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 [57]. Since there is considerable evidence that oxytocin plays a role in the initiation or propagation of human labor, there is rationale for using oxytocin antagonists to treat preterm labor. Atosiban (Tractocile, 1-(3-mercaptopropanoic acid)-2-((O-ethyl-D-tyrosine)-4-threonine-8-L-ornithine-oxytocin, Ferring) is a synthetic oxytocin receptor antagonist that binds preferentially to the vasopressin V₁A receptor in addition to binding to the oxytocin receptor [58]. It specifically inhibits myometrial contractions, as opposed to causing relaxation in smooth muscle [59]. Furthermore, the drug has a limited ability to cross the placenta, minimizing the potential for fetal accumulation and toxicity. 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, and atosiban is more specific for the oxytocin receptor.

In a large randomized controlled trial comparing atosiban with a variety of β-agonists, the oxytocin antagonist was found to be equally as efficacious as the β-agonists and much better tolerated by pregnant women [43]. Unfortunately, in a large, randomized placebo-controlled American trial, several difficulties occurred that prevented gaining a true understanding of the value of the receptor antagonist [60]. Women were stratified into the placebo or atosiban groups based on centre of enrolment, without consideration of gestational age at time of enrollment. In the final analysis of the 491 women, there was a biased distribution in which the atosiban group had significantly more women at lower gestational ages (< 26 weeks). Interestingly, the atosiban group suffered a higher rate of fetal-infant deaths overall, 4.5% (13/288) compared to 1.7% (5/295) in the placebo group [61]. For the subgroup of babies who were delivered at < 26 weeks gestation, the death rates were much higher in the atosiban group. Ten babies died of the 27 (37%) whose mothers were treated with atosiban with or without rescue and were < 26 weeks gestation at admission compared to 0 of 16 in the placebo group. For those treated with atosiban at < 24 weeks gestation and a birth weight of < 650 g, 7 of 10 newborns died. Conversely, when preterm birth occurred at > 28 weeks gestational age, atosiban was superior to placebo at prolonging gestation by > 48 h. As a consequence, the FDA did not approve atosiban for use in the United States, and questioned its effects on amniotic fluid volume and consequent potential adverse effects on lung and kidney development [61]. While individual studies have found atosiban superior to salbutamol [62], ritodrine [63], and other β-agonists [43] in terms of maternal tolerance, a recent Cochrane review did not find atosiban superior to placebo or β-mimetics in efficacy [61]. Also, in common with essentially all other literature on tocolysis, there was no significant effect on neonatal outcome. Atosiban was not approved by the FDA as a consequence, and it was not presented to the Health Products and Food Branch in Canada for approval. But in Europe, atosiban was registered as a tocolytic agent in 2000, and it is currently recommended as a first choice agent for tocolysis [53,54].

Ferring next developed barusiban, an analog of oxytocin (FE 200 440), which has a much higher specificity for the oxytocin receptor than atosiban. It crosses the placenta of fetal rabbits and marmosets but there are no adverse maternal side effects, fetal neurobehavioral effects, or changes in the fetal immune system, renal function, or organ development [64]. When given to monkeys in mid-second trimester to mimic the longest probable exposure in humans, no effect on milk production was detected in contrast to studies on rats. In vivo studies comparing barusiban to atosiban demonstrated that both drugs acted immediately in
600 pregnant cynomolgus monkeys; however, barusiban was
601 four times more potent, had up to a five times longer
duration of action, and maintained low intrauterine pressure
in the presence of daily oxytocin challenges [63]. It
inhibits oxytocin-induced contractions with a 10-fold lower
dosage than atosiban. Interestingly, it has no effect on
normal term birth, while able to delay preterm delivery in
cynomolgus monkeys [64].

In a recent double-blind, multicenter, placebo-controlled
clinical trial conducted by Ferring, patients were administered
a single intravenous bolus of barusiban or placebo. In contrast
to expectations based on animal models, there were no
statistically significant differences in uterine contractions or
time of delivery between the placebo and barusiban
groups [65]. Further development as a tocolytic agent has
been abandoned.

6.5 Calcium channel blockers
Increased intracellular Ca\(^{++}\) is essential for uterine contractions.
Since this increase relies partly on influx of Ca\(^{++}\) 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.

Studies show that nifedipine is as effective as \(\beta\)-agonists,
but with fewer maternal side effects [66,67]. Women with
cardiovascular disease, including hypertension, congenital
cardiac malformations and pulmonary hypertension, have
a higher risk of developing adverse effects when using
nifedipine for tocolysis. A recent Cochrane review concluded
that when tocolysis is indicated for women in preterm labor,
calcium channel blockers are preferable to other tocolytics
compared, mainly betamimetics [67]. Calcium channel blockers
are shown to be a more effective tocolytic agent, since fewer
births occurred within 7 days of receiving treatment and
prior to 34 weeks of gestation. Also, treatment with
calcium channel blockers was associated with less respiratory
distress syndrome, intraventricular hemorrhage, necrotizing
enterocolitis, and fewer maternal side effects.

6.6 Nitric oxide donors
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 [68]. 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 [69]. These data suggest that this
class of tocolytic agents also will be severely restricted
by nonspecific cardiovascular side effects. Overall, there is
insufficient evidence for any conclusions about the effects of
nitric oxide donors, and they should not be used until data
from future trials become available [70].

6.7 Indomethacin
Given the rapidly accumulating evidence that prostaglandins
are involved in the mechanism of parturition, it is not
surprising that PGHS inhibitors were proposed early on as
potentially useful tocolytic agents [55]. On first glance, it
appears that this drug would be a good choice for tocolytic
therapy in women; overall, however, there is insufficient
evidence for any firm conclusions about any effect on delay
of labor [71]. Moreover, it was becoming clear that inhibition
of prostaglandin synthesis could have serious negative conse-
fquences in the fetus. This is due to the importance of fetal
prostaglandins in development of the kidney, fetal hemostasis
and fetal vascular regulation. Some studies found a possible
increased risk of premature closure of the ductus, renal and
cerebral vasoconstriction and necrotizing enterocolitis
associated with high dose and prolonged exposure to
indomethacin. However, re-evaluation of the available evidence
has led to doubts about the causal role of indomethacin
in the development of necrotizing enterocolitis and intra-
ventricular hemorrhage [71]. Indeed, indomethacin is currently
the most used tocolytic agent in Canada [72], despite the
lack of good evidence on which to base decisions to use it.
Clearly, short- and long-term follow-up studies on neonatal
outcomes are warranted.

7. Current research goals and
emerging treatments
The current approach for administering tocolytics is to give
them to women who are in active preterm labor in an
attempt to reduce or eliminate myometrial contractions and,
it is hoped, to prevent further effacement of the cervix.
However, this approach has been likened to trying to stop a
speeding locomotive by standing on the tracks. Once the
physiological and biochemical processes of labor have begun,
with their redundant systems for assuring birth of the fetus,
it is very difficult to delay birth for long. Hence current
thinking is focused around trying to maintain pregnancy in
Phase 0 (Figure 2) and preventing it from entering Phase I,
activation for labor.

This entails preventing the expression of the uterine
activation proteins (UAPs). Data support PGHS-2 and the
PGF\(_{2\alpha}\) receptor, FP, as being the critical activators; but
inhibitors of PGHS-2 are not favored by clinicians. Hence,
specifically targeting FP, which has few physiological func-
tions in the mother outside of myometrial contraction and
decidual activation, and even fewer in the fetus, is a strategy
for arresting uterine activation. Our studies have suggested
three controls of FP expression or action. The first is proges-
sterone; high levels of progesterone suppress FP expression,
whereas low levels of progesterone or its receptor allow FP
expression. Secondly, cytokines, especially interleukin (IL)-1\(\beta\)
and IL-6, promote FP expression, and IL-1\(\beta\) works via
nuclear factor kappa B (NF\(\kappa\)B). Inhibiting NF\(\kappa\)B blocks
IL-1\(\beta\)-induced FP expression [73]. And last, direct antagonism
of FP blocks PGF₂α action, attenuates uterine contractions and prolongs pregnancy [74].

7.1 Progesterone

Progesterone is the primary hormone that maintains pregnancy in Phase 0 (Figure 2). In most animal species, a decrease in circulating or placental progesterone leads to uterine activation and delivery. However, in women, there is no decrease in circulating levels of progesterone at term. Almost 50 years ago, Csapo proposed the see-saw theory, which argued that there is a balance between progesterone, PGs, oxytocin and estradiol that either maintains or terminates pregnancy [75]. However, the mechanisms responsible for tipping the balance in women from pregnancy maintenance to termination are still unknown, especially in the face of high plasma proges-
teron concentrations. Some evidence promotes the idea of a progesterone withdrawal as the trigger for parturition, which can mean either a decrease in its local concentrations in the uterus or decreased uterine sensitivity to it. For instance, the progesterone receptor antagonist, mifepristone (RU486), causes abortion in early pregnancy and increased uterine activation at term. Or a change in the progesterone receptor (PR) isoform ratio could occur at term or preterm such that increasing PR-A prevents the normal isoform, PR-B, from dimerizing in the cytosol and binding to the progesterone response element on the chromatin. A change in progesterone metabolism might lead to fewer metabolites that bind the pregnane X receptor (PXR) and constitutive androstane receptor (CAR), which promote expression of inducible nitric oxide synthase (iNOS) and subsequent uterine relaxation [76].

Thus, the new tocolytic strategy that has evolved is to continue Phase 0 of pregnancy by administering progesterone to women at risk of preterm delivery and preventing transition to Phase I. According to the update of January 2008 from the Society of Obstetricians and Gynecologists of Canada (SOGC), the best candidates for this therapy are women who have had a previous preterm labor and/or short cervix (< 15 mm at 22 – 26 weeks’ gestation) on transvaginal ultrasound [77].

Some recent progesterone trials have garnered considerable attention because of their apparent success. Weekly i.m. injection of 17-α hydroxyprogesterone caproate (17P) in a multicenter, double-blind, placebo-controlled trial in women with a documented history of spontaneous preterm birth significantly reduced the risk of delivery at < 37 weeks of gestation (relative risk [RR] 0.66, 95% confidence interval [CI] 0.54 – 0.81) [78]. In another American study among women with singleton pregnancies and a history of spontaneous preterm birth using weekly injection of 17P, the preterm birth rate was reduced from 12.1% to 11.8%, which questions the significance of progesterone therapy [79].

There are also two clinical trials among women with a short cervix at high risk of preterm birth. In the first, the rate of preterm birth at < 32 weeks of gestation was significantly lower for those who received vaginal progesterone than the placebo group (p = 0.014) [80]. The other study also showed the reduction of the preterm birth rate at < 34 weeks of gestation in the progesterone group (RR 0.56, CI 0.36 – 0.86) [81]. According to the Cochrane database, intramuscular 17P is associated with a reduction in the risk of preterm birth at < 37 weeks of gestation (RR 0.65, CI 0.54 – 0.79) [82]. On the other hand, proges-
terone therapy does not appear to be effective in twin pregnan-
ces. An American multicenter, randomized, double-blind, placebo-controlled trial found that delivery before 35 weeks occurred in 41.5% of pregnancies in the 17P group (RR 1.1, CI 0.9 – 1.3) [83]. Another twin trial in the Netherlands has not yet reported any results [84]. It is thought that the mechanism for preterm delivery in twins is different in most cases than in singleton pregnancies.

As for neonatal outcome, only one study found fewer admissions into the NICU with progesterone treatment (p = 0.016) and shorter NICU stays (p = 0.013) [80]. Therefore, under the SOGC technical update, counseling the patient at increased risk for preterm labor should include consideration of the potential benefits of progesterone use, but also our lack of knowledge of neonatal outcomes and optimal dosing [77].

7.2 Nuclear factor-kappa B (NFkB) inhibitors

The NFkB family of transcription factors is associated with inflammation and can be activated by pro-inflammatory cytokines. Considerable evidence accumulated since 1999 has shown that NFkB is involved with many aspects of PG synthesis and action in the intrauterine tissues, especially in association with labor. This mechanism includes stimulation by TNF-α [85], IL-1β [86], and lipopolysaccharide (LPS) [87]. It appears that NFkB mediates IL-1β action at several levels of the PG synthesis-receptor cascade, including secretory type II PLA₂ [88], PGHS-2 [89], and FP [73]. Our laboratory cloned and characterized the human FP promoter and found it to contain several response elements that bind the transcription factors NFkB, C/EBPβ (CCAAT/enhancer binding protein beta) and AP-1 (activator protein-1), which are associated with inflammatory cytokine action [90]. It also contains both repressor and enhancer regions, suggesting that FP is highly regulated. The possibility exists that NFkB may also regulate specific PG synthases and PG metabolism (PG dehydrogenase), but there is no evidence at present to support or refute this notion.

An inhibitor of NFkB, SN-50, was able to delay preterm birth when administered into the amniotic fluid of mice [91], and infusion of sulfasalazine, an anti-inflammatory and NFkB inhibitor, decreased uterine electromyographic activity in pregnant ewes induced to enter preterm labor with RU486, the progesterone receptor blocker (I.R. Young, pers. commun.). These studies suggest the participation of NFkB in preterm labor.

7.3 FP receptor antagonists

Theratechnologies’ THG113 is an octapeptide inhibitor of the FP receptor that binds the receptor to its G protein,
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thereby inhibiting its signal transduction action. It is effective in delaying LPS-induced preterm delivery in mice [92]; only about 1% crosses the placenta to the fetus, and it is rapidly metabolized. We tested a more efficacious derivative, THG113,31, in sheep [74], and determined that it suppressed uterine electromyographic activity (a measure of myometrial contractility) by up to 70% and delayed preterm delivery induced by the progesterone receptor antagonist, RU486. After cessation of infusion of THG113,31, animals quickly delivered due, most likely, to its rapid metabolism. This may be an ideal tocolytic, as there are potentially very few adverse side effects of the inhibitor in adult women, and because it crosses the placenta poorly to the fetus where PGE₂, and not PGF₂α, is the primary prostaglandin. In January 2007, the inhibitor was licensed to the Pharmaceutical Development Corporation for further development and renamed PDC113.

7.4 Tyrophostins

Tyrophostins, inhibitors of kinases that phosphorylate tyrosine residues in regulatory proteins, decrease inflammatory reactions and reduce cytokines [93,94]. Several have been developed to arrest cell proliferation and angiogenesis in cancer therapy. We demonstrated a few years ago that tyrophostins inhibited PGHS-2 expression in cultured human amnion cells [95]. In mice, we showed they were as or more effective than the PGHS-2 inhibitor, NS-398, in preventing LPS-induced preterm birth in mice [96]. Much more work studying their mechanisms of action is required.

8. Expert opinion

Preterm birth is an important problem globally, and the rate is increasing in both developed and developing countries. The major derivative problem is not mortality, but the long-term morbidity that derives from an infant being born before its time. The cost to society in terms of medical care, special education, lost productivity and trauma to the extended family is enormous and continues throughout a lifetime. Clearly, this is a problem that society needs to deal with. Unfortunately, it is an extremely complex one, because it is difficult to predict which pregnant women will deliver early, there are two patients to consider, and it is a relatively small market for pharmaceutical investment. We offer some suggestions for consideration that may lead to hope for the future that will improve pregnancy outcomes.

First, the entire problem of preterm birth needs a more coordinated societal approach. This includes healthcare providers, governments and communities, the scientific community, investors, and the pharmaceutical companies. Good antenatal healthcare must be available to every pregnant woman. Good care, reduced stress, appropriate lifestyle, and a strong support system may have a more positive impact on pregnancy outcomes than any drug treatment or other intervention. Governments need to become more involved, not only to help alert society to the scope of the problem but to provide resources and incentives for scientific and pharmaceutical involvement in understanding and preventing preterm delivery and by enacting legislation that will reduce the risk of litigation to investors and practitioners who develop new therapeutic interventions.

Next, we need better strategies for developing therapeutic interventions to delay preterm delivery. Rather than trying to stop uterine contractions after they have started, it is better to prevent the activation of the uterus and to keep it in pregnancy maintenance or Phase 0. For this, investment into predicting which women are at the greatest risk of delivering early is worthwhile so that healthcare practitioners know who to watch and to reduce the cost or potential adverse effects of a treatment given to women who do not need it. Making use of the new ‘omics’ technologies and assessing biomarkers of risk is one direction for investigation and investment. More research into how the uterus is activated for labor is needed and, along with that, the identification of new targets for therapeutic intervention that will maintain pregnancy.

Several barriers exist to developing new therapeutics, such as high cost, small market, potential adverse effects, etc. (see Section 4). One way to reduce or eliminate these barriers is to find new uses for old drugs. Indeed, many of the tocolytics currently in use around the world were originally developed for other applications. Indomethacin, magnesium sulfate, betamimetics, calcium channel blockers and nitric oxide donors were initially developed for pain or cardiovascular problems. More investment into drugs such as tyrosine kinase inhibitors and inhibitors of transcription factors may prevent activation of the uterus and maintain pregnancy.

Highly important is that better evidence is needed about the efficacy of existing and new therapeutic interventions. Clearly, once a new therapeutic is identified, very careful thought about the trial design and outcomes is imperative. Avoidance of biasing is necessary. Outcomes will vary according to gestational age. For example, at 24 weeks the goal will be to improve survival. However, at 32 weeks, when survival is no longer the major consideration, the goal will be to improve newborn and longer-term health outcomes.

Better evidence about the effectiveness of progesterone treatment is required. No clear preference exists for 17P (which must be injected) versus vaginal progesterone. More trials are necessary because the control or placebo groups in the original trials had extraordinarily large preterm delivery rates. It is still uncertain for which pregnancies (e.g., singleton vs multiple) progesterone therapy is effective. Soon it will become more difficult to test progesterone because many physicians in the United States will be prescribing it as routine antenatal care for their patients without convincing evidence.

Considerable need and opportunity exists in the field of therapeutic preterm birth prevention, although considerable challenges also exist. Successful development of new treatments
to prolong pregnancy and delay preterm delivery will require coordinated efforts from a wide swath of society.

**Declaration of interest**

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