Anti-inflammatory prostaglandins for the prevention of preterm labour

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Abstract

Preterm birth occurs in 10–12% of pregnancies and is the primary cause of neonatal mortality and morbidity. Tocolytic therapies have long been the focus for the prevention of preterm labour, yet they do not significantly improve neonatal outcome. A direct causal link exists between infection-induced inflammation and preterm labour. As inflammation and infection are independent risk factors for poor neonatal outcome, recent research focus has been shifted towards exploring the potential for anti-inflammatory strategies. Nuclear factor kappa B (NFκB) is a transcription factor that controls the expression of many labour-associated genes including PTGS2 (COX2), prostaglandins (PGs) and the oxytocin receptor (OXTR) as well as key inflammatory genes. Targeting the inhibition of NFκB is therefore an attractive therapeutic approach for both the prevention of preterm labour and for reducing neonatal exposure to inflammation. While PGs are considered to be pro-labour and pro-inflammatory, the cyclopentenone PG 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) exhibits anti-inflammatory properties via the inhibition of NFκB in human amniocytes, myocytes and peripheral blood mononuclear cells in vitro. 15d-PGJ2 also delays inflammation-induced preterm labour in the mouse and significantly increases pup survival. This review examines the current understanding of inflammation in the context of labour and discusses how anti-inflammatory PGs may hold promise for the prevention of preterm labour and improved neonatal outcome.

Introduction

The timing of human birth is a carefully regulated event that normally occurs following 37–42 weeks gestation. Being born outside this timeframe has immense consequences for both the mother and neonate. In 2010, an estimated 14.9 million babies worldwide were born preterm (before 37 weeks gestation), equating to 11.1% of all live births (Blencowe et al. 2012). Prematurity remains the leading cause of neonatal death, and the second most common cause of death after pneumonia in children under 5 years of age (Liu et al. 2012). In the vast majority of countries, including the UK, the rate of preterm birth is on the increase (Sykes et al. 2011, Blencowe et al. 2012). Although survival rates have improved over the last few decades due to advancements in neonatal care, morbidity rates remain unaltered (Fanaroff et al. 2003). This has led to a growing sense of urgency from governments and research bodies to prioritise research directed towards understanding the mechanisms underpinning preterm birth as well as the development of new treatment strategies for its prevention (Howson et al. 2012).

While a proportion of preterm births is medically indicated or ‘caregiver initiated’ (Goldenberg et al. 2012), the majority results from spontaneous labour and/or premature preterm rupture of membranes (Gravett et al. 2010). In absolute numbers, the increasing rate of preterm birth appears to be derived equally from increasing numbers of both spontaneous and caregiver-initiated preterm births (Norman et al. 2009). The main challenge in developing preventative strategies to reduce the incidence of preterm birth lies in the heterogeneous nature of the aetiologies of spontaneous preterm birth, often referred to as a syndrome, inferring that no single strategy could cover all causes (Villar et al. 2012). In order to overcome this challenge, it is essential to develop a preventative strategy that targets the activation of common biological pathways leading to cervical remodelling, foetal membrane rupture and uterine contractility.

Normal term labour is often described as a pro-inflammatory state. There is mounting evidence that the presence of inflammation in the absence of infection is sufficient to cause preterm labour (Shim et al. 2004, Christiaens et al. 2008). Moreover, inflammation is a risk
factor for adverse neonatal outcomes independent of gestational age of delivery. Taking this into account, anti-inflammatory therapies are attractive not only for the prevention of preterm labour but also as a means to confront associated adverse neonatal outcomes. This review summarises the key pathways involved in term and preterm labours, with particular emphasis on the role of inflammation, and provides evidence supporting the future potential use of anti-inflammatory prostaglandins (PGs) for the treatment of preterm labour and improved neonatal outcome.

The role of PGs in labour and inflammation

**PG synthesis**

PGs play a central role in the processes involved in term and preterm labour, contributing to uterine contractility, membrane rupture and cervical ripening. PGs are unsaturated fatty acids, containing 20 carbon atoms derived from membrane phospholipids under the action of phospholipases, primarily phospholipase A2, that release arachidonic acid into the cytoplasm (Simmons et al. 2004). Arachidonic acid is then converted to PGH2 by the cyclo-oxygenase enzymes COX1 and COX2 (also known as PG endoperoxide H synthases 1 and 2 respectively). PGH2 is the substrate for the specific synthase enzymes that produce the four principal PGs: PGE2, PGF2α, PGD2 and prostacyclin (PGI2). Subsequent conversion of PGE2 and PGD2 by non-enzymatic dehydration reactions leads to the production of the cyclopentenone PGs: PGA2, 15-deoxy-Δ12,14-PGA2, PGJ2 and 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) as shown in Fig. 1.

PG synthesis is regulated at several steps to ensure quiescence for the maintenance of pregnancy, and increased production in preparation for labour. Expression of sPLA2G1B (PLA2-III A) is increased in human myometrium in preterm and term labour compared with non-labouring subjects (Slater et al. 2004).

Although no labour-associated changes in PLA2 are seen in amnion with the onset of labour (Bennett et al. 1994), an increase in maternal plasma immunoreactive PLA2 has been shown in term and preterm labour (Rice et al. 1992). Production of PGs is also regulated by the activity of the cyclo-oxygenase enzymes. Although PTGS1 (COX1) is constitutively expressed in most cell types, PTGS2 (COX2) is an inducible enzyme which is particularly responsive to inflammatory stimuli (Morita 2002). Expression of PTGS2 but not PTGS1 is increased at term in amnion, choriodecidua and myometrium, with a further increase after labour in foetal membranes (Slater et al. 1999a, b). PG production can be further modulated by PG synthases. For example, the onset of human labour is accompanied by an increase in microsomal PGES1 and PGES2 in the myometrium (Astle et al. 2007) but not cytosolic PGES. During pregnancy, PG synthesis is negatively regulated by the presence of prostaglandin dehydrogenase. 15-Hydroxy-prostaglandin dehydrogenase is the primary enzyme metabolising PGs, and during pregnancy it is found at high levels in the chorion, decidua, placenta, myometrium and cervix (Sangha et al. 1994, Ramirez et al. 1995, Giannoulias et al. 2002), with levels decreasing with the onset of term and preterm labour (Olson & Ammann 2007).

**The role of PGs in labour**

Successful birth involves uterine activation, cervical ripening and foetal membrane remodelling. PGs play an important role in the coordinated activation of these processes. The principal site of production of PGs in labour is the amnion where there is increased production of PGE2 and PGF2α with the onset of labour (Olson et al. 1983). PGE2 and PGF2α are potent inducers of spontaneous uterine contractility (Crankshaw & Dyal 1994). Upon stimulation of the PTGER1 (EP1) and PTGER3 (EP3) receptors, PTFS1 (PGE2) leads to calcium

![Figure 1](image-url)
release via inositol triphosphate and adenylate cyclase and cAMP inhibition respectively (Blanks et al. 2007). However, recent data have suggested that spontaneous and PGE2-induced myometrial contractility is via the PTGER3 rather than the PTGER1 receptor (Arulkumaran et al. 2012). PGF2α also stimulates myometrial contractility through the FP receptor (PTGFR) likely by increasing intracellular calcium mobilisation (Parkington et al. 1999). PGE2 aids cervical ripening by decreasing the concentration of collagen (Ekman et al. 1986), and increasing the synthesis of proteoglycans (Norman et al. 1993). Evidence is also emerging of a direct role of PGE2 in foetal membrane rupture, via the remodelling of the extracellular matrix through increased expression of matrix metalloproteinases (MMPs), for example MMP9 (McLaren et al. 2000). Similarly, PGF2α increases the production of MMP9 in decidua (Ulug et al. 2001).

**The role of PGs in inflammation**

PGs are typically thought of as having predominantly pro-inflammatory effects. Their biosynthesis is increased in inflamed tissue and they contribute to the development of the cardinal signs of acute inflammation: dolor (pain), calor (heat), rubor (redness) and tumor (swelling). Pain sensation occurs due to PGE2-mediated action on peripheral sensory neurones and centrally within the spinal cord and brain (Funk 2001). PGE2 is also responsible for redness and swelling caused by increased blood flow in inflamed tissue through the augmentation of arterial dilatation and increased microvascular permeability (Funk 2001). Moreover, PGs can induce the expression and release of various chemokines, leading to infiltration of the inflammatory cell at the site of inflammation (Aoki & Narumiya 2012).

The release of cytokines by these cells can further drive PG synthesis by increasing PTGS2 expression (Aoki & Narumiya 2012). Consistent with this role, PGs contribute to the physiological inflammatory response seen at the time of labour. For example, PGE2 enhances migration of leukocytes towards the cervix, which in turn leads to an increased production of interleukin 8 (IL8; Hertelendy & Zakar 2004), and PGF2α indirectly leads to the activation of IL1β in the decidua and consequently the increased production of MMP9 (Schonbeck et al. 1998, Christiaens et al. 2008). PGF2α has also been shown to increase CON43 and PTGS2 expression in myocytes, the effect of which is enhanced by IL1β (Xu et al. 2013).

**The role of nuclear factor kappa B in labour and inflammation**

It is increasingly recognised that labour onset represents the culmination of numerous converging signalling pathways that are both maternal and neonatal in origin. These pathways lead to a common node that involves the activation of inflammation in gestational tissues. A key regulator of these pathways is the transcription factor nuclear factor kappa B (NFκB; Lindstrom & Bennett 2005a, Lappas & Rice 2009). NFκB is a protein complex consisting of five family members: NFκB1 (p105/p50), NFκB2 (p100/52), Rel A (p65), Rel B and c-Rel (Lindstrom & Bennett 2005a). NFκB subunits are typically localised in the cytoplasm as inactive homo or hetero dimers, where they are complexed with the inhibitor protein, IκB as shown in Fig. 2. Phosphorylation of IκB by IκB kinase (IKK) at specific serine residues (Zandi et al. 1997) results in the attachment of ubiquitin residues (Chen et al. 1995) and subsequent cleavage by

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**Figure 2** Downstream effects of NFκB activation in gestational tissues and implications for the foetus. The transcription factor NFκB is key to control the expression of labour-associated genes and inflammatory response genes. IKKβ phosphorylates IκB in response to a pro-inflammatory stimulus. This results in the attachment of ubiquitin residues which are then cleaved by the 26s proteosome, allowing the free NFκB subunits to translocate into the nucleus to bind to NFκB response elements. The resulting effect is the release of pro-inflammatory cytokines and the production of contractile-associated proteins in foetal membranes, myometrium and cervix, which ultimately lead to membrane rupture, uterine contractility and cervical dilation. As a result of an exaggerated inflammatory response, the blood–brain barrier becomes more permeable making the neonate more susceptible to brain injury, and the lung is also targeted resulting in BPD. PVL, periventricular leukomalacia; BPD, bronchopulmonary dysplasia.
the 26S proteasome (Karin & Ben-Neriah 2000). Liberated NFκB subunits undergo activation by phosphorylation, permitting their translocation into the nucleus where they bind to the DNA response elements of target genes leading to their transcriptional activation.

Consistent with a role in the activation of inflammation before the onset of human labour, increased NFκB activity can be detected in the amnion from preterm labour patients (Lim et al. 2012). Similarly, nuclear translocation of p65 is evident in the upper and lower segments of the uterus at term, consistent with NFκB playing a central role in the events preceding labour (Khanjani et al. 2011). As further evidence supporting the importance of NFκB in the processes leading up to labour and delivery, several NFκB inhibitors SN-50, sulphasalazine and 15d-PGJ2 have been shown to delay preterm labour in the mouse (Condon et al. 2004, Pirianov et al. 2009, Nath et al. 2010).

NFκB regulation of labour-associated and inflammatory response genes

NFκB plays a pivotal role in the transcriptional regulation of numerous genes associated with the activation of labour. Figure 2 summarises the downstream effects of NFκB activation in gestational tissues and their resulting potential implications for the foetus. Key genes involved in the synthesis and expression of PGs including sPLA2G1B-IIA, cPLA2 (Lappas & Rice 2007), PTGS2 (Allport et al. 2001) and PGF2α receptor expression (Zaragoza et al. 2006, Liang et al. 2008) have been shown to be NFκB regulated. Similarly, the oxytocin receptor (OXTR (OTR)) gene responsible for propagating uterine oxytocin stimulation contains several NFκB response elements in its promoter region, and thus activation of NFκB likely contributes to the observed increase in OXTR expression at term (Terzidou et al. 2006). NFκB is also involved in the transcriptional regulation of MMPs, including MMP9, which are required for remodelling of the extracellular matrix (Choi et al. 2007), leading to both cervical ripening and foetal membrane rupture.

NFκB also plays a central role in modulating the expression of key inflammatory genes associated with parturition. NFκB activity is highly inducible by pro-inflammatory stimuli such as lipopolysaccharide (LPS), tumour necrosis factor alpha (TNFα) and IL1β via the canonical pathway. Considering many cytokines that contain NFκB recognition elements within their promoter regions (e.g. TNFα, IL1β, CXCL8 (IL8) and IL6 (Lindstrom & Bennett 2005a)), NFκB activation by inflammatory stimuli can lead to the formation of a feed forward loop and activation of persistent inflammatory pathway. Although immune cells are recognised as major producers of pro-inflammatory cytokines, myocytes, amniocytes and placental cells also contribute to pro-inflammatory environment during term and preterm labour (Keelan et al. 2003). Overexpression of NFκB in myocytes leads to the upregulation of a cassette of genes that are principally involved in immunity and inflammation (Khanjani et al. 2011). Furthermore, the transcription of chemokines, such CXCL8, by myocytes, amniocytes and cervical cells is regulated at least in part by NFκB (Elliott et al. 2001, Soloff et al. 2004).

The role of infection and inflammation in preterm labour

Spontaneous labour at term is characterised by a molecular inflammatory signature in the foetal membranes, myometrium and cervix (Blank et al. 2008). Mathematical modelling of the three main pathways thought to be involved in labour onset; inflammatory stimulation, functional progesterone withdrawal or OXTR activation, strongly points to inflammation as being the primary driver for the activation of the myometrium for labour to ensue (Bisits et al. 2005). It therefore follows that premature activation of inflammatory pathways could lead to the untimely activation of myometrial contractions and labour. Consistent with this, infection and/or inflammation are the only well-defined pathological processes at the molecular level in which a firm causal link with preterm labour has been established. Although between 25 and 40% of preterm births are thought to be attributed to intrauterine microbial infection (Goldenberg et al. 2000), inflammation in the absence of infection can be sufficient to cause preterm labour (Shim et al. 2004, Christiaens et al. 2008).

Gestational tissue-level inflammation

In response to an intrauterine infection, the maternal system mounts an immune response, whereby infiltrating leukocytes and decidual cells secrete pro-inflammatory cytokines including IL1α (IL1), IL6, CXCL8 and TNFα (Casey & MacDonald 1988, Romero & Mazor 1988, Hunt 1989). These cytokines are elevated in the amniotic fluid and foetal membranes in preterm labour patients (Keelan et al. 2003), where they likely induce PG production in the foetal membranes and decidua, leading to the premature initiation of foetal membrane remodelling manifested in the form of preterm premature ruptures of membranes (PPROM) (Romero et al. 1989, Mitchell et al. 1990). Supporting evidence for this comes from in vitro experimentation which shows that exposure of amnio–chorion to TNFα increases the activity of MMPs, drives IL1β and TNFα production and induces apoptosis – all characteristic processes of foetal membrane rupture (Menon & Fortunato 2004).

An elevated immune signature in the cervix is also associated with preterm labour. Both IL6 and IL8 levels are significantly higher in cervical secretions of women destined to deliver preterm (Becher et al. 2009). IL8 may

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play a role in cervical ripening by chemo-attracting leukocytes such as neutrophils, which produce MMPs and PGE$_2$ (Ogawa et al. 1998, Wright et al. 2010). The cervix plays both a mechanical and biochemical protective role during pregnancy in the prevention of ascending infection as demonstrated by the higher incidence of microbial invasion of the amniotic fluid in women with a shorter cervix (Gomez et al. 2005a, Hassan et al. 2006).

While often considered to be the ‘engine’ of parturition, the myometrium also responds to and propagates inflammatory signalling during parturition. During preterm labour, the myometrium experiences marked leukocyte invasion (Thomson et al. 1999), along with increased levels of pro-inflammatory cytokines such as CXCL8 and IL1$\beta$ (Tattersall et al. 2008). IL1$\beta$ stimulates the production of PGs (Pollard & Mitchell 1996a, b, Todd et al. 1996) by stimulating the release of arachidonic acid (Molnar et al. 1993) and increasing PTGS2 expression (Belt et al. 1999). IL1$\beta$ also increases oxytocin production, which also leads to increased uterine contractility (Frieben-Hoffmann et al. 2001).

**Systemic inflammation**

NFkB activity and T helper 1 (Th1) cytokine production in peripheral blood mononuclear cells (PBMCs) are suppressed in pregnancy compared with non-pregnant controls (McCarricken et al. 2003, Sykes et al. 2012a). The reduction in pro-inflammatory cytokine production in PBMCs is likely to be as a result of the downregulation of the NFkB/IkB signalling pathway (McCarricken et al. 2004, 2007). Detecting signs of an overt systemic inflammatory reaction is rare in women presenting in threatened preterm labour in the clinical setting with conventional tests. However, several groups have been able to demonstrate an association between the expression of pro-inflammatory cytokines in serum or PBMCs and women at risk and/or in preterm labour (Raghupathy et al. 2001, Makhseed et al. 2003, Curry et al. 2007).

**Inflammation and adverse neonatal outcome**

The presence of infection and/or inflammation is now considered an independent risk factor for the adverse neonatal outcome. For example, amniotic fluid and cord blood pro-inflammatory cytokines IL1$\beta$, IL6 and TNF$\alpha$ are associated with radiological evidence of cerebral damage (Yoon et al. 1997, Duggan et al. 2001) and chronic lung disease (Yoon et al. 1999). It is likely that the same transcription factors activated in preterm labour also play a role in inflammation-induced brain damage (Yuan et al. 2010). Preventative therapies should therefore focus on targeting these transcription factors to prevent both preterm labour and the associated neonatal sequelae.

**Current strategies for the prevention of preterm labour**

Regardless of great research effort, there has been little advancement in the therapeutic approaches aimed to tackle preterm labour. Clinical management strategies can be roughly divided into acute management and prophylactic therapies.

Historically, tocolytics have been used in an attempt to delay preterm labour by inhibiting uterine contractility. These include oxytocin antagonists (atosiban), calcium channel blockers (nifedipine), β-mimetics (e.g. terbutaline) and non-steroidal anti-inflammatory agents (e.g. indomethacin). A short course of tocolysis is given to women in threatened preterm labour to allow time for the corticosteroids administered to stimulate foetal maturation; however, no long-term neonatal benefits from tocolysis have been demonstrated. This may partly be due to the inclusion of mostly women who are not destined to labour despite presenting in threatened preterm labour. In the case of women who genuinely require tocolysis, the pathways leading to labour are likely, in many cases, to be well established to be overcome by transient inhibition of the contractions. Another limitation of tocolysis is the targeting of only one late aspect of preterm labour – uterine contractions, while having no effect on the causal aspects or on the aetiologies of the adverse effect of the neonate.

Prophylactic treatment of preterm labour is therefore an attractive treatment strategy as it theoretically offer the means to prevent the activation of myometrial contractions but also distally inhibit associated inflammation and other causal pathways of neonatal injury. One such approach is cervical cerclage. A meta-analysis of 208 women with a short cervix reported on a statistically significant reduction in rate of preterm delivery <35 weeks with cerclage placement (relative risk 0.61; 95% CI 0.4–0.92; Berghella et al. 2010). There is also evidence that cervical cerclage in women at high risk of preterm labour results in a statistically significant reduction of a composite of perinatal mortality and morbidity (Berghella et al. 2011).

Similarly, several large trials have now been conducted to examine the effect of progesterone prophylaxis in women at high risk of preterm labour. While not unambiguous, there is a general consensus that progesterone treatment leads to a reduction in rates of preterm birth (da Fonseca et al. 2003, Meis et al. 2003, Fonseca et al. 2007, Hassan et al. 2011). However, the primary outcome of the majority of these studies was gestation length rather than neonatal outcome and thus limited evidence is available for short and long-term benefits to the neonate/infant with progesterone supplementation.

**Anti-inflammatory effects of PGs**

Although the pro-inflammatory effects of PGs are well documented, there is also evidence that PGs may also act to resolve inflammation.


**Prostaglandin E₂**

PGE₂ can act as an immunosuppressant by creating a Th2 bias over Th1 cytokine production. This occurs by both enhancing Th2 cytokine production while repressing the differentiation of Th1 cells (Hilkens et al. 1996, Aoki & Narumiya 2012). PGE₂ can significantly reduce IL1β-mediated IL8 production in cultured human myocytes, which is likely to be via the EP₂/EP₄ receptors (Slater et al. 2006). Data also support a neuroprotective role in models of inflammation by limiting cytokine release and further PG synthesis in a negative feedback mechanism through the inhibition of mPGES1 (Ricciotti & FitzGerald 2011). PGE₂ has been shown to inhibit the translocation of the p50/p65 subunit, thus inhibiting NFκB activity in stimulated rheumatoid synovial fibroblasts (Gomez et al. 2005b). As NFκB activation leads to COX2 synthesis and ultimately PG synthesis, it seems plausible that this inhibition by PGE₂ serves an important role of negative feedback in regulating its own synthesis.

**Prostaglandin D₂**

The placenta produces considerable amounts of PGD₂, which has been shown *in vitro* to have anti-inflammatory effects in villous trophoblast cells through the inhibition of IL6 production (Helliwell et al. 2006). PGD₂ production by the placenta is thought to be responsible for the anti-inflammatory Th2 cytokine predominance at the maternal foetal interface via the chemoattraction of CRTH2⁺Th2 cell interface (Saito et al. 2002).

**Cyclopentenone PGs**

PGs of the A and J series contain a cyclopentenone ring which is characterised by the presence of a α,β-unsaturated carbonyl group. The electrophilic nature of the carbonyl group allows it to form Michael adducts with nucleophilic amino acid residues of proteins such as cysteine, histidine and lysine (Gharbi et al. 2007). Unlike most PGs that mainly act via G protein-coupled receptors, cyclopentenone PGs are capable of targeting intracellular proteins such as signalling molecules and transcription factors.

**PGA₁ and PGA₂**

PGA₁ and PGA₂ are formed from PGE₁ and PGE₃, respectively, through a non-enzymatic dehydration reaction. The resulting α,β-unsaturated carbonyl group located within the cyclopentenone ring is thought to largely determine bioactivity capacity to inhibit different components of the NFκB signalling pathway in both the cytosol and the nucleus by forming covalent adducts with nucleophiles (Diez-Dacal & Perez-Sala 2012). For example, PGA₁ has been shown to inhibit NFκB dependent PTGS2 expression in 3T3 fibroblasts (Mandal et al. 2005), whereas PGA₂ has been shown to possess potent anti-inflammatory properties in murine microglia and astrocytes through inhibition of pro-inflammatory cytokine and chemokine production (Storer et al. 2005).

**Prostaglandin J₂**

The anti-inflammatory effects of PGD₂ are further enhanced via the non-enzymatic generation of the PGJ₂ family. The J₂ series of PGs were first discovered in the 1980s (Santoro et al. 1987) when Fukushima et al. showed that PGD₂ undergoes spontaneous dehydrogenation to PGJ₂ in aqueous solutions, and when in the presence of albumin, PGJ₂ isomerises to form Δ¹²-J₂. This can be further metabolised by dehydrogenation to form the most studied of the PGJ₂ metabolites, 15d-PGJ₂. As well as the reactive carbon in the α,β-unsaturated carbonyl group, Δ¹²-J₂ and 15d-PGJ₂, features a second reactive electrophilic carbon on one of their side chains (Straus & Glass 2001).

**15-Deoxy-Δ¹²,14-PGJ₂**

15d-PGJ₂ has cell surface receptors as targets, DP1 and PTGSR2 (CRTH2); however its anti-inflammatory properties can be attributed mainly to its intracellular targets: NFκB, AP1 and PPARγ nuclear receptor. It is still not known how 15d-PGJ₂ enters the cell or nucleus to act upon these receptors; however, it is likely that an active transport mechanism facilitates entry similar to the entry of other cyclopentenone PGs (Narumiya & Fukushima 1987). In addition, the action of endogenous 15d-PGJ₂ may be through its production within the cell from locally produced PGD₂.

Endogenous 15d-PGJ₂ can be detected in inflammatory fluid, with levels increasing in the resolution phase, supporting its anti-inflammatory role (Straus & Glass 2001). *In vitro* studies show that 15d-PGJ₂ can reduce neutrophil migration (Napimoga et al. 2008) and inhibit the secretion of IL6, IL1β, IL12 and TNFα from macrophages (Scher & Pillinger 2009). Repression inflammatory response gene transcription in macrophages has been shown to be via inhibition of NFκB and AP1 activation in a PPARγ dependent mechanism (Ricote et al. 1998). However, evidence suggests that 15d-PGJ₂ can also act in a receptor-independent manner to inhibit NFκB. The highly reactive ring of 15d-PGJ₂ can form a covalent interaction with multiple components of the NFκB signalling pathway (IkB kinase complex B, p50 and p65 subunits) via the Michael reaction, resulting in impaired nuclear entry and DNA-binding activity (Cernuda-Morollón et al. 2001) as shown in Fig. 3. Similarly, 15d-PGJ₂ can act through a receptor-independent mechanism by directly binding to c-jun, a component of the AP1 signalling pathway leading to inhibition of DNA-binding activity (Perez-Sala et al. 2003). Since AP1 contributes to the induction of
expression of inflammatory response genes such as PTGS2 and CXCL8, it is plausible that 15d-PGJ2 and other cyclopentenone PGs also elicit their anti-inflammatory effects via the AP1 signalling pathway, yet this remains to be investigated.

Application of anti-inflammatory PGs for the prevention of preterm labour

To date, the most researched anti-inflammatory PG in the context of the prevention of preterm labour is 15d-PGJ2, yet these studies are limited to in vitro experimentation in human cells and explants or in vivo animal studies. In either case, the collective consensus is that 15d-PGJ2 attenuates pathways associated with labour onset through the inhibition of the expression of labour-associated and inflammatory response genes. Therefore this section will focus upon how 15d-PGJ2 may be potentially used as a therapeutic agent for the treatment of preterm labour and birth.

Human studies

Our group have previously shown that 15d-PGJ2 inhibits NFκB activity in IL1β-stimulated cultured human amniocytes and myocytes at concentrations between 16 and 32 μM (Lindstrom & Bennett 2005b). The cyPG acts at multiple steps in the NFκB signalling pathway, preventing IL1β-stimulated IkBα degradation, IKK phosphorylation and by directly targeting the p65 subunit. The inhibition of NFκB activity is dependent on the electrophilic carbon, and although PGA1 was also able to reduce nuclear translocation of p65, this effect was seen at concentrations sixfold as that of 15d-PGJ2, which is likely to be attributable to the presence of one, and not two, electrophilic carbons. This study also demonstrated that NFκB inhibition in amniocytes and myocytes is independent of the PPAR nuclear receptors. We have also excluded the role of the G protein-coupled receptor, PTGSR2, in 15d-PGJ2-mediated NFκB inhibition in amniocytes and myocytes (Sykes et al. 2012b). Both cyclopentenone PGs, 15d-PGJ2 and PGA1, inhibit IL1β-induced PTGS2 expression in amniocytes and myocytes, again, independently of the PPAR nuclear receptors because agonists of PPARγ and PPARα failed to replicate this effect (Lindstrom & Bennett 2005b). Consistent with these results, 30 μM of 15d-PGJ2 has been shown to significantly reduce TNFα-stimulated PTGS2 and PGE2 synthesis in amniocytes in a PPARγ-independent mechanism (Ackerman et al. 2005).

At 10 μM 15d-PGJ2 significantly reduces the production of the pro-inflammatory cytokines, IL6 and CXCL8, from amnion-derived WISH cells in a PPARγ-independent manner (Berry et al. 2005). Inhibition of LPS-stimulated IL6, IL8 and TNFα in amnion, chorionic and placental explants can also be demonstrated albeit at higher concentrations (30 μM; Lappas et al. 2002). Similar anti-inflammatory effects can be seen in PBMCs isolated from women at different stages of pregnancy and from those in labour. In these cells, 15d-PGJ2 significantly reduces the PMA/ionomycin-stimulated phosphorylation of p65 and the downstream production of IFNγ and TNFα (Sykes et al. 2012c).
**Animal studies**

In exploring the plausibility of using 15d-PGJ$_2$ as a therapeutic treatment for preterm birth in an in vivo system, we reported that 4 μg of intrauterine 15d-PGJ$_2$ delays LPS-induced preterm labour in the mouse (Pirianov et al. 2009). Examination of uterine tissue harvested from dams 6 h post-treatment confirmed the inhibition of IKKβ activity, as well as reduced the levels of p65 phosphorylation and PTGS2.

**Application of anti-inflammatory PGs for neuroprotection**

Together with the direct adverse effects of prematurity on the neonate, inflammation also increases the risk of neonatal brain injury. There is mounting evidence that 15d-PGJ$_2$ and other anti-inflammatory cyclopentenone PGs have neuroprotective effects. In our murine model of inflammation-induced preterm labour, 15d-PGJ$_2$ increased pup survival in LPS-treated mice from 30 to 95% (Pirianov et al. 2009). This was associated with the inhibition of IKKβ and NFkB activity in the foetal brain. As LPS treatment leads to increased cytokine production by the foetal brain (Bell et al. 2004, Elovitz et al. 2006), inhibition of NFkB should conceptually repress cytokine production within the brain thus providing neuroprotection to the neonate.

Several animal studies have reported a neuroprotective role for 15d-PGJ$_2$ in the presence of inflammation. Petrova et al. (1999) demonstrated in rats that 15d-PGJ$_2$ reduces NFkB activity in LPS-stimulated microglia and IL1β-stimulated astrocytes. Moreover, 15d-PGJ$_2$ also provides significant neuroprotection in a rodent model of ischaemic white matter injury, which is associated with an inhibition of p65 nuclear translocation, TNFα and IL1β production (Nicholson et al. 2012).

Despite much research indicating beneficial effects of anti-inflammatory cyclopentenone PGs in in vitro and in vivo animal models of preterm birth, clinical studies have yet to be undertaken. There is a problem relating to the general instability of these compounds and their insolvability in water; however, studies are underway investigating liposome suspension formulations (Fukushima et al. 2000). In addition, clinical studies of new drugs in pregnancy, particularly in the context of preterm labour, have been historically challenging. Although concentrations used in vitro are higher, it must not be forgotten that these are endogenous compounds, and that 15d-PGJ$_2$ is detectable in amniotic fluid (Helliwell et al. 2006) and placenta (Jawerbaum et al. 2004). Therefore, it is not beyond possibility that these anti-inflammatory PGs may soon reach the clinical setting.

**Conclusion**

Targeting NFkB with cyclopentenone PGs is a potential therapeutic strategy for the prevention of inflammation-induced preterm labour. 15d-PGJ$_2$ holds the most promise because it has higher potency due to the presence of two electrophilic carbons in its chemical structure. There is now abundant evidence supporting both is anti-inflammatory effects and its potential inhibitory effects on labour-associated genes. It therefore follows that in addition to it potentially delaying inflammation-induced preterm labour, it may also serve as a neuroprotective treatment for the unborn foetus.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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