(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 June 2005 (16.06.2005)

PCT

(10) International Publication Number
WO 2005/053705 A1

(51) International Patent Classification[7]: A61K 31/5575, A61P 15/06

(21) International Application Number: PCT/GB2004/001380

(22) International Filing Date: 29 March 2004 (29.03.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0327975.9 2 December 2003 (02.12.2003) GB

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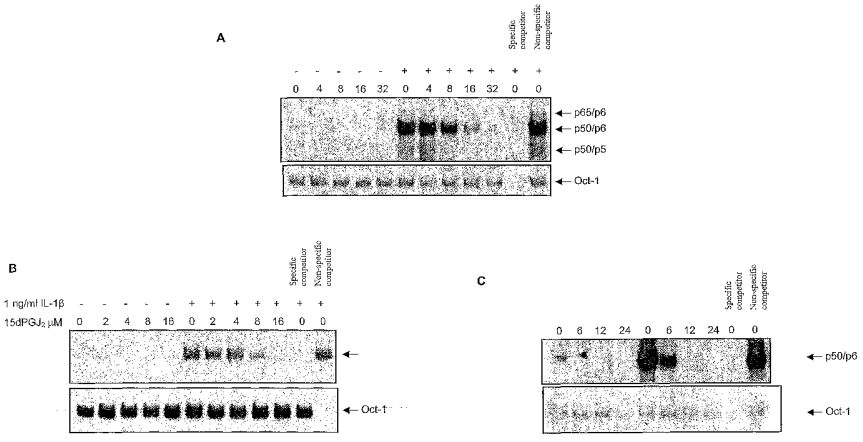
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: USE OF A CYCLOPENTENONE PROSTAGLANDIN FOR DELAYING THE ONSET AND/OR PREVENTING THE CONTINUATION OF LABOUR



(57) Abstract: The present invention provides the use of a cyclopentenone prostaglandin in the manufacture of a medicament for delaying the onset and/or preventing the continuation of labour in a female. Preferably the cyclopentenone prostaglandin prevents and/or reduces an inflammatory response in the reproductive system of a female. Preferably, the cyclopentenone prostaglandin is 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, or prostaglandin A_1 or a precursor thereof. The invention further provides a pharmaceutical composition comprising cyclopentenone prostaglandin and methods of use thereof.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

USE OF A CYCLOPENTENONE PROSTAGLANDIN FOR DELAYING THE ONSET AND/OR PREVENTING THE CONTINUATION OF LABOUR

The present invention relates to agents for improving perinatal outcome in pre-term labour. In particular, the present invention relates to the use of prostaglandins to prevent and/or reduce an inflammatory response in the reproductive system of a female, thereby delaying the onset of labour.

Human pre-term labour, defined as spontaneous labour occurring prior to 37 weeks of gestation (with 39 weeks being term) continues to be a major problem, particularly in developed countries. Preterm birth occurs in 5-10% of all pregnancies but is associated with 70% of all neonatal deaths and up to 75% of neonatal morbidity (Rush *et al.,* 1976). Premature neonates are at high risk of cerebral palsy, developmental delay, visual and hearing impairment and chronic lung disease.

During pregnancy, the uterus is maintained in a state of non-contractile quiescence whilst the cervix remains firm and closed. With the onset of labour, the cervix needs to become softer and to offer low resistance to force applied and have fibres which move under tension. The uterus also needs to begin contracting.

Both at term and preterm, the biochemistry of labour resembles an inflammatory reaction and there is accumulating evidence pointing to a pivotal role for pro-inflammatory cytokines and prostaglandins (PGs) in the labour process. Interleukin-1β (IL-1β) levels are elevated in amniotic fluid (Romero *et al.*, 1990), gestational membranes (Keelan *et al.,* 1999; Elliot *et al.*, 2001) and the lower uterine segment (Maul *et al.*, 2002) at term labour, and may contribute to labour onset by stimulating IL-8 and PG synthesis (Mitchell *et al.*, 1990; Brown *et al.*, 1998). PGs increase in maternal urine and blood and in fetal membranes in association with labour (Satoh *et al.*,

1979; Skinner and Challis, 1985). PGE_2 stimulates uterine contractions (Dyal and Crankshaw, 1985), indirectly increases fundamentally dominant myometrial contractility by upregulation of oxytocin receptors and synchronisation of contractions (Garfield *et al.*, 1990), and acts in concert with IL-8 to remodel the cervix (reviewed in Kelly, 2002).

The onset of labour is associated with activation of the Nuclear Factor Kappa B (NFκB) transcription factor system in the amnion which plays a role in the expression of pro-inflammatory genes such as interleukin-8 (IL-8), interleukin-6 (IL-6) and cyclo-oxygenases 1 and 2 (COX-1 and COX-2). COX genes are also referred to as prostaglandin H synthase or PG synthase. The resulting inflammatory infiltrate (mediated by the cytokines) and increase in prostaglandin synthesis (mediated by the cyclo-oxygenases) leads to cervical ripening, fetal membrane rupture and myometrial contractions.

Five members of the NF-κB/Rel family have been identified in mammals: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p100), p65 (RelA), c-rel, and Rel B. These proteins share a structurally conserved amino-terminal region termed the Rel homology domain (RHD). The RHD is responsible for dimerisation, DNA binding, and interaction with the inhibitors of kappa B (IκB) proteins. It also contains a nuclear localisation signal (NLS). In its active DNA-binding form NF-κB consists of heterogeneous dimers of various combinations of NF-κB subunits: each member of the NF-κB family, except for Rel B, can form homodimers, as well as heterodimers with one another. The p65, c-rel and Rel B proteins contain a carboxy-terminal non-homologous transactivation domain, which activates transcription from κB sites in target genes; in contrast, p50 and p52 proteins lack a transactivation domain. The various NF-κB dimers exhibit different binding affinities for specific κB sites (Kunsch *et al.*, 1992,

Phelps *et al.*, 2000), and differentially stimulate transcription through distinct κB elements (Lin *et al.*, 1995).

In resting cells, NF-κB dimers are normally sequestered in an inactive form in the cytoplasm by association with the inhibitory IκB proteins, which include IκBα, IκBβ and IκBε. The IκBs are characterised by the presence of multiple ankyrin repeats which mediate binding to the RHD and mask the NLS of NF-κB.

The major NF-κB signalling pathway, which is activated by pro-inflammatory stimuli and LPS, targets IκBα- and IκBβ-bound NF-κB (for review see Li and Verma 2002). p50/p65 dimers are the most abundant form of NF-κB in most cell types, and activation of IκBα-bound p50/p65 dimers is the best characterised pathway. In this 'classical' pathway, diverse stimuli trigger signal transduction cascades that ultimately converge on the activation of a specific IκB kinase (IKK). The IKK complex consists of several proteins, the main ones being IKKα (IKK1), IKKβ (IKK2), and NF-κB essential modulator (NEMO or IKKγ). The activated IKK complex phosphorylates IκBα at serines 32 and 36, which results in the poly-ubiquitination of IκBα at lysines 21 and 22. This modification targets IκBα for rapid degradation by the 26S proteasome. The degradation of the IκB inhibitor exposes the NLS of NF-κB resulting in translocation of the p50/p65 dimer to the nucleus where it can bind to κB sites in the promoter of target genes and promote transcription.

Most stimuli cause only the transient activation of NF-κB. The critical inhibitory step in NF-κB inactivation involves binding of newly synthesised IκBα to NF-κB in the nucleus. IκBα is quickly resynthesised following its degradation. The newly synthesised IκBα is localised in the nucleus and displaces NFκB from its DNA binding sites. IκBα contains leucine-rich

nuclear export sequences (NES) (Johnson *et al* 1999), which then enable it to transport NF-κB back to the cytoplasm, thereby completing an autoregulatory post-induction repression.

In many cells nearly half of the NF-κB is sequestered by the other major IκB isoform, IκBβ (Whiteside *et al.*, 1997). In contrast to IκBα, IκBβ is not NF-κB inducible and does not exert a rapid post-induction repression of NF-κB activity. Rather, IκBβ has been implicated in persistent NF-κB activation. Prolonged exposure to certain stimuli, such as LPS, leads to the long-term induction of NF-κB activity despite high levels of newly synthesised IκBα. Following stimulus-induced degradation, the newly synthesised IκBβ is un-phosphorylated and, in contrast to IκBα or the constitutively phosphorylated IκBβ, can interact with NF-κB bound to target promoters without displacing it from the DNA (Suyang *et al.,* 1996). This interaction of un-phosphorylated IκBβ with DNA-bound NF-κB is thought to protect NF-κB from nuclear export, and thus inhibition, by IκBα, and the outcome is a sustained NF-κB response.

PGs are a family of biologically active molecules having a diverse range of actions depending on the prostaglandin type and cell target. There is considerable evidence to support a central role for PGs in human parturition. Labour is associated with increased PG synthesis within the uterus (Turnbull 1977) particularly from the fetal membranes (Skinner and Challis 1985). PGs act to mediate cervical ripening and to stimulate uterine contractions (Cranckshaw and Dyal 1994) and indirectly to increase fundamentally dominant myometrial contractility by up-regulation of oxytocin receptors and synchronisation of contractions (Garfield *et al* 1990). PG synthesis in amnion, chorion-decidua and myometrium increases with labour (for a review, see Bennett and Slater 1996). Chorion prostaglandin dehydrogenases are thought to protect the uterus from basal

prostaglandin synthesis during pregnancy but are down-regulated at term. Deficiency of prostaglandin dehydrogenase in chorion has been associated with pre-term labour (van Meir 1996, 1997).

Accordingly, inhibition of prostaglandin synthesis is an effective method of preventing or arresting pre-term labour (Keirse, 1995). Conversely, prostaglandins have been administered to induce labour as a means to terminate pregnancy (Ganstrom *et al.,* 1987).

Most PGs bind to prostanoid receptors localised on the cell surface and act through second messenger systems (Narumiya, 1995). However, PGD_2 metabolites are actively incorporated into the nuclei of cells (Narumiya *et al.*, 1987) and can exert their effects through direct interactions with nuclear receptors. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. They exist in three distinct forms, PPAR-α, PPAR-δ, and PPAR-γ, which form heterodimers with the retinoic X receptor (RXR) and bind to PPAR response elements (PPREs) in the promoter of target genes to induce transcription. PPAR-γ can also repress gene transcription by negatively interfering with the NF-κB, AP-1, STAT and C/EBP pathways (Zhou *et al.,* 1999; Subbaramaiah *et al.*, 2001; Takata *et al.*, 2002; Suzawa *et al.*, 2003).

The aetiology of pre-term labour is multi-factorial but bacterial infection is believed to play an important role, especially at earlier gestational ages (for review see Romero *et al.,* 2002). A growing body of epidemiological data suggests that intrauterine infection is an important cause of brain injury in infants born before 32 weeks of gestation. During ascending intrauterine infection, micro-organisms can stimulate the production of pro-inflammatory cytokines, such as tumour necrosis factor α (TNFα) and IL-1β, as well as PGs and other inflammatory mediators, resulting in the

premature onset of labour. Intrauterine infection/inflammation has also been identified as a key contributor to the development of cerebral palsy (CP) and schizophrenia (Urakubo *et al.*, 2001; Gibson *et al.*, 2003), and, although CP does occur in term infants, the risk of CP is strongly associated with prematurity (Dammann *et al.*, 1999).

In addition, inflammatory responses caused by mechanical stretching of the uterus may contribute to the onset of labour. Mechanical stretching of the uterus occurs to an extent as a normal part of pregnancy and may be responsible for some of the biochemical changes which occur near to term and which cause the normal onset of labour at term. In the context of preterm labour, mechanical stretch may occur where the uterus is overdistended by multiple pregnancy or by excess amniotic fluid (clinically termed hydramnios or polyhydramnios). There may also be more local stretch of the lower segment of the uterus, the cervix and overlying fetal membranes in cases where there is cervical weakness (clinically termed cervical incompetence). Stretch leads to an increase in the production of a series of 'labour-associated' proteins including COX-2 (which then increases prostaglandin synthesis), cytokines such as IL-8 and IL-1b and the oxytocin receptor. Increased prostaglandin and cytokine productions causes cervical ripening or further cervical ripening (and may lead to neonatal brain injury). Prostaglandins and OTR receptor lead to uterine contractions.

Obstetric management of pre-term labour is still largely reactive and centred on the use of drugs intended to inhibit contractions to delay delivery. This was thought to be principally dependent upon gestational age leading to the concept that prolongation of the pregnancy will always improve outcome. However, there is now growing evidence that the mechanisms leading to pre-term birth also cause fetal cerebral damage. Characteristically, damage is localised to the white matter, involving both a diffuse astrogliosis with subsequent loss of myelin-producing oligodendrocytes, as well as

multifocal necroses resulting in cystic change (periventricular leucomalacia, PVL). Such lesions lead to cerebral palsy in 60–90% of affected infants (described in Vlope, 2001).

There are currently no drugs available which will safely and effectively inhibit pre-term contractions. The most commonly used agents, β-sympathomimetics such as Ritodrine, Salbutamol and Terbutaline, cause significant maternal cardiovascular, respiratory and metabolic side effects and may lead to pulmonary oedema, cardiac failure and maternal death. Furthermore they are subject to tachyphylaxis and become ineffective after 24 to 48 hours. Meta-analysis of randomised controlled trials has shown that the value of β-sympathomimetics is only in the temporary delay of labour to allow *in utero* transfer or administration of steroid to improve fetal lung surfactant production.

Other than the antenatal administration of corticosteroids, no obstetric interventions affect neonatal outcome although improvements in neonatal intensive care have dramatically increased survival rates. Commonly used agents are dexamethasone or betamethasone. Antenatal administration of corticosteroids improves the outcome for the pre-term neonate since it reduces the incidence and severity of respiratory distress syndrome, intracranial haemorrhage and necrotising enterocolitis. One function of corticosteroids is to mature the fetal lung, which leads to an increase in surfactant production and therefore prevents or reduces the severity of neonatal respiratory problems. Such agents are known to those skilled in the art.

Current obstetric management of pre-term labour (or threatened pre-term labour or pre-term premature rupture of membranes) is to attempt to delay delivery using 'tocolytic' drugs to allow time for steroid administration.

Typically, effective tocolytic drugs are oxytocin receptor antagonists, calcium channel blockers, sympathomimetics and nitric oxide donors.

A commonly used oxytocin receptor antagonist is Atosiban, that functions by blocking the oxytocin receptor, thereby preventing activation of the receptor by endogenous oxytocin that stimulates uterine contractions. A commonly used calcium channel blocker is Nifedipine, that functions to block the influx of calcium into the myometrial cells, which is a requirement for contractions to take place. A commonly used sympathomimetic is Ritodrine, that functions by activating adrenergic receptors on the myocyte cell membrane leading to phosphorylation and down-regulation of the activity of myosin light chain kinase, an enzyme essential for contractions. A commonly used nitric oxide donor is glyceryl trinitrate, that functions by increasing myocyte cGMP thereby down-regulating the activity of myosin light chain kinase, an enzyme essential for contractions.

Indomethacin, a cyclo-oxygenase inhibitor, is effective in preventing the contractions of pre-term labour. It is more effective in short term prolongation of pregnancy than the β-sympathomimetics and, unlike β-sympathomimetics, it can reduce the risk of delivery pre-term (Keirse 1995). The use of indomethacin is limited by fetal side effects. Indomethacin reduces fetal urine output and constriction of the ductus arteriosus (Moise *et al* 1995). Clinically significant ductal constriction occurs only in a proportion, increasing with gestational age from 10% at 26 weeks to 50% at 32 weeks. Accordingly the use of indomethacin is limited in clinical practice to use $\leq$ 32 weeks, and to short courses ($\leq$ 48 hours) after which any effects on the constriction of the ductus have been shown to be reversible (Tulzer *et al* 1991; Moise *et al* 1993; Respondek *et al* 1995).

Because of these side effects some obstetricians now use Sulindac, which appears to be equally good as a tocolytic (Carlon *et al* 1992) in place of indomethacin. Sulindac produces a smaller reduction in fetal urine output and minimal effect on ductal patency (Carlon *et al* 1992; Rasanen and Jouppila 1995). However, Sulindac is far from an ideal choice of tocolytic agent.

Accordingly, new agents or regimens capable of reducing and/or preventing an inflammatory response in the reproductive system of a female are highly desired. Such medicaments or approaches would allow the treatment of pathogenic infection within the reproductive system of a female and/or delay pre-term delivery without causing injury to the fetus/neonate.

In light of the above, the present inventors have surprisingly discovered that prostaglandins can be used to delay the onset and/or prevent the continuation of labour in a female.

Thus, in a first aspect, the present invention provides the use of a cyclopentenone prostaglandin in the manufacture of a medicament for delaying the onset and/or preventing the continuation of labour in a female.

Preferably, this is achieved by preventing and/or reducing an inflammatory response in the reproductive system of a female.

The invention stems from the unexpected finding that the cyclopentenone prostaglandins, such as 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 (15-dPGJ$_2$) and prostaglandin A_1 (PGA$_1$), inhibit and/or reduce NFκB activity within uterine cells of the female reproductive system. Thus, cyclopentenone prostaglandins provide a means for the inhibition and/or reduction of NFκB activity in the reproductive system of a female. Medicaments of the invention are believed to inhibit cytokine synthesis and inhibit the

biochemical processes of labour, thereby safely prolonging pregnancy. Accordingly, the present invention will improve obstetric management of pre-term labour as the onset of labour may be delayed without injuring the fetus/neonate.

The cyclopentenone prostaglandins are naturally-occurring substances that contain a cyclopentenone ring structure. The cyclopentenone ring is characterised by the presence of a chemically-reactive α,β-unsaturated carbonyl and is formed by dehydration of the cyclopentane ring of a precursor prostaglandin.

Generally, the first step in the biosynthesis of prostaglandins involves the intracellular release of arachidonic acid from plasma membrane phospholipids via the action of phospholipase A_2. Arachidonic acid is then converted sequentially to PGG_2 and PGH_2 by the cyclo-oxygenase and peroxidase activities of the PGH synthases, PGH 1 and 2. The prostaglandins PGE_2, PGD_2 and $PGF_{2\alpha}$ are subsequently synthesised from PGH2 via the action of the PGE_2, PGD_2 and $PGF_{2\alpha}$ synthase, respectively. The cyclopentenone prostaglandins, prostaglandin A_2 (PGA_2), prostaglandin A_1 (PGA_1) and prostaglandin J_2 (PGJ_2) are formed by dehydration of prostaglandin E_2 (PGE_2), prostaglandin E_1 (PGE_1) and prostaglandin D_2 (PGD_2), respectively. PGJ_2 is metabolised further to Δ^{12}-prostaglanding J_2 (Δ^{12}-PGJ_2), and 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 (15-$dPGJ_2$).

Other unnatural or synthetic prostaglandins can be made by chemical synthesis. Total synthesis of prostaglandins was first accomplished by Corey in the 1960s (reviewed in Corey, 1991), and subsequently simplified by Suzuki *et al.* (1990). This latter scheme uses a C8 organometallic reagent for one side chain and a C7 acetylenic halide for the other side chain which are added to the desired chemical head-group. This synthesis is versatile and allows the synthesis of a variety of natural and unnatural

prostaglandins including the cyclopentenone prostaglandins. A general pathway for natural and chemical synthesis of prostaglandins and cyclopentenone prostaglandins is described in Straus and Glass (2001), the disclosure of which is incorporated herein.

Chemical modification of cyclopentenone prostaglandins using techniques known in the art of chemistry may alter the clinical effectiveness of the molecule. Such alterations may, for example, increase or decrease the stability or another characteristic of the cyclopentenone prostaglandin, to give a desired change in activity. For example, modification of the 15C residue of cyclopentenone prostaglandins will reduce the metabolism of the compound, thereby increasing its half-life *in vivo*. Such modifications will be appreciated by those skilled in the art.

Thus, by "cyclopentenone prostaglandin", we include any natural, unnatural or chemically-modified prostaglandin which has a cyclopentenone ring. Cyclopentenone prostaglandin is often abbreviated to "cyPG". Especially preferred cyclopentenone prostaglandins include prostaglandin D_2 (PGD_2) and its metabolite 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 (15-$dPGJ_2$). Also preferred is prostaglandin A_1 (PGA_1).

15-$dPGJ_2$ may be obtained from Cayman Chemical, 1180 East Ellsworth Road, Ann Harbour, MI 48108 USA (catalogue number 18570). PGA_1 may be obtained from Alexis Biochemicals Ltd, PO Box 6757, Bingham, Nottingham, NG13 8LS, UK (catalogue number 340-045-M005).

By "onset of labour" and/or "continuation of labour" we include the biochemical and/or physiological changes associated with preparation of the tissues of the female reproductive system for delivery. For example, the uterus increases in contractility and undergoes contractions. The cervix also ripens in readiness for delivery. Such changes are well known in the arts of

obstetrics, gynaecology and midwifery and, for example, the Bishop's score indicates the degree of cervical ripening (described in Herman *et al.*, 1993). By "delaying the onset of labour in a female and/or preventing the continuation of labour in a female" we include the meaning that at least one of these biochemical and/or physiological changes are delayed or prevented.

By "female" we include any female mammal such as human, or a domesticated mammal, preferably of agricultural significance including a horse, pig, cow, sheep, dog and cat. It is preferred if the female is a human female.

In a second aspect, the present invention provides the use of a cyclopentenone prostaglandin in the manufacture of a medicament for preventing and/or reducing an inflammatory response in the reproductive system of a female. Such medicaments are able to inhibit and/or reduce NFκB activity in uterine cells.

By "NFκB" we include homo- and heterodimers of RelA (p65), RelB, NFκB1 (p50), NF_KB2 (p52) and cRel. The RelA (p65), RelB, NFκB1 (p50), NFκB2 (p52), and cRel genes and the sequence of the polypeptide products are described in Li *et al.* (2002).

By "NFκB activity" we include the activities of NFκB associated with the expression of genes controlled by any homo- or heterodimer of RelA (p65), RelB, NFκB1 (p50), NF_KB2 (p52) or cRel of the NFκB transcription factor family. In particular, we include: nuclear translocation of NFκB which can be measured, for example, by Western blotting analysis of nuclear and cytosolic cellular fractions for the protein of interest (described in Sambrook *et al.*, 1989; Lee *et al.*, 2003); binding of NFκB to target nucleic acid sequences (such as specific regions and sequences of DNA), which can be measured, for example, by Electro-Mobility Shift Assay (EMSA, as

described in Dignam *et al.*, 1983; Lee *et al.*, 2003); and NFκB-mediated expression of target genes which can be measured, for example, by northern blotting and/or Western blotting (Sambrook *et al.*, 1989; Lee *et al.*, 2003). Methods for measuring these activities of NFκB are well known by those skilled in the art of biochemistry and molecular biology.

By "uterine cells" we include any cells within the uterus of a female, or cells derived from the uterus of a female, particularly placental cells, amnion cells, myocytes, uterine and cervical fibroblasts, and maintained as a primary or transformed cell culture or line. These cell types are typically referred to as "gestational tissues".

Cultures of amnion cells may be prepared from tissue by separating the entire amnion, except for the part overlying the placenta, from the chorion, followed by separating amnion epithelial cells from fibroblasts and maintaining the epithelial cells using mammalian cell culture techniques (Lee *et al.*, 2003). Myometrial cell culture may be prepared from tissue from the lower uterine segment, separating cells by incubation with Dispase and collagenase/elastase/DNAase solution and maintaining the myometrial cells using mammalian cell culture techniques (Pieber *et al.*, 2001). Techniques for the generation and maintenance of primary and transformed mammalian cell cultures will be well known to those skilled in the relevant art.

By "reproductive system of a female", we include any cells and/or tissues and/or organs of a female directly or indirectly involved in the formation, nourishment, maintenance and development of a neonate, embryo or fetus at any gestational stage during pregnancy. In particular we include the cells and/or tissues of the uterus, placenta, amnion, chorion, decidua, cervix and vagina.

Preferably, the medicament is for preventing and/or reducing an inflammatory response in the reproductive system of a female that is pregnant.

By "inflammatory response" we include biochemical and physiological changes associated with inflammation mediated by cells of the host's immune system. Such changes are known in the arts of human and veterinary medicine, immunology, molecular biology and biological science.

If a patient is detected clinically at being at high risk of preterm delivery, because of detection of fibronectin in the vagina, identification of cervical shortening on ultrasound, the identification on clinical examination of cervical dilatation, or the onset of contractions then there is a high risk that there may be inflammation within the uterus. Other clinical measures of inflammation within the uterus are maternal temperature, white blood cell count, serum c-reactive protein concentrations and amniotic cytokine concentrations (taken at amniocentesis) which suggest a high risk of inflammation within the uterus if abnormal. Methods for measuring such changes will be well known to those skilled in the art.

By "pregnant", we include the meaning that the female is carrying a fertilised egg in the uterus, or an embryo or neonate or fetus at any stage of gestational development.

Preferably, the present invention provides a use wherein the female is human and the duration of pregnancy is more than approximately 13 weeks of human pregnancy. More preferably, the duration of pregnancy is approximately between 20 and 32 weeks.

Preferably, the medicament reduces and/or prevents an inflammatory response in the reproductive system of a female associated with the onset or continuation of labour. The biochemical and physiological changes associated with the onset or continuation of labour have been mentioned above.

There are many situations where it is useful to substantially prevent or reduce at least one of the changes in the female reproductive system associated with the onset or continuation of labour. For example, it is well known that certain groups of pregnant females are at high risk of pre-term labour. Females that have had one or more instances of pre-term labour previously are at considerably higher risk of a further pre-term labour when pregnant. An increased risk of pre-term labour can also be determined by measuring oncofetal fibronectin levels and by cervical examination using methods well known in the art.

It is also useful to prevent or reduce at least one of the changes in the female reproductive system associated with the continuation of labour, particularly uterine contractions, temporarily in circumstances where this is desirable. For example, it may be desirable temporarily to inhibit uterine contractions during labour in order to clear the fetal lungs or in order to transfer the female from one place to another. It is often desirable to transfer the female to a more suitable place where better care is available for her and the offspring.

It is also useful to substantially prevent for a considerable duration pre-term labour using the method of the invention. In particular, it is useful to inhibit pre-term uterine contractions from the time when they first occur (or soon thereafter) until the normal time of delivery.

Preferably, the medicament reduces and/or prevents an inflammatory response in the reproductive system of a female associated with infection by a pathogenic agent

More preferably, the pathogenic agent is viral, bacterial or fungal.

Preferably, the medicament reduces and/or prevents an inflammatory response in the reproductive system of a female associated with stretch of the uterus.

By "stretch of the uterus" we include mechanical stretching of the uterus occurring where the uterus is overdistended by multiple pregnancy or by excess amniotic fluid (clinically termed hydramnios or polyhydramnios). There may also be more local stretch of the lower segment of the uterus, the cervix and overlying fetal membranes in cases where there is cervical weakness (clinically termed cervical incompetence).

Preferably, the medicament reduces and/or prevents one or more of the following conditions: pre-term labour; pathogenic infection; cervical ripening, uterine contractions.

By "pre-term labour", we include the meaning of spontaneous labour occurring before the usual calculated time for delivery. In humans, pre-term labour is defined as spontaneous labour occurring before 37 weeks of gestation (with 39 weeks being term). The usual calculated time of delivery for females as defined by the invention will be well known in the arts of human and veterinary medicine.

Preferably, the medicament reduces and/or prevents fetal or neonatal damage.

More preferably, the medicament reduces and/or prevents one or more of the following conditions: astrogliosis; loss of myelin-producing oligodendrocytes; multifocal necroses resulting in cystic change (periventricular leucomalacia, PVL).

By "astrogliosis" we include the meaning of hypertrophy (i.e. increasing cell size) of the astroglia, that usually occurs in response to injury. Astroglia are the largest and most numerous neuroglial cells in the brain and spinal cord. Astrocytes (from "star" cells) are irregularly shaped with many long processes, including those with "end feet" which form the glial (limiting) membrane and directly and indirectly contribute to the blood-brain barrier. They regulate the extracellular ionic and chemical environment, and "reactive astrocytes" (along with microglia) respond to injury. Astrocytes can release neuro-transmitters, but their role in signaling (as in many other functions) is not well understood.

By "oligodendrocytes" we include the meaning of neuroglial cell of the central nervous system (CNS) in vertebrates whose function is to myelinate CNS axons. "Loss of myelin-producing oligodendrocytes" means that there a reduction in the number of these cells.

By "multifocal necroses" we include the meaning of death of tissue occurring at more than one site. By "cystic change" we include the meaning of the development of fluid filled spaces in the region where necrosis has taken place. By "periventricular leucomalacia" or "PVL" we include the meaning of damage to the periventrical cerebral white matter which is seen following cytokine induced or hypoxia/ischeamia induced necroses and which can go on to become cystic change.

A particularly preferred embodiment of the invention is the use of the cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and/or prostaglandin A_1.

Alternatively, the cyclopentenone prostaglandin is provided in the form of a prodrug of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and/or prostaglandin A_1.

It will be appreciated by those skilled in the art that certain metabolic precursors of cyclopentenone prostaglandins, may not possess pharmacological activity as such, but may, in certain instances, be administered to a patient and thereafter metabolised in the body to form compounds of the invention which are pharmacologically active. Such derivatives may therefore be described as "prodrugs".

All prodrugs of the cyclopentenone prostaglandins, particularly those of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and/or prostaglandin A_1, are included within the scope of the invention.

Preferably, the prodrug is PGD_2 (the precursor of 15-$dPGJ_2$) or PGE_1 (the precursor of PGA_1).

Preferably, the medicament further comprises a pharmaceutically acceptable excipient, diluent or carrier.

By "pharmaceutically acceptable" we mean that the carrier does not have a deleterious effect on the recipient. Typically, the carrier will be sterile and pyrogen free.

Preferably the medicament is in a form adapted for delivery by mouth, intravenous injection or intra-amniotic injection.

Preferably, the medicament is in a form which is compatible with the amniotic fluid. More preferably, the medicament is in a form which has substantially the same pH and/or osmotic tension as amniotic fluid.

The amniotic fluid has a distinct pH and a distinct osmotic tension. The amniotic fluid pH and osmotic tension are well known to, or can be readily measured by, the person skilled in the art.

Preferably, the medicament further comprises an agent for treating a female who has or is at risk of one or more of the following conditions: pre-term labour; pathogenic infection; cervical ripening, uterine contractions.

By an "agent for treating a female who has or is at risk of one or more of the following conditions: pre-term labour; pathogenic infection; cervical ripening, uterine contractions" we include corticosteroids, tocolytic agents and anti-inflammatory prostaglandins.

Preferably, the agent is a corticosteroid.

More preferably, the agent is capable of preventing and/or reducing respiratory distress syndrome.

One function of corticosteroids is to mature the fetal lung, which leads to an increase in surfactant production and therefore prevents or reduces the severity of neonatal respiratory problems

More preferably, the agent is selected from dexamethasone or betamethasone. Such agents are known to those skilled in the art. Administration of such agents may be two doses of 12mg intra-muscular (IM), 12 or 24 hours apart.

Preferably, the agent is capable of delaying delivery.

More preferably, the agent capable of delaying delivery is selected from: oxytocin receptor antagonists; calcium channel blockers; sympathomimetics; nitric oxide donors

Preferably, the agent is a tocolytic agent.

By "tocolytic" we include the meaning of a drug whose action is to stop uterine contractions.

More preferably, the tocolytic agent is selected from: oxytocin receptor antagonists, calcium channel blockers, sympathomimetics, nitric oxide donors.

More preferably, the oxytocin receptor antagonist is Atosiban. More preferably, the calcium channel blocker is Nifedipine. More preferably, the sympathomimetic is Ritodrine. More preferably, the nitric oxide donor is glyceryl trinitrate.

Preferably, the inflammatory response is mediated by NFκB in uterine cells.

More preferably, the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB DNA-binding in uterine cells.

More preferably, the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB-mediated transcriptional regulation in uterine cells.

More preferably, the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB production in uterine cells.

A further aspect of the invention is to provide a pharmaceutical composition comprising a cyclopentenone prostaglandin and a pharmaceutically acceptable carrier or exipient, the cyclopentenone prostaglandin being present in an amount effective to prevent and/or reduce an inflammatory response in the reproductive system of a female.

A further aspect of the invention is a method of treating inflammation within the reproductive system of a female, the method comprising administering an effective amount of a medicament of the invention.

Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1 : *15dPGJ$_2$ inhibition of NF-κB DNA binding*

Electro-mobility shift assay (EMSA) analysis of NF-κB DNA binding in nuclear protein extracts from *(A) m*yometrial cells, *(B)* L+ amnion cells, and *(C)* L- amnion cells treated with 15dPGJ$_2$ or vehicle for 2 h +/- IL-1b stimulation (15 min). Consensus kB probe used to assess NF-kB DNA binding, and consensus Oct-1 probe used as control.

Figure 2 : *PPAR-γ protein expression*

Western immunoblots of *(A)* nuclear and cytosolic protein extracts from myometrial and amnion cells with or without 15 min IL-1β stimulation, and *(B)* nuclear extracts of myometrial cells treated with 15d-PGJ$_2$ +/- IL-1b. Probing with antibody to PPARγ.

Figure 3 : *PPAR-α protein expression*

Western immunoblots of nuclear and cytosolic protein extracts from myometrial and amnion cells with or without 15 min IL-1b stimulation. Probing with antibody to PPARα.

Figure 4 : *PPAR-γ agonists do not inhibit NFkB DNA binding*

EMSA analysis of nuclear protein extracts from myometrial cells treated with *(A)* troglitazone, *(B)* GW1929 or vehicle for 2 h +/- IL-1b stimulation (15 min). Consensus κB probe used to assess NF-κB DNA binding, consensus Oct-1 probe used as control. For supershift analysis, extracts were preincubated with antibodies against p50 or p65.

Figure 5 : *Troglitazone and WY-14643 do not inhibit NFκB DNA binding*

EMSA analysis of nuclear protein extracts from myometrial cells treated with (A) WY-14643 or vehicle, and (B) high doses of troglitazone, WY-14643 or vehicle for 2 h followed by IL-1b stimulation (15 min). Consensus κB probe used to assess NFκB DNA binding, consensus Oct-1 and Sp-1 probes used as controls.

Figure 6 : *PPAR-γ antagonist GW9662 does not alleviate 15dPGJ$_2$ inhibition of NFκB DNA binding*

EMSA analysis of nuclear protein extracts from amnion cells treated with 15dPGJ$_2$ +/- GW9662 or vehicle for 2 h followed by IL-1β stimulation (15 min). Consensus kB probe used to assess NFκB DNA binding, consensus Oct-1 probe used as control.

Figure 7 : *PGA_1 inhibition of NFκB DNA binding*

EMSA analysis of nuclear protein extracts from *(A)* myometrial cells, and *(B)* amnion cells treated with PGA_1 or vehicle for 2 h followed by IL-1b stimulation (15 min). Consensus κB probe used to assess NFκB DNA binding, consensus Oct-1 probe used as control. For supershift analysis, extracts were preincubated with antibodies against p50 or p65.

Figure 8 : *Effect of cyPGs and PPAR agonists on NFκB transcriptional activity in amnion*

Amnion cells derived from L- or L+ placentas were transiently transfected with the NFκB-dependent reporter construct κB.BG.Luc, treated with $15dPGJ_2$, PGA_1, troglitazone, WY-14643, or vehicle for 2 h, and then stimulated with IL-1β (1 ng/ml) for 6 h. The mutated κBmut.Luc construct was used as a control to confirm NFκB-mediated transactivation. Values are normalised for b-gal reporter activity.

Figure 9 : *$15dPGJ_2$ inhibition of NFκB transcriptional activity in myometrium*

Myometrial cells were transiently transfected with the NFκB-dependent reporter construct κB.BG.Luc, treated with $15dPGJ_2$ or vehicle for 2 h, +/- IL-1β (1 ng/ml) for 6 h. The mutated κBmut.Luc construct was used as a control to confirm NFκB-mediated transactivation. Values are normalised for b-gal reporter activity. *(NS* = nonstimulated*)*.

Figure 10 : *Effect of PGA_1 and PPAR agonists on NFκB transcriptional activity in myometrium*

Myometrial cells were transiently transfected with the NFκB-dependent reporter construct κB.BG.Luc, treated with troglitazone, WY-14643, PGA_1 or vehicle for 2 h, +/- IL-1b (1 ng/ml) for 6 h. The mutated κBmut.Luc construct was used as a control to confirm NFκB-mediated transactivation. Values are normalised for CMV-Renilla reporter activity. (*NS* = nonstimulated).

Figure 11 : *PPAR-γ agonist GW1929 does not inhibit NFκB transcriptional activity*

Myometrial cells were transiently transfected with the NFκB-dependent reporter construct κB.BG.Luc, treated with GW1929 or vehicle for 2 h, +/- IL-1b (1 ng/ml) for 6 h. Values are normalised for CMV-Renilla reporter activity. (*NS* = nonstimulated).

Figure 12 : *Troglitazone and GW1929 potentiate PPAR-γ activation of a PPRE reporter*

Myometrial cells were cotransfected with 0.4 mg of the PPAR-γ-dependent reporter construct 3-PPRE-TK.pGL3 and 100 ng, 200 ng or 300 ng of a PPAR-γ expression construct. Cells were treated with 10 mM or 20 mM of *(A)* troglitazone or *(B)* GW1929, or vehicle for 24 h. Values are normalised for CMV-renilla reporter activity. Similar results were obtained with transfection of amnion cells.

Figure 13 : *Troglitazone does not inhibit NFκB transcriptional activity in PPAR-γ-transfected cells*

Myometrial cells were transfected with 0.4 mg κB.BG.Luc reporter and 200 ng PPAR-γ expression vector and treated with 10 mM troglitazone or vehicle for 7 h +/- IL-1b (1 ng/ml) for 17 h. Values are normalised for CMV-renilla reporter activity. (*NS* = nonstimulated).

Figure 14 : *PPAR-γ overexpression does not potentiate 15d-PGJ$_2$ inhibition of NFkB activity*

Myometrial cells were transfected with 0.4 mg κB.BG.Luc reporter and 200 ng PPAR-γ expression vector and treated with 15d-PGJ$_2$ for 2h followed by IL-1β (1 ng/ml) for 6 h. Values are normalised for β-galactosidase reporter activity. (*NS* = nonstimulated).

Figure 15 : *15dPGJ$_2$ inhibition of p65 nuclear localisation, p50 phosphorylation, and IκBα degradation*

Western immunoblots of nuclear or cytosolic protein extracts from *(A)* myometrial cells, *(B)* L- amnion cells, and *(C)* L+ amnion cells treated with 15dPGJ$_2$ or vehicle for 2 h +/- IL-1β stimulation (15 min). Blots probed with antibodies against p65, p50 or IκBα.

Figure 16 : *PGA$_1$ inhibition of p65 nuclear localisation and IκBα degradation*

Western immunoblot of nuclear or cytosolic protein extracts from myometrial cells treated with PGA$_1$ or vehicle for 2h followed by IL-1β stimulation (15 min). Blots probed with antibodies against p65or IκBa.

Figure 17 : *PGE$_2$ does not inhibit TNFα- and IL-1β-induced NFκB activation*

Analysis of nuclear protein extracts from myometrial cells treated with PGE_2 or vehicle for 2 h +/- TNFα or IL-1b stimulation (15 min). (*A*) EMSA using consensus κB probe. (*B*) Western immunoblot probing for nuclear p65.

Figure 18 : *PGE_2 does not induce NFκB DNA binding*

EMSA analysis of nuclear protein extracts from *(A)* L- amnion cells and *(B)* myometrial cells treated with vehicle, PGE_2 or IL-1β. Consensus κB probe used.

Figure 19 : *$15dPGJ_2$ inhibits IκBα phosphorylation*

Western immunoblots of cytosolic extracts from myometrial cells *(A)* treated with $15dPGJ_2$ for 2h +/- IL1b for 15min; probed for IKK, and *(B)* treated with 30mM $15dPGJ_2$, 40mM MG132 or vehicle for 2h, +/- IL-1β for 15min; probed for IκBα.

Figure 20 : *Effect of $15dPGJ_2$ and PPAR agonists on IL-1β-induced COX-2 protein expression*

Western immunoblot of cytosolic protein extracts from myometrial cells treated with $15dPGJ_2$, troglitazone, WY-14643 or vehicle for 2h, followed by IL-1β stimulation for 6h. Probed with antibodies to *(A)* COX-2, and *(B)* a smooth muscle actin.

Figure 21 : *Schematic of the structure of (A) prostaglandin A_1 (PGA_1) and (B) 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 ($15\text{-}dPGJ_2$)*

Figure 22 : *Effect of LPS and 15d-PGJ_2 on inflammatory responses – IL-1β levels*

Concentrations of IL-1β in placental homogenates collected from gestation day 16 mice 6 hours after intrauterine injection of 250μg LPS + vehicle or 250μg LPS + 4μg 15d-PGJ_2. * denotes statistically significant difference (t-test ($p<0.05$)).

Figure 23 : *Effect of LPS and 15d-PGJ_2 on inflammatory responses – phospho-p65 levels*

Relative concentrations of phospho-p65 in placental homogenates collected from gestation day 16 mice 6 hours after intrauterine injection of 250μg LPS + vehicle or 250μg LPS + 4μg 15d-PGJ_2. * denotes statistically significant difference (t-test ($p<0.05$)).

EXAMPLE 1 – Experimental data

Methods

Abbreviations

EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethyleneglycol bis-aminoethyltetra acetic acid
DTT	Dithiotreitol
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
NP-40	Nonidet P-40
SDS-PAGE	Sodium dodecyl sulphate – Polyacrylamide gel electrophoresis
PVDF	Polyvinylidene difluoride
PBS-T	Phosphate Buffered Saline plus Tween
HRP	Horseradish peroxidase
PBS	Phosphate Buffered Saline
FCS	Foetal Calf Serum
DMEM	Dulbecco's modified eagle's medium

Tissue biopsies and cell culture

Local Ethics committee approval was obtained for the collection of these tissues and patients gave informed consent.

Human myometrial cell culture

Myometrial tissue was collected at term from the upper margin of uterine incision at the time of lower segment caesarean section either prior to the onset of labour (L-) or during fetal distress (L+). L+ samples were collected by Dr Mark Johnson and Dr S Soorrana at Chelsea & Westminster Hospital. Myometrial tissue was dissected, rinsed in PBS, and digested in serum-free DMEM containing 15mg/ml collagenase 1A (Sigma), 15mg/ml collagenase X, and 50mg/ml bovine serum albumin for 45min at 37°C. The cell suspension was filtered through a cell strainer, centrifuged at 400g for 5min, and the pellet re-suspended and plated out in DMEM, 10% FCS (Helena BioScience), 1% L-glutamine, 1% penicillin-streptomycin. Cells were used between passage numbers 1-4.

Human Amnion Cell Culture

Placentae were obtained from patients at term either at elective Caesarean section prior to labour (L-) or following spontaneous labour onset and vaginal delivery (L+). Amnion cells were prepared as described in Bennett *et al.*, (1989). Briefly, the amnion was separated from the placenta, washed 3x in PBS, cut into strips, and incubated in 0.5mM EDTA in PBS for 15min. The strips were washed in PBS 2x and digested with 2.5mg/ml dispase in serum-free DMEM for 35min at 37°C. The amnion was then shaken vigorously in DMEM, 10% FCS to dissociate the cells, the remaining strips discarded, and the cell suspension pelleted at 175g for

10min and cultured in DMEM, 10% FCS (Sigma), 1% L-glutamine, 1% penicillin-streptomycin.

Protein Extracts from cultured cells

Nuclear and cytosolic protein extracts were obtained from cultured amnion cells as described by Schreiber *et al* (1989). For nuclear/cytosolic fractionation, confluent cell monolayers were scraped and lysed using a buffer containing 10mM HEPES, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA, 2mM DTT, 1% (v/v) NP-40 and complete protease inhibitor tablets (CPIs, Roche), diluted to manufacturer's instructions. Cell lysates were incubated on ice for 10min and NP-40 added to a final concentration of 1% (v/v). Lysates were vortexed for 10secs and centrifuged for 30secs at 4°C, 12000g. The supernatants were retained as the cytosolic protein extracts. The pellets were resuspended in buffer containing 10mM HEPES, 10mM KCl, 0.1mM EDTA, 0.1mM EGTA, 2mM DTT, 400mM NaCl, 1% NP-40 (v/v) and CPIs. Samples were shaken vigorously for 15min in an ice bath. The nuclear protein extracts were obtained in the supernatant following a 5min centrifugation at 4°C, 12000g.

For whole cell lysates, confluent cell monolayers were scraped and lysed in a high-salt extraction buffer containing 0.4M KCl, 20mM HEPES, 20% (v/v) glycerol, 1mM DTT, and CPIs.

Protein Extracts from fresh tissue biopsies

Tissue samples were rinsed in ice-cold PBS, dissected, flattened between aluminium foil, flash-frozen in liquid nitrogen, and stored at -80°C. Samples were reduced to powder in liquid nitrogen using a pestle and mortar. Powdered tissue was homogenized in a Dounce homogeniser on ice in a buffer containing 0.6% (v/v) NP-40, 150mM HEPES, 1mM EDTA,

0.5mM PMSF and any unbroken tissue was removed by centrifugation for 30sec at 2000rpm at 0°C. The supernatant was incubated on ice for 5min, centrifuged for 10min at 4000rpm at 0°C, and the nuclear pellets resuspended in 25% (v/v) glycerol 20mM HEPES, 0.42M NaCl, 1.2 mM $MgCl_2$, 0.2mM EDTA, 0.5mM DTT, and CPIs.

All extracts were aliquoted, frozen on dry ice and stored at -80°C. The extracts were processed for protein quantitation by the Lowry method using Bio-Rad protein assay reagents (Bio-Rad Laboratories) according to manufacturer's instructions.

Electro-mobility shift assay (EMSA)

Oligonucleotide labelling

Sense and antisense strands (175nmole/ml each) were incubated in annealing buffer (10mM Tris-HCl pH7.5, 100mM NaCl, 1mM EDTA) for 10min at 65°C, and allowed to cool at room temperature for 2h. 3.5 pmole double-stranded oligonucleotides were end-labelled with 0.37MBq $^{32}P(\gamma ATP)$ by incubating for 30min at 37°C with T4 polynucleotide kinase. Labelled oligonucleotides were recovered by centrifugation at 3000rpm for 2min through MicroSpin G-25 or G-50 sephadex columns (Amersham Biosciences).

EMSA

3-5µg protein extracts were incubated on ice for 1h with non-radiolabelled non-specific oligonucleotide (poly(dI-dC) or Oct-1) in a binding buffer (20% (v/V) glycerol, 5mM $MgCl_2$, 2mM EDTA, 50mM Tris-HCl pH7.5, 250mM NaCl, 2mM DTT), followed by a 45min incubation with 0.035pmole $^{32}P(\gamma ATP)$-end labelled oligonucleotide probes:

consensus NF-κB:	5'-AGT TGA GGG GAC TTT CCC AGG C-3'
consensus Oct-1:	5'-TGT CGA ATG CAA ATC ACT AGA A-3'
consensus SP-1:	5'-ATT CGA TCG GGG CGG GGC GAG
upstream COX-2 κB:	5'-CGG GAG AGG GGA TTC CCT GCG C-3'
downstream COX-2 κB:	5'-AGA GTG GGG ACT ACC CCC TCT-3'

Oct-1 or SP-1 consensus sequences were used as a controls for a NF-κB-specific effect. The resulting protein/DNA complexes were separated in a 4% acrylamide gel, the gel dried under vacuum at 80°C and exposed to X-ray film. For supershift analysis, samples were incubated with 2μg antibodies for 30min on ice prior to incubation with oligonucleotides. Non-radio-labelled oligonucleotides were used at 100-fold molar excess for specific and non-specific competition for DNA binding. Reagents for EMSA were obtained from Promega Life Sciences, Delta House, Chilworth Research Centre, Southampton SO16 7NS, United Kingdom.

SDS-PAGE and Western blotting analysis

Protein samples (20-70μg) were mixed with Laemmli sample buffer (1:1) containing β-mercaptoethanol (5%), and boiled for 5min. Proteins were then separated by SDS-PAGE (12-14% gels) and transferred onto PVDF membrane (Amersham Pharmacia Biotech). The membranes were blocked overnight in 5% non-fat milk prepared in PBS-T buffer, at 4°C. The blots were incubated with the primary antibody in 1% non-fat milk in PBS-T buffer for 1h, and washed three times (10min each) in PBS-T with vigorous shaking. The blots were then incubated with HRP-conjugated secondary antibody (diluted 1:2000 in 1% non-fat milk in PBS-T buffer) for 1h and washed three times (10min each) in PBS-T. Signal detection was achieved using enhanced chemi-luminescence (ECL plus system, Amersham Pharmacia Biotech) according to manufacturer's instructions.

To re-probe a membrane, blots were incubated for 30min in 50°C stripping buffer (2% SDS, 62.5mM Tris-HCl pH6.7, 100mM 2-mercaptoethanol), washed 2x in PBS-T, placed in blotto overnight, and then probed with a new antibody as above.

30-50μg protein extracts were subjected to SDS-PAGE and Western immuno-blotting. Secondary antibodies were IgG-HRP and ECL Plus detection kit (Amersham Pharmacia Biotech, Amersham Place, Little Chalfont, Bucks, HP7 9NA) was used for visualisation.

Transfections and luciferase assay

Cells at 70-80% confluence in 24-well plates were transfected using the liposome Transfast (Promega). 0.5μg per well of luciferase reporter construct was transfected using a 1:1 ratio of transfection (i.e., 3μl Transfast per 1μg DNA) in serum-free DMEM for 1h. DMEM, 10% FCS was then added and the cells were incubated at 37°C for 24h. The medium was replaced with DMEM, 2% FCS for a further 24h, and the cells treated with various agonists/inhibitors or vehicle for 6-8h. Transfections were analysed in a dual firefly/renilla (Packard BioSciences/Calbiochem) luciferase assay or firefly/β-galactosidase (Promega/Galacton) assay using a luminometer.

pGL3.6κB.BG.luc was the reporter construct used to assess NF-κB-mediated transcription, while the mutant pGL3.6κBmut.luc and empty pGL3.BG.luc were used as controls (Schwarzer *et al.*, 1998).

pGL3.6κB.BG.luc: a NF-κB-dependent reporter construct with 6 copies of the NF-κB binding site. It contains two tandem repeats of the sequence 5'-GGG GAC TTT C CC TGG GGA CTT TCC CTG GGG ACT TTC CC-3', which contains three copies of the decameric NF-κB binding site

(underlined) upstream of a minimal β-globin promoter driving a luciferase gene.

pGL3.6κBmut.luc: this reporter construct is as above except that the core NF-κB binding site is mutated to 5'-GCC ACT TTC C-3' (mutated bases underlined).

pGL3.BG.luc: this reporter construct contains only the minimal β-globin promoter.

Cells were co-transfected with the renilla vector pRL-CMV or a β-galactosidase vector pCH110 as internal controls for transfection efficiencies.

In vitro translation and plasmid preps

For recombinant production of p65, a pSG5/p65 expression construct was transcribed and translated using a TNT Coupled Reticulocyte Lysate System (Promega), according to manufacturer's instructions. QIAGEN Maxi Prep kits were used for plasmid isolation from transformed JM109 *E. coli* cells, and all constructs were subsequently precipitated with polyethylene glycol.

Reagents/Antibodies

Recombinant cytokine IL-1β and TNFα from R&D Systems; 15d-PGJ_2, PGA_1, troglitazone, GW-9662, and 16,16-dimethyl-PGE_2 from Cayman Chemical; WY-14643, MG132 proteasome inhibitor, and PG490 (triptolide) from Calbiochem; HRP-conjugated secondary antibodies and antibodies to p50, p65, c-rel, Rel B, COX-2, IκBα, IκBβ, and PPARγ from Santa Cruz; antibodies to p52, Bcl-3 and smooth muscle actin from Upstate

Biotechnologies. Antibody to PPAR-γ from Affinity BioReagents, to phospho-p65 from Cell Signaling, to COX-1 from Alexis Biochemicals, and to lamin B from Oncogene Research Products.

Mouse model of preterm labour

Surgery was performed on timed pregnant MF1 mice at day 16 of gestation. After deep maternal anaesthesia was attained, a minilaparotomy was performed in the lower abdomen. The uterine horns were exposed through the incision and preterm labour was induced by the intrauterine injection of 250μg lipopolysaccharide (LPS, Sigma) into the gravid horn. This was immediately followed by injection of 4μg 5d-PGJ_2 (Cayman), or an equal volume of vehicle (methyl acetate), at the same site. The uterus was then returned to the abdomen and the fascia and skin were closed with continuous vicryl sutures.

Effect of LPS and 15d-PGJ_2 on inflammatory responses

Mice were sacrificed 6 hours after injection of LPS ± 15d-PGJ2. Placentae were washed in phosphate buffered saline (PBS), flash frozen in liquid nitrogen and stored at -80°C until further processing. Fetuses were washed in PBS, then immediately fixed in 4% paraformaldehyde for 24h and then stored in 70% ethanol until further processing. Placentae were homogenized for 1 minute in the presence of lysis buffer comprising 400mM KCl, 20mM HEPES pH7.4, 1mM dithiothreitol, 20% glycerol and 5% (v/v) protease inhibitor cocktail.

Homogenate levels of Interlekin-1β (IL-1β) and tumour necrosis factor α (TNFα) were determined in placental lysates by ELISA (R and D systems) according to manufacturers instructions. Total protein concentrations were determined for each homogenate and IL-1β and TNFα levels were

expressed as pg/mg total protein. Homogenates were also subjected to polyacrylamide gel electrophoresis. Loading volumes were adjusted according to the protein content of each homogenate such that a constant amount of protein was run in each lane. Phosphorylated p65 (phospho-p65) was detected by western immunoblotting using a specific antibody (Santa Cruz) and quantified by densitometric analysis.

Results

CyPGs, but not PPAR agonists, inhibit NF-κB DNA binding in amnion and myometrial cells.

15d-PGJ_2 inhibited IL1-β-induced NF-κB DNA binding in a dose-dependent manner in myometrial cells, as well as in L- and L+ amnion cells *(Fig. 1)*. Protein binding to a consensus Oct-1 or Sp-1 probe was unaffected by either IL-1β or 15d-PGJ_2 treatment, confirming that the effects observed are NF-κB-specific.

Since PPAR-γ is the putative endogenous receptor for 15d-PGJ_2, and PPAR expression may be affected by cytokines (Tontonoz *et al.*, 1998, Tanaka *et al.*, 1999), PPAR-γ protein expression was examined in myometrial and amnion cells. PPAR-γ was shown to be expressed predominantly in the nucleus of both cell types, and its expression was not affected by IL-1β or 15d-PGJ_2 treatment *(Fig. 2)*. 15d-PGJ_2 can also transactivate PPAR-α, though more weakly than PPAR-γ (Forman *et al.*, 1995). PPAR-α expression in myometrial and amnion cells was found to be predominantly cytoplasmic *(Fig. 3)*.

The ability of synthetic PPAR agonists to mimic the inhibitory effects of 15d-PGJ_2 was examined. The PPAR-γ agonist troglitazone had no effect on

NF-κB DNA binding at 10-50μM doses, although it did cause a slight reduction at 100μM (*Fig. 4, 5*). Troglitazone can transactivate PPAR-γ at 1μM and induces weak interactions between PPAR-γ and the co-activators p300 and steroid receptor co-activator (SRC-1) at 10μM doses; adipogenesis is positively regulated by PPAR-γ, and troglitazone can induce expression of adipogenic markers at 5-10μM doses (Prusty *et al.*, 2002). Thus, at 100μM concentrations, it is unlikely that troglitazone is exerting a specific effect through PPAR-γ. Since structurally distinct PPAR ligands may differentially affect coactivator/corepressor recruitment, a new potent PPAR-γ agonist, which lacks the TZD moiety, was also used. This GW1929 ligand failed to inhibit NF-κB DNA binding *(Fig. 6)*. The synthetic PPAR-α agonist WY-14643 can transactivate PPARα at 5-25μM doses in a GAL4 chimera transfection system (Kehrer *et al.*, 2001), but WY-14643 had no effect on NF-κB DNA binding, at 10-100μM concentrations. To further investigate a potential role for PPAR-γ in mediating the inhibitory effects of 15d-PGJ_2, NF-κB DNA binding was assessed in cells treated with 15d-PGJ_2 in the presence of the selective PPAR-γ inhibitor GW-9662. GW9662 binds irreversibly to PPAR-γ through covalent modification of Cys^{285} in the ligand-binding domain (Leesnitzer *et al.*, 2002). GW-9662 failed to alleviate 15d-PGJ_2 inhibition of NF-κB (*Fig. 6*).

In contrast, PGA_1, which does not act as a PPAR ligand but does contain a cyclopentenone ring, was able to inhibit NF-κB DNA binding in amnion and myometrial cells, albeit at much higher doses than 15d-PGJ_2 (*Fig. 7*).

CyPGs, but not PPAR agonists, inhibit NF-κB transcriptional activity.

To determine whether the cyPG effects on NF-κB DNA binding extend to inhibition of NF-κB transactivation potential, amnion cells were transfected

with the NF-κB-dependent reporter κB.BG.Luc and treated with 15d-PGJ_2, PGA_1, troglitazone, WY-14643 or vehicle, followed by IL-1β stimulation (*Fig. 8*). Constitutive reporter activity was seen in both L- and L+ amnion cells, although the levels were lower and showed a greater increase with IL-1β. in L- cells, in agreement with previous studies by Allport *et al* (2001). Both 15d-PGJ_2 and PGA_1 inhibited IL-1β-induced NF-κB transcriptional activity, whereas troglitazone and WY-14643 did not.

In myometrial cells, 15d-PGJ_2 inhibited IL-1β-induced NF-κB transcriptional activity in a dose-dependent manner, reducing reporter activity to basal levels (*Fig. 9*). IL-1β-induced NF-κB transcriptional activity was also reduced to basal levels by PGA_1, but not troglitazone, GW1929 or WY-14643 (*Fig. 10, 11).*

GW1929 and troglitazone were shown to be functional as PPAR-γ ligands, potentiating PPAR-γ-mediated transcription of a PPRE-dependent reporter in both cell types. Endogenous PPAR-γ levels were not sufficient to drive the PPRE reporter in the transfection system used, with transcription requiring overexpression of the receptor. Troglitazone was also unable to inhibit a NF-κB-dependent reporter in PPARγ-transfected cells, and PPARγ overexpression did not promote 15d-PGJ_2 inhibition of NF-κB transcriptional activity *(Fig. 12, 13, 14).*

CyPGs, but not PGE_2, inhibit NF-κB activation and IκB degradation.

15d-PGJ_2 inhibited IL-1β-induced p65 nuclear translocation and p50 phosphorylation in myometrial cells and in L-, L+ amnion cells in a dose-dependent manner (*Fig. 15).* This was paralleled by inhibition of IL-1β-induced IκBα and IκBβ degradation. Similarly, PGA_1 inhibited p65 nuclear translocation and IκBαdegradation in myometrial cells (*Fig. 16).*

16,16-Dimethyl-PGE_2, a PGE_2 analogue with increased half-life, did not inhibit NF-κB DNA binding (controlled for with Oct-1 binding) or IL-1β-induced p65 nuclear translocation in myometrial and amnion cells *(Fig. 17).* This is not unexpected, since, in contrast to the cyPGs, PGE_2 is known to be pro-inflammatory, does not contain a cyclopentenone ring, and does not activate PPAR-γ (Forman *et al.*, 1995). 16,16-dimethyl-PGE_2 did not inhibit NF-κB DNA binding or p65 nuclear translocation in myometrial cells *(Fig. 18).* However, neither did it stimulate NF-κB activity as reported in T cells (Dumais *et al.*, 1998), nor did it synergise with IL-1βor TNFα.

Effect of 15-dPGJ_2 on NF-κB upstream activators and downstream targets.

In contrast to the proteasome inhibitor MG132, which prevented IL-1β-induced IκBα degradation and resulted in the accumulation of undegraded, phosphorylated IκBα, accumulation of phosphorylated IκBα was not detected following 15-dPGJ_2 treatment, suggesting that 15-dPGJ_2 may be affecting IKKs or other upstream kinases *(Fig. 19).* Both IL-1β and 15-dPGJ_2 treatment had no effect on IKKα or IKKβ protein expression, although it is more likely that 15d-PGJ_2 would inhibit the kinase activity of the IKKs.

Since COX-2 is an important target gene for NF-κB in labour, the effect of 15-dPGJ_2 and PPAR agonists on COX-2 expression was assessed. IL-1β-induced COX-2, expression was inhibited by 15-dPGJ_2, but not by troglitazone or WY-14643 *(Fig. 20).* Similar results were obtained in L- and L+ amnion cells.

Effect of LPS on pre-term delivery.

Pre-term delivery of pups occurred by 16 hours after injection of LPS using the mouse model of preterm labour, as set out in the methods above.

Effect of LPS and 15d-PGJ_2 on inflammatory responses

In all mice, levels of TNFα and IL-1β were significantly higher in the placentae proximal to the injection site compared to those in the opposite horn. Levels of IL-1β were approximately 40% lower in proximal placentae injected with LPS + 15d-PGJ_2 compared to those given LPS + vehicle (Fig. 22). This difference was statistically significant ($p<0.05$). In contrast, TNFα levels were not significantly altered according to drug treatment.

Significantly, placental levels of IL-1β were not altered according the proximity of the placenta to the site of injection, indicating that inflammatory response can be distributed throughout the uterus, irrespective of the site of infection. However, phospho-p65 levels were approximately 35% lower in proximal placentae injected with LPS + 15d-PGJ_2 compared to those given LPS + vehicle (Fig. 23) and this difference was statistically significant ($p<0.05$).

Conclusions

NF-κB inhibition by cyPGs

15d-PGJ_2 inhibited IL1-β-induced NF-κB DNA binding and NF-κB-mediated transactivation in myometrial cells, as well as in L- and L+ amnion cells. 15d-PGJ_2 inhibited the nuclear translocation and activation of NF-κB, at least in part, by preventing the degradation of IκBα by IL-1β.

In myometrial and amnion cells, which expressed both PPAR-α and PPAR-γ receptors, neither PPAR-γ nor PPAR-α agonists were able to inhibit IL-1β-induced NF-κB DNA binding or NF-κB transcriptional activity at doses shown to inhibit NF-κB in other cell types (Chinetti *et al.*, 1998; Gupta *et al.*, 2001), or even at higher concentrations. In a study investigating the potential functional interactions between PPAR-γ and NF-κB in adipocytes, PPAR-γ agonists did not impair TNFα-induced NF-κB activation, nuclear translocation, or DNA binding activity; rather, they antagonised the transcriptional regulatory activity of NF-κB, and PPAR-γ overexpression was required to demonstrate such inhibition (Ruan *et al.*, 2003). In the present study, while PPAR-γ overexpression potentiated transactivation of a PPRE, it did not enable the PPAR-γ agonists to inhibit NF-κB transcription. In addition, 15d-PGJ_2 was able to inhibit NF-κB transcription in the absence of exogenous PPAR-γ and overexpression of this receptor did not promote inhibition.

IL-1β-induced COX-2 expression was inhibited by 15d-PGJ_2 but not by PPAR agonists. While PPAR agonists are known to be anti-inflammatory and can inhibit COX-2 expression (Staels *et al.*, 1998; Subbaramaiah *et al.*, 2001), they have also been reported to enhance COX-2 expression in certain cell types (Meade *et al.*, 1999; Ikawa *et al.*, 2001; Pang *et al.*, 2003).

CyPGs such as 15d-PGJ_2 are characterised by the presence of a cyclopentenone ring system containing an electrophilic carbon. This ring can react covalently with nucleophiles such as the free sulfhydryls of glutathione and cysteine residues in cellular proteins. Receptor-independent actions of 15d-PGJ_2 have been attributed to its cyclopentenone ring. NF-κB proteins contain a conserved cysteine residue in their DNA-binding domain (DBD) and alkylation of this cysteine impairs DNA binding (Toledano *et al.*, 1993). In the present study, PGA_1, a cyPG that does not act as a PPAR-

γ ligand, was able to inhibit NF-κB DNA binding and transactivation, albeit at higher concentrations than 15d-PGJ_2. This ability of PGA_1, but not PGE_2 or PPAR agonists, to mimic the effects of 15d-PGJ_2 suggests that these cyPGs may inhibit NF-κB in amnion and myometrial cells by virtue of their cyclopentenone ring. While direct modification of NF-κB cysteines has not been addressed in this study, both 15d-PGJ_2- and PGA_1-mediated inhibition of NF-κB was shown to involve the inhibition of IκBα degradation, suggesting that events further upstream in the NF-κB cascade are being targeted.

Thus, while PPAR activation may not be effectively anti-inflammatory in amnion and myometrium, the use of cyPGs should prove useful in repressing NF-κB, and therefore an array of pro-inflammatory and labour-associated genes, in these tissues. CyPG administration offers an attractive alternative approach to anti-inflammatory treatment since a potential specificity of cyPGs for IKKβ/IκBα would spare other potentially beneficial pathways of NF-κB activation (e.g., the processing of p105 and formation of p50 homodimers), which might be disrupted by more broad-spectrum NF-κB inhibitors. The use of the cyPGs, able to simultaneously trigger the inhibition of the pro-inflammatory NF-κB and harness the anti-inflammatory activities of endogenous cytoprotective molecules represents a novel therapeutic approach in the treatment of preterm labour and neurodevelopmental disorders of the neonate.

This study provides evidence that the mouse model used is an effective model for the study of preterm delivery and agents that may delay the onset of preterm delivery. The finding of lower levels of IL-1β and phospho-p65 in mice treated with the cyclopentenone prostaglandin 15d-PGJ_2 suggests that this compound is effective at blocking the inflammatory pathway induced by LPS treatment *in vivo*.

EXAMPLE 2 – Preferred pharmaceutical formulations and modes and doses of administration.

The compounds of the present invention may be delivered using an injectable sustained-release drug delivery system. These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period.

The compounds of the present invention can be administered by a surgically implanted device that releases the drug directly to the required site. For example, Vitrasert releases ganciclovir directly into the eye to treat CMV retinitis. The direct application of this toxic agent to the site of disease achieves effective therapy without the drug's significant systemic side-effects.

Electroporation therapy (EPT) systems can also be employed for administration. A device which delivers a pulsed electric field to cells increases the permeability of the cell membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

Compounds can also be delivered by electroincorporation (EI). EI occurs when small particles of up to 30 microns in diameter on the surface of the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

An alternative method of administration is the ReGel injectable system that is thermosensitive. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly erodes and dissolves into known, safe, biodegradable polymers. The active drug is delivered over time as the biopolymers dissolve.

The compounds of the invention can also be delivered orally. The process employs a natural process for oral uptake of vitamin B_{12} in the body to co-deliver proteins and peptides. By riding the vitamin B_{12} uptake system, the protein or peptide can move through the intestinal wall. Complexes are synthesised between vitamin B_{12} analogues and the drug that retain both significant affinity for intrinsic factor (IF) in the vitamin B_{12} portion of the complex and significant bioactivity of the drug portion of the complex.

Compounds can be introduced to cells by "Trojan peptides". These are a class of polypeptides called penetratins which have translocating properties and are capable of carrying hydrophilic compounds across the plasma membrane. This system allows direct targeting of oligopeptides to the cytoplasm and nucleus, and may be non-cell type specific and highly efficient (Derossi *et al.,* 1998).

Preferably, the pharmaceutical formulation of the present invention is a unit dosage containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of the active ingredient.

The compounds of the invention can be administered orally or by any parenteral route, in the form of a pharmaceutical formulation comprising the active ingredient, optionally in the form of a non-toxic organic, or inorganic, acid, or base, addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated, as well

as the route of administration, the compositions may be administered at varying doses.

Formulations in accordance with the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (*e.g.* povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (*e.g.* sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethylcellulose in varying proportions to provide desired release profile.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouth-washes comprising the active ingredient in a suitable liquid carrier.

In human therapy, the compounds of the invention can be administered alone but will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice.

For example, the compounds of the invention can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The compounds of the invention may also be administered *via* intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose, sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (preferably corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC), sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Preferred excipients in this regard include lactose, starch, cellulose, milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

The compounds of the invention can also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intra-thecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well-known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Generally, in humans, oral or parenteral administration of the compounds of the invention is the preferred route, being the most convenient.

For veterinary use, the compounds of the invention are administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

The formulations of the pharmaceutical compositions of the invention may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose or an appropriate fraction thereof, of an active ingredient.

A preferred delivery system of the invention may comprise a hydrogel impregnated with a compound of the invention, which is preferably carried on a tampon which can be inserted into the cervix and withdrawn once an appropriate cervical ripening or other desirable affect on the female reproductive system has been produced.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavouring agents.

EXAMPLE 3 – Exemplary pharmaceutical formulations

Whilst it is possible for a compound of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the compound of the invention and not deleterious to

the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen-free.

The following examples illustrate pharmaceutical formulations according to the invention in which the active ingredient is a compound of the invention.

Example 3A: Tablet

Active ingredient	100 mg
Lactose	200 mg
Starch	50 mg
Polyvinylpyrrolidone	5 mg
Magnesium stearate	4 mg
	359 mg

Tablets are prepared from the foregoing ingredients by wet granulation followed by compression.

Example 3B: Ophthalmic Solution

Active ingredient	0.5 g
Sodium chloride, analytical grade	0.9 g
Thiomersal	0.001 g
Purified water to	100 ml
pH adjusted to	7.5

Example 3C: Tablet Formulations

The following formulations A and B are prepared by wet granulation of the ingredients with a solution of povidone, followed by addition of magnesium stearate and compression.

Formulation A

	mg/tablet	mg/tablet
(a) Active ingredient	250	250
(b) Lactose B.P.	210	26
(c) Povidone B.P.	15	9
(d) Sodium Starch Glycolate	20	12
(e) Magnesium Stearate	5	3
	500	300

Formulation B

	mg/tablet	mg/tablet
(a) Active ingredient	250	250
(b) Lactose	150	-
(c) Avicel PH 101®	60	26
(d) Povidone B.P.	15	9
(e) Sodium Starch Glycolate	20	12
(f) Magnesium Stearate	5	3
	500	300

Formulation C

	mg/tablet
Active ingredient	100
Lactose	200
Starch	50
Povidone	5
Magnesium stearate	4
	359

The following formulations, D and E, are prepared by direct compression of the admixed ingredients. The lactose used in formulation E is of the direction compression type.

Formulation D

	mg/capsule
Active Ingredient	250
Pregelatinised Starch NF15	150
	400

Formulation E

	mg/capsule
Active Ingredient	250
Lactose	150
Avicel ®	100
	500

Formulation F (Controlled Release Formulation)

The formulation is prepared by wet granulation of the ingredients (below) with a solution of povidone followed by the addition of magnesium stearate and compression.

	mg/tablet
Active Ingredient	500
Hydroxypropylmethylcellulose (Methocel K4M Premium)®	112
Lactose B.P.	53
Povidone B.P.C.	28
Magnesium Stearate	7
	700

Drug release takes place over a period of about 6-8 hours and was complete after 12 hours.

Example 3D: Capsule Formulations

Formulation A

A capsule formulation is prepared by admixing the ingredients of Formulation D in Example C above and filling into a two-part hard gelatin capsule. Formulation B (*infra*) is prepared in a similar manner.

Formulation B

	mg/capsule
Active ingredient	250
Lactose B.P.	143
Sodium Starch Glycolate	25
Magnesium Stearate	2
	420

Formulation C

	mg/capsule
Active ingredient	250
Macrogol 4000 BP	350
	600

Capsules are prepared by melting the Macrogol 4000 BP, dispersing the active ingredient in the melt and filling the melt into a two-part hard gelatin capsule.

Formulation D

	mg/capsule
Active ingredient	250
Lecithin	100
Arachis Oil	100
	450

Capsules are prepared by dispersing the active ingredient in the lecithin and arachis oil and filling the dispersion into soft, elastic gelatin capsules.

Formulation E (Controlled Release Capsule)

The following controlled release capsule formulation is prepared by extruding ingredients a, b, and c using an extruder, followed by spheronisation of the extrudate and drying. The dried pellets are then coated with release-controlling membrane (d) and filled into a two-piece, hard gelatin capsule.

	mg/capsule
Active ingredient	250
Microcrystalline Cellulose	125
Lactose BP	125
Ethyl Cellulose	13
	513

Example 3E: Injectable Formulation

Active ingredient	0.200 g
Sterile, pyrogen free phosphate buffer (pH7.0) to	10 ml

The active ingredient is dissolved in most of the phosphate buffer (35-40°C), then made up to volume and filtered through a sterile micropore filter into a sterile 10 ml amber glass vial (type 1) and sealed with sterile closures and overseals.

Example 3F: Intramuscular injection

Active ingredient	0.20 g
Benzyl Alcohol	0.10 g
Glucofurol 75®	1.45 g
Water for Injection q.s. to	3.00 ml

The active ingredient is dissolved in the glycofurol. The benzyl alcohol is then added and dissolved, and water added to 3 ml. The mixture is then filtered through a sterile micropore filter and sealed in sterile 3 ml glass vials (type 1).

Example 3G: Syrup Suspension

Active ingredient	0.2500 g
Sorbitol Solution	1.5000 g
Glycerol	2.0000 g
Dispersible Cellulose	0.0750 g
Sodium Benzoate	0.0050 g
Flavour, Peach 17.42.3169	0.0125 ml
Purified Water q.s. to	5.0000 ml

The sodium benzoate is dissolved in a portion of the purified water and the sorbitol solution added. The active ingredient is added and dispersed. In the glycerol is dispersed the thickener (dispersible cellulose). The two dispersions are mixed and made up to the required volume with the purified water. Further thickening is achieved as required by extra shearing of the suspension.

Example 3H: Suppository

	mg/suppository
Active ingredient (63 μm)*	250
Hard Fat, BP (Witepsol H15 - Dynamit Nobel)	1770
	2020

*The active ingredient is used as a powder wherein at least 90% of the particles are of 63 μm diameter or less.

One fifth of the Witepsol H15 is melted in a steam-jacketed pan at 45°C maximum. The active ingredient is sifted through a 200 μm sieve and added to the molten base with mixing, using a silverson fitted with a cutting head, until a smooth dispersion is achieved. Maintaining the mixture at 45°C, the remaining Witepsol H15 is added to the suspension and stirred to ensure a

homogenous mix. The entire suspension is passed through a 250 μm stainless steel screen and, with continuous stirring, is allowed to cool to 40°C. At a temperature of 38°C to 40°C 2.02 g of the mixture is filled into suitable plastic moulds. The suppositories are allowed to cool to room temperature.

Example 3I: Pessaries

	mg/pessary
Active ingredient	250
Anhydrate Dextrose	380
Potato Starch	363
Magnesium Stearate	7
	1000

The above ingredients are mixed directly and pessaries prepared by direct compression of the resulting mixture.

Example 3J: Creams and ointments

Described in *Remington.*

Example 3K: Microsphere formulations

The compounds of the invention may also be delivered using microsphere formulations, such as those described in Cleland (1997; 2001).

Example 3L: Dry Powder Inhalation

The compounds of the invention may be delivered by inhalation, with the aid of a dry powder inhaler delivering micronised particles in metered quantities as described in Ansel (1999).

Example 3M: Aerosol Inhalation

The compounds of the invention may be delivered by inhalation, with the aid of a suitable inhaler delivering micronised particles in metered quantities employing a non CFC propellant as described in Ansel (1999).

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CLAIMS

1. Use of a cyclopentenone prostaglandin in the manufacture of a medicament for delaying the onset and/or preventing the continuation of labour in a female.

2. Use of a cyclopentenone prostaglandin in the manufacture of a medicament for preventing and/or reducing an inflammatory response in the reproductive system of a female.

3. A use according to Claim 2 wherein the female is pregnant.

4. A use according to Claim 1 or 3 wherein the female is human and the duration of pregnancy is more than approximately 13 weeks.

5. A use according to Claim 4 wherein the duration of pregnancy is approximately between 20 and 32 weeks.

6. A use according to any preceding claim wherein the medicament reduces and/or prevents an inflammatory response in the reproductive system of a female associated with the onset or continuation of labour.

7. A use according to any preceding claim wherein the medicament reduces and/or prevents an inflammatory response in the reproductive system of a female associated with infection by a pathogenic agent.

8. A use according to Claim 7 wherein the pathogenic agent is viral, bacterial or fungal.

9. A use according to Claim 6 wherein the inflammatory response is activated by stretch of the uterus.

10. A use according to any preceding claim wherein the medicament reduces and/or prevents one or more of the following conditions: pre-term labour; pathogenic infection; cervical ripening, uterine contractions.

11. A use according to any preceding claim wherein the medicament reduces and/or prevents fetal or neonatal damage.

12. A use according to Claim 11 wherein the fetal or neonatal damage is brain damage.

13. A use according to Claim 12 wherein the fetal or neonatal damage is one or more of the following conditions: astrogliosis; loss of myelin-producing oligodendrocytes; multifocal necroses resulting in cystic change (periventricular leucomalacia, PVL).

14. A use according to any preceding claim wherein the cyclopentenone prostaglandin is 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and/or prostaglandin A_1 and/or is a prodrug of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and/or prostaglandin A_1.

15. A use according to Claim 14 wherein the prodrug is PGD_2 or PGE_1.

16. A use according to any preceding claim wherein the medicament further comprises a pharmaceutically acceptable excipient, diluent or carrier.

17. A use according to any preceding claim wherein the medicament is in a form adapted for delivery by mouth.

18. A use according to any preceding claim wherein the medicament is in a form adapted for delivery by intravenous injection.

19. A use according to any preceding claim wherein the medicament is in a form adapted for delivery by intra-amniotic injection.

20. A use according to any preceding claim wherein the medicament is in a form which is compatible with the amniotic fluid.

21. A use according to any preceding claim wherein the medicament further comprises an agent for treating a female who has or is at risk of one or more of the following conditions: pre-term labour; pathogenic infection; cervical ripening, uterine contractions.

22. A use according to Claim 21 wherein the agent is a corticosteroid.

23. A use according to Claim 21 or 22 wherein the agent is capable of preventing and/or reducing respiratory distress syndrome in the neonate.

24. A use according to Claim 23 wherein the agent is selected from dexamethasone or betamethasone.

25. A use according to Claim 21 wherein the condition is preterm labour and the agent is capable of delaying delivery.

26. A use according to Claim 21 wherein the condition is uterine contractions and the agent is a tocolytic agent.

27. A use according to Claim 26 wherein the tocolytic agent is selected from oxytocin receptor antagonists, calcium channel blockers, sympathomimetics, nitric oxide donors.

28. A use according to Claim 27 wherein the oxytocin receptor antagonist is Atosiban.

29. A use according to Claim 27 wherein the calcium channel blocker is Nifedipine.

30. A use according to Claim 27 wherein the sympathomimetic is Ritodrine.

31. A use according to Claim 27 wherein the nitric oxide donor is glyceryl trinitrate.

32. A use according to any preceding claim wherein the inflammatory response is mediated by NFκB in uterine cells.

33. A use according to Claim 32 wherein the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB DNA-binding in uterine cells.

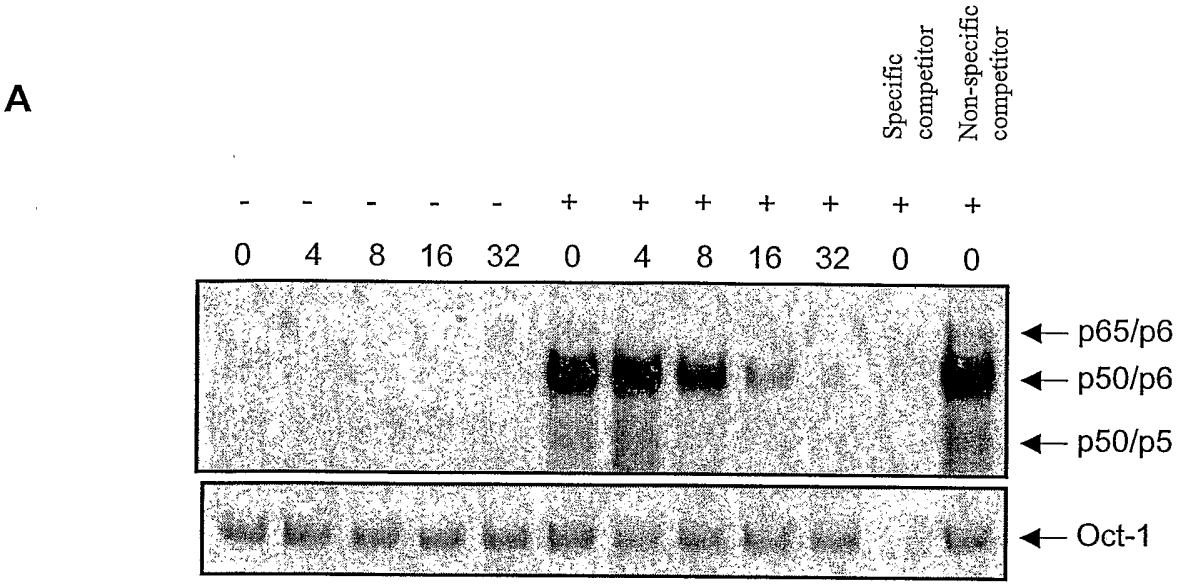
34. A use according to Claim 33 wherein the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB-mediated transcriptional regulation in uterine cells.

35. A use according to Claim 34 wherein the cyclopentenone prostaglandin is capable of inhibiting and/or reducing NFκB activity by preventing and/or reducing NFκB production in uterine cells.

36. A pharmaceutical composition comprising a cyclopentenone prostaglandin and a pharmaceutically acceptable carrier or exipient, the cyclopentenone prostaglandin being present in an amount effective to prevent and/or reduce an inflammatory response in the reproductive system of a female.

37. A method of treating inflammation within the reproductive system of a female, the method comprising administering an effective amount of a medicament as defined in any one of the preceding claims to a subject in need thereof.

FIGURE 1


A
Specific competitor
Non-specific competitor
- - - - - + + + + + + +
0 4 8 16 32 0 4 8 16 32 0 0
p65/p6
p50/p6
p50/p5
Oct-1

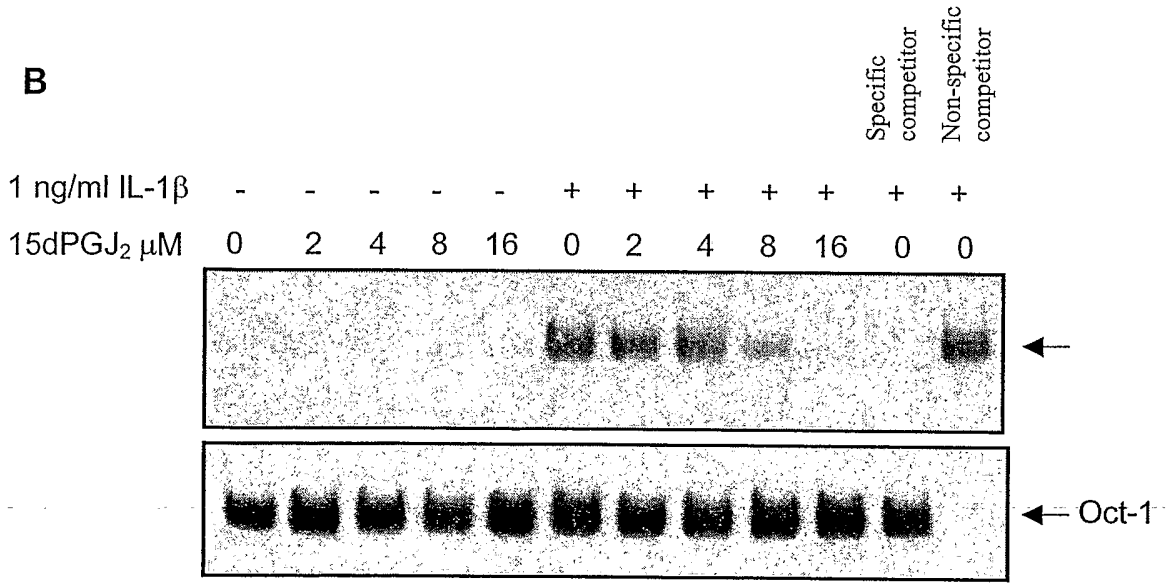

B
Specific competitor
Non-specific competitor
1 ng/ml IL-1β - - - - - + + + + + + +
15dPGJ2 μM 0 2 4 8 16 0 2 4 8 16 0 0
Oct-1

FIGURE 1 - *continued*

C

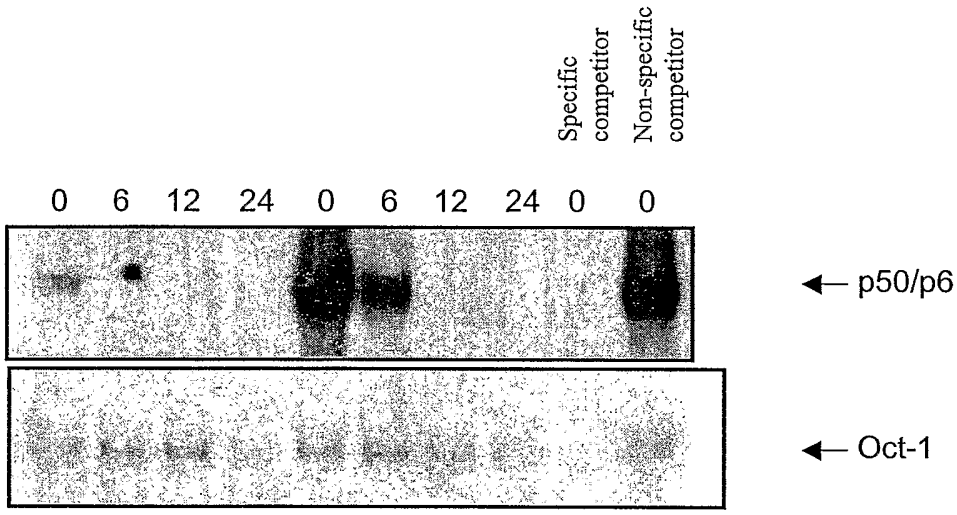


FIGURE 2

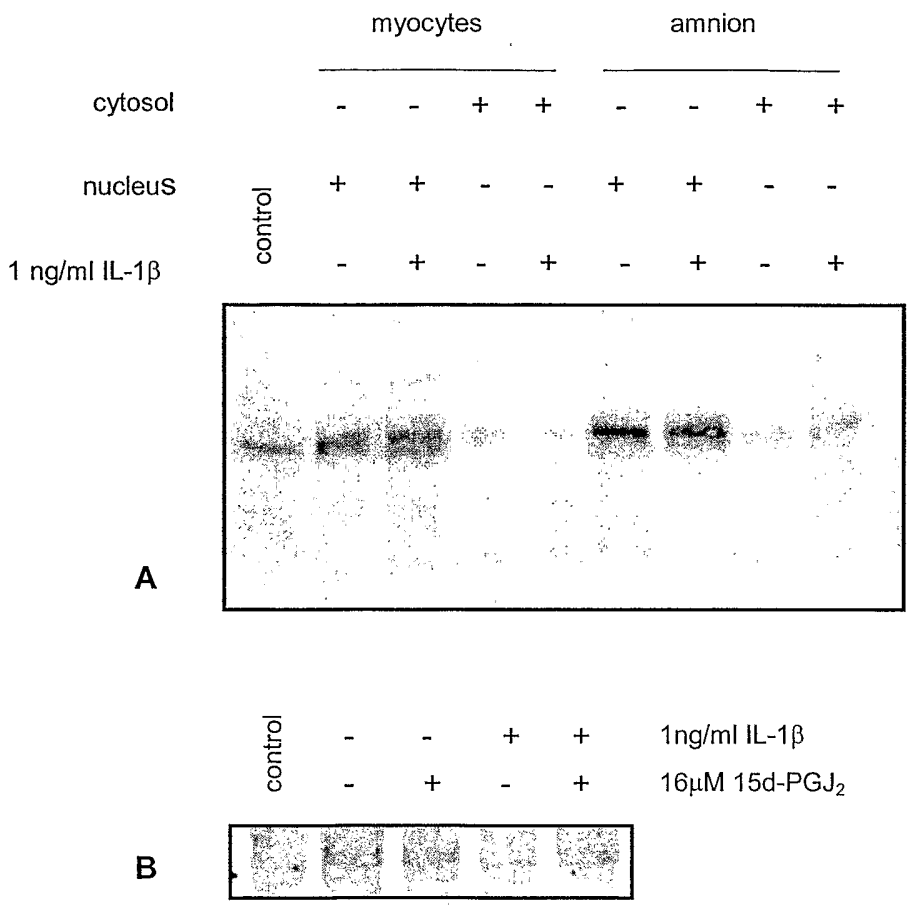

myocytes
amnion
cytosol
nucleus
1 ng/ml IL-1β
control
- - + + - - + +
+ + - - + + - -
- + - + - + - +
A
control
- - + + 1ng/ml IL-1β
- + - + 16μM 15d-PGJ2
B

FIGURE 3

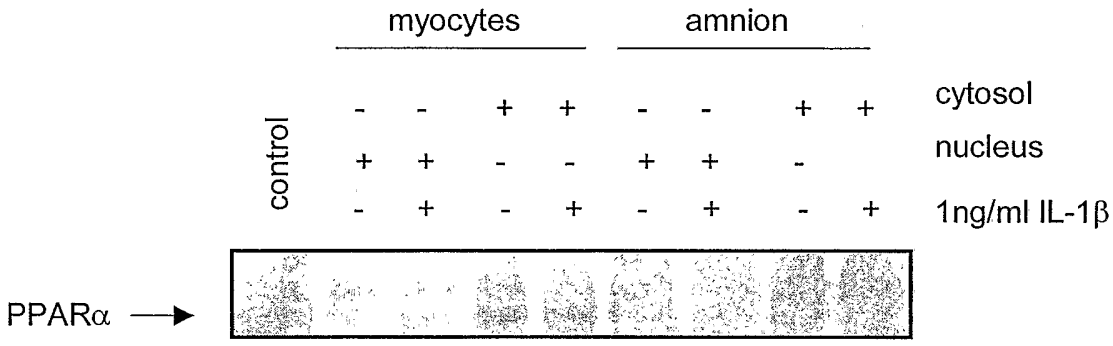
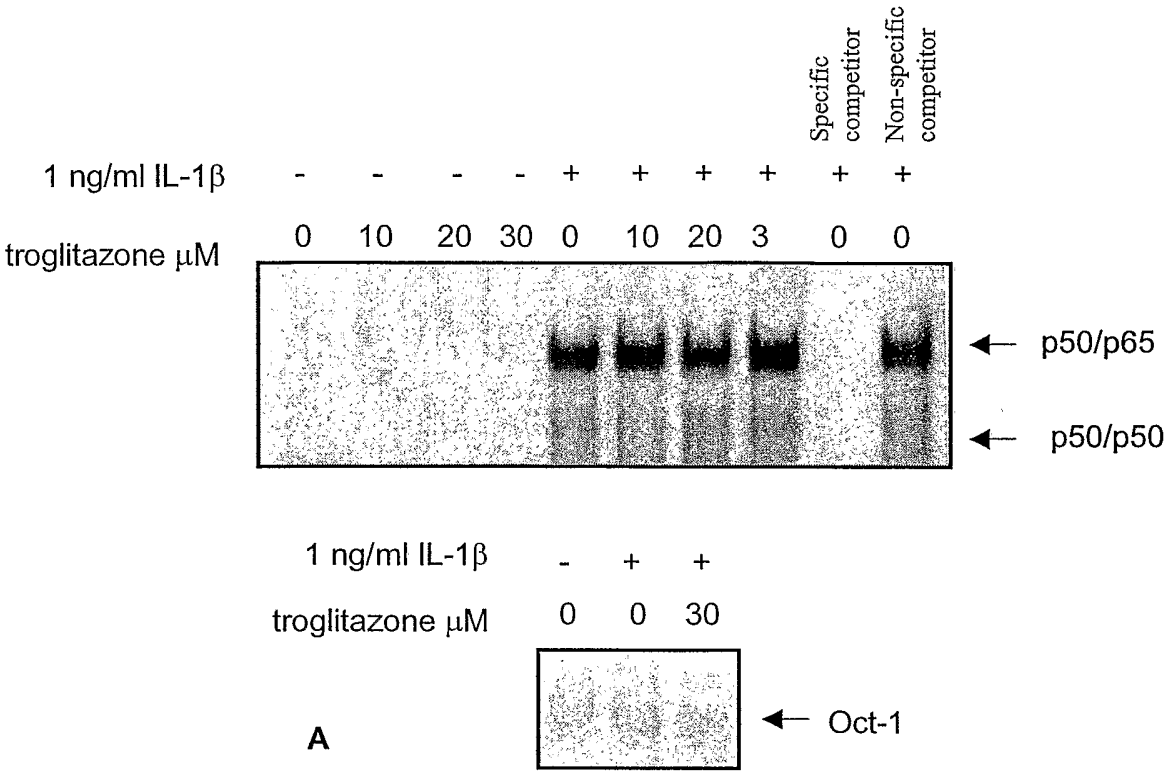


FIGURE 4


Specific competitor
Non-specific competitor
1 ng/ml IL-1β - - - - + + + + + +
troglitazone μM 0 10 20 30 0 10 20 3 0 0
p50/p65
p50/p50
1 ng/ml IL-1β - + +
troglitazone μM 0 0 30
Oct-1
A

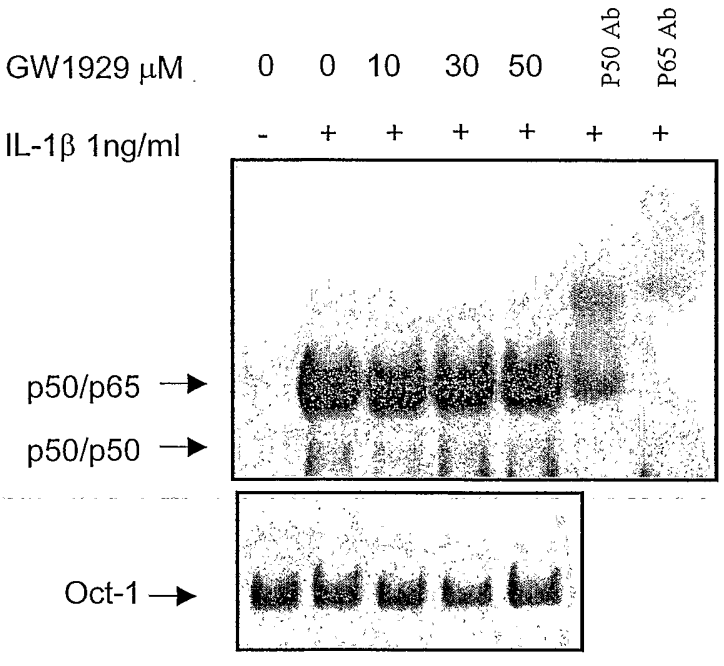
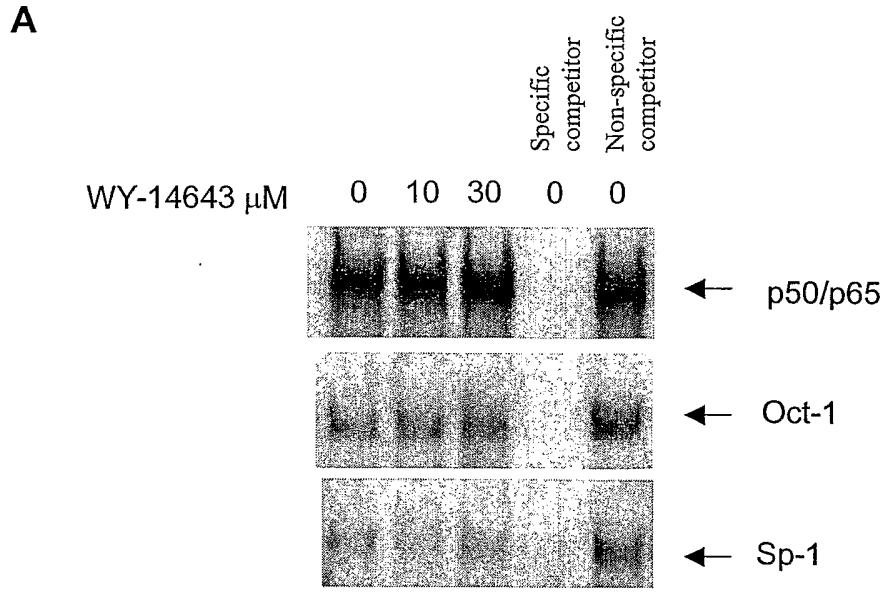

P50 Ab
P65 Ab
GW1929 μM 0 0 10 30 50
IL-1β 1ng/ml - + + + + + +
p50/p65
p50/p50
Oct-1
B

FIGURE 5


A
Specific competitor
Non-specific competitor
WY-14643 μM
0 10 30 0 0
p50/p65
Oct-1
Sp-1

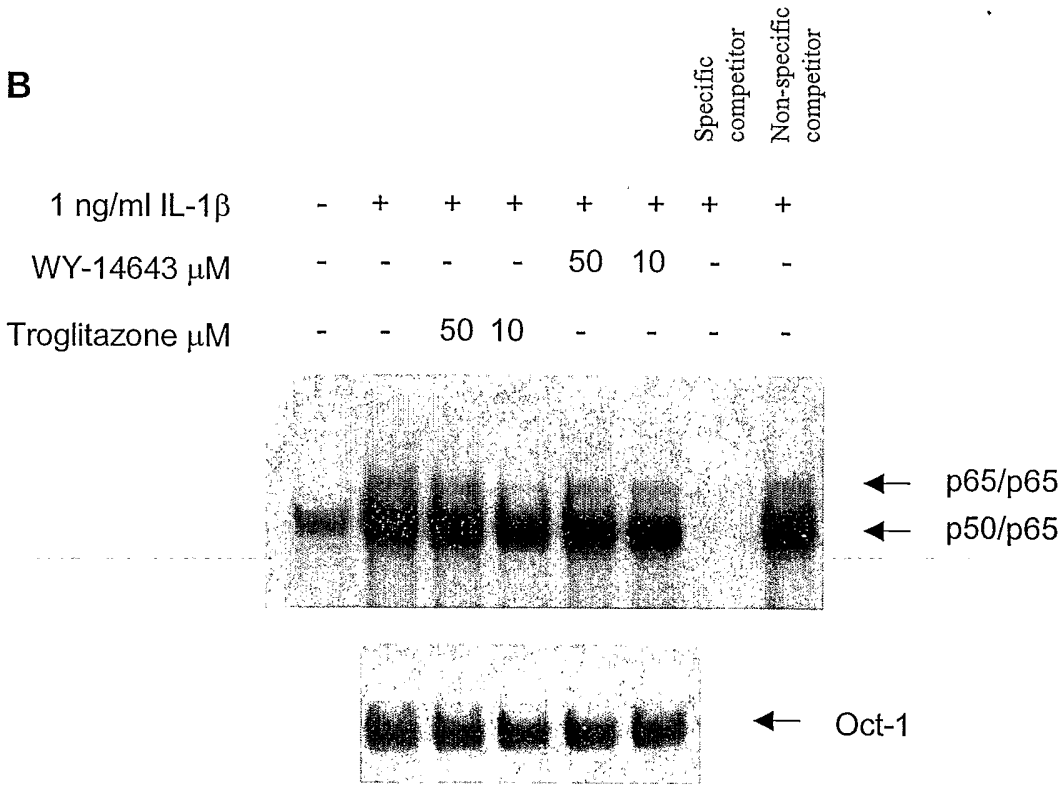

B
Specific competitor
Non-specific competitor
1 ng/ml IL-1β
- + + + + + + +
WY-14643 μM
- - - - 50 10 - -
Troglitazone μM
- - 50 10 - - - -
p65/p65
p50/p65
Oct-1

FIGURE 6

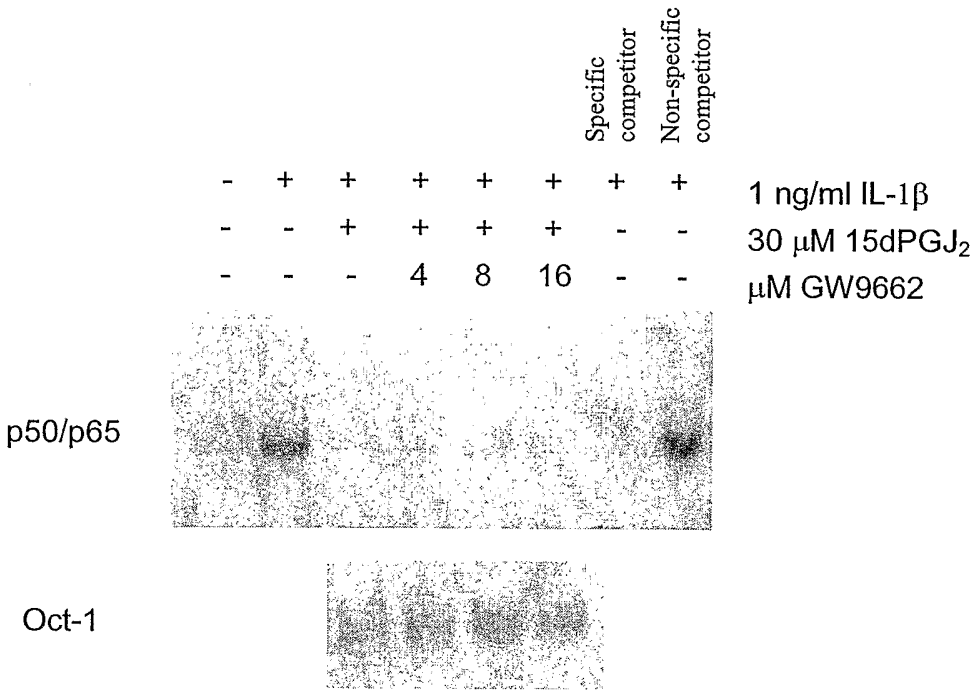
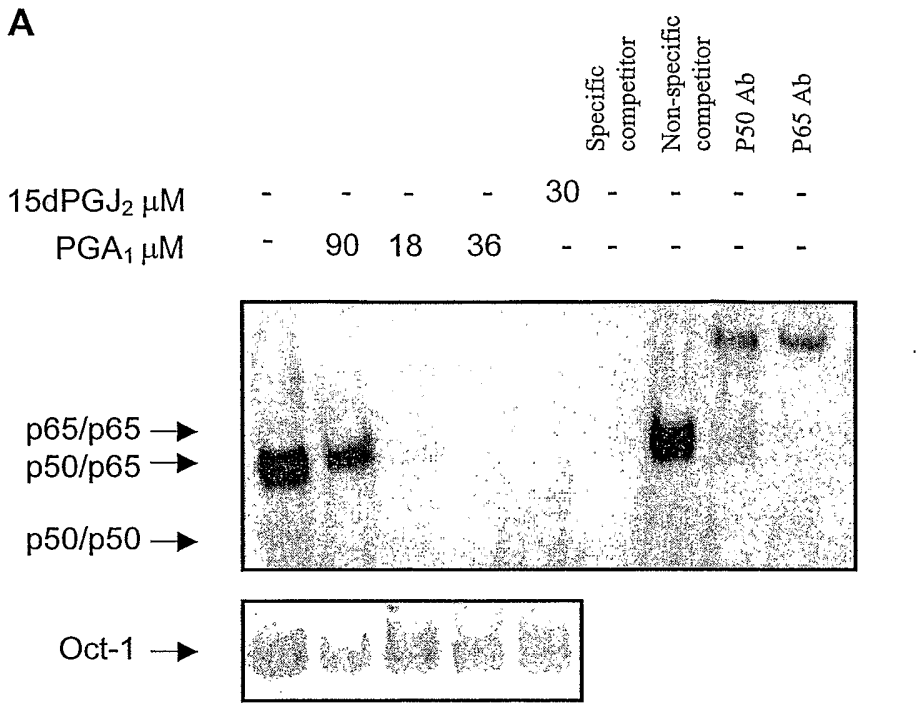

Specific competitor
Non-specific competitor
- + + + + + + +
- - + + + + - -
- - - 4 8 16 - -
1 ng/ml IL-1β
30 μM 15dPGJ2
μM GW9662
p50/p65
Oct-1

FIGURE 7


A
Specific competitor
Non-specific competitor
P50 Ab
P65 Ab
15dPGJ2 μM - - - - 30 - - - -
PGA1 μM - 90 18 36 - - - - -
p65/p65
p50/p65
p50/p50
Oct-1

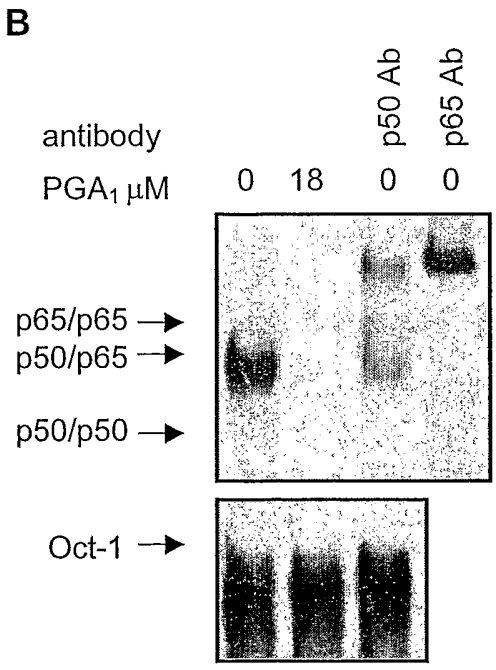

B
antibody
p50 Ab
p65 Ab
PGA1 μM 0 18 0 0
p65/p65
p50/p65
p50/p50
Oct-1

FIGURE 8

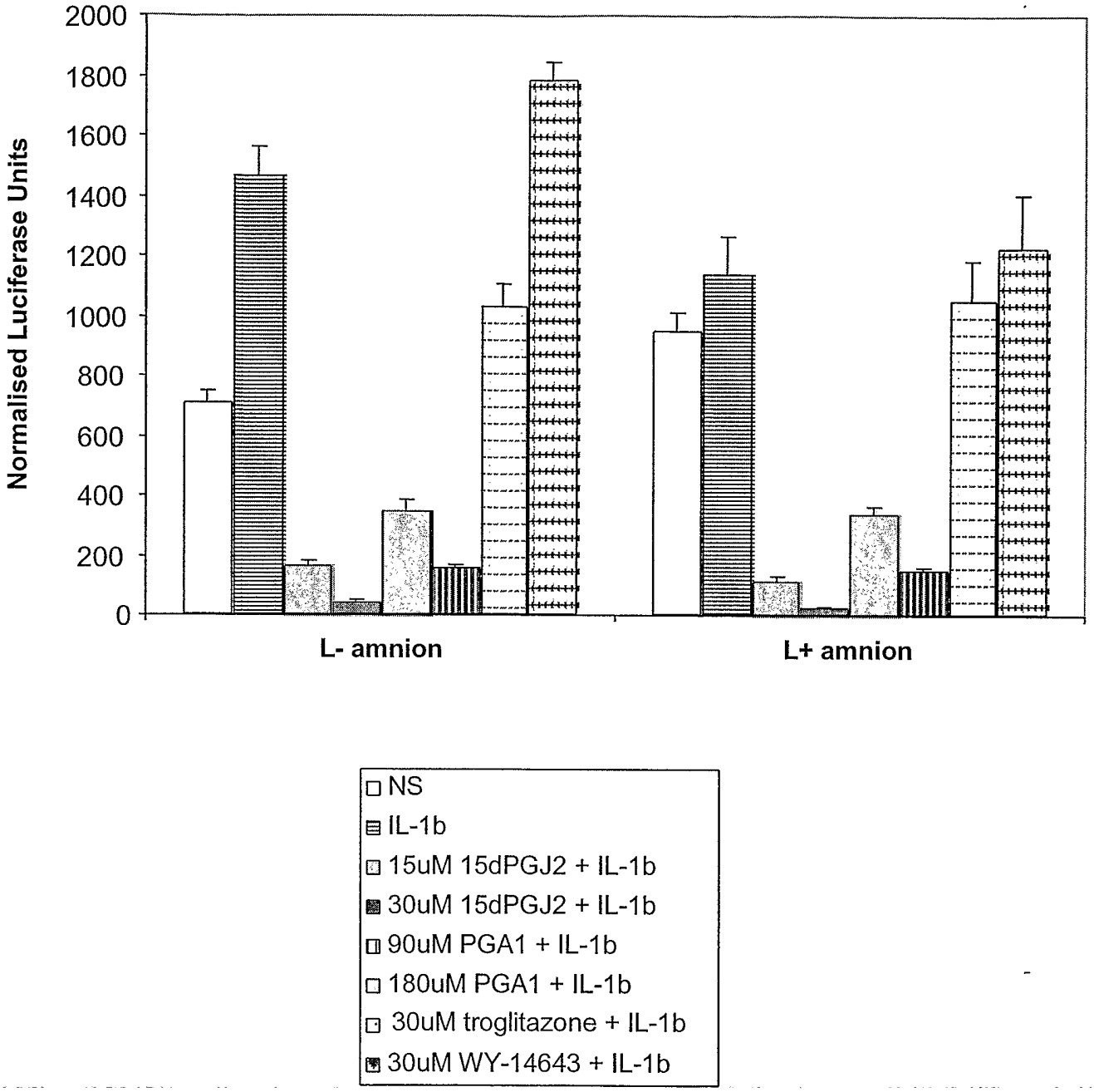

Normalised Luciferase Units
2000
1800
1600
1400
1200
1000
800
600
400
200
0
L- amnion
L+ amnion
NS
IL-1b
15uM 15dPGJ2 + IL-1b
30uM 15dPGJ2 + IL-1b
90uM PGA1 + IL-1b
180uM PGA1 + IL-1b
30uM troglitazone + IL-1b
30uM WY-14643 + IL-1b

FIGURE 9

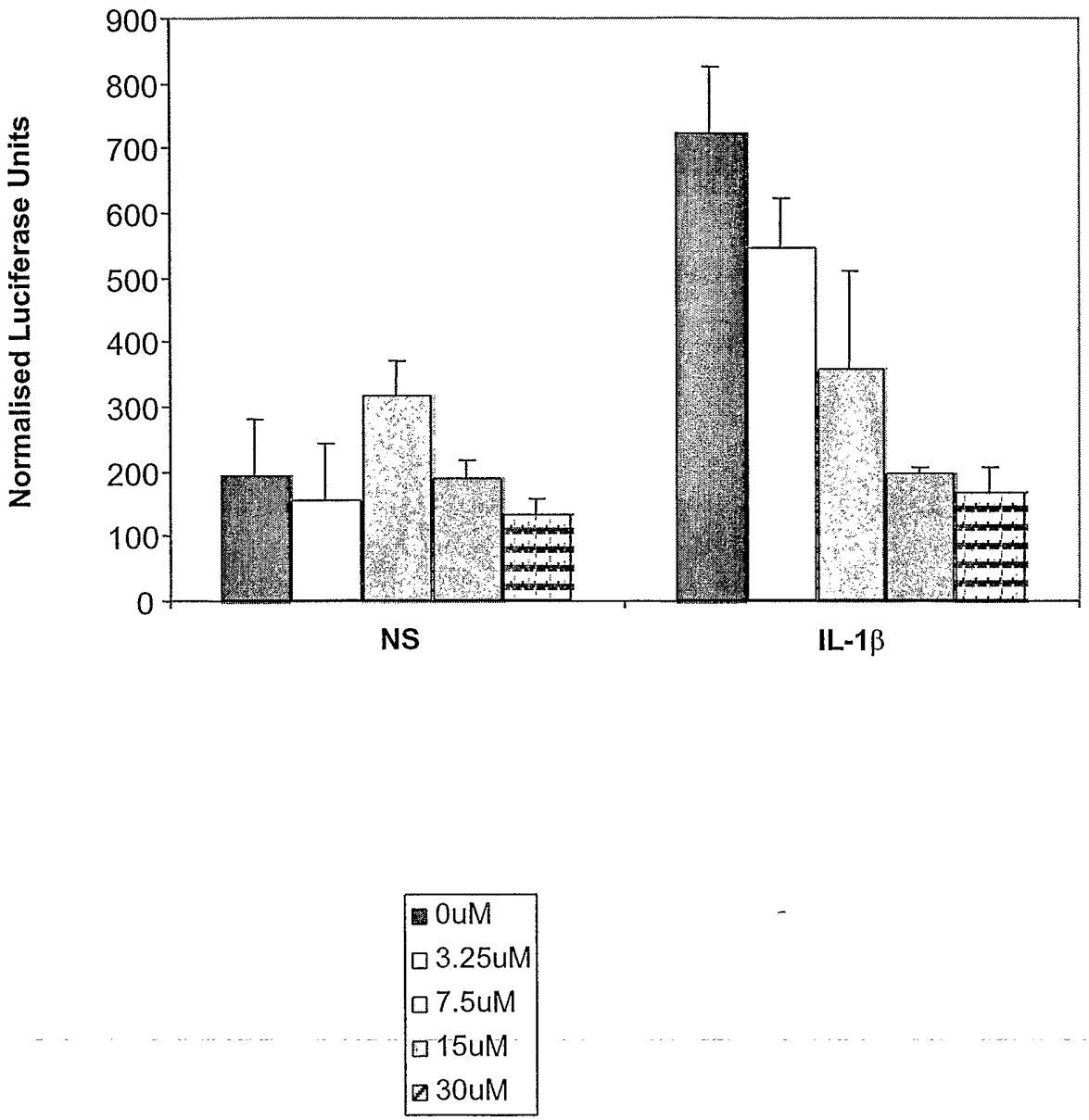

Normalised Luciferase Units
900
800
700
600
500
400
300
200
100
0
NS
IL-1β
0uM
3.25uM
7.5uM
15uM
30uM

FIGURE 10

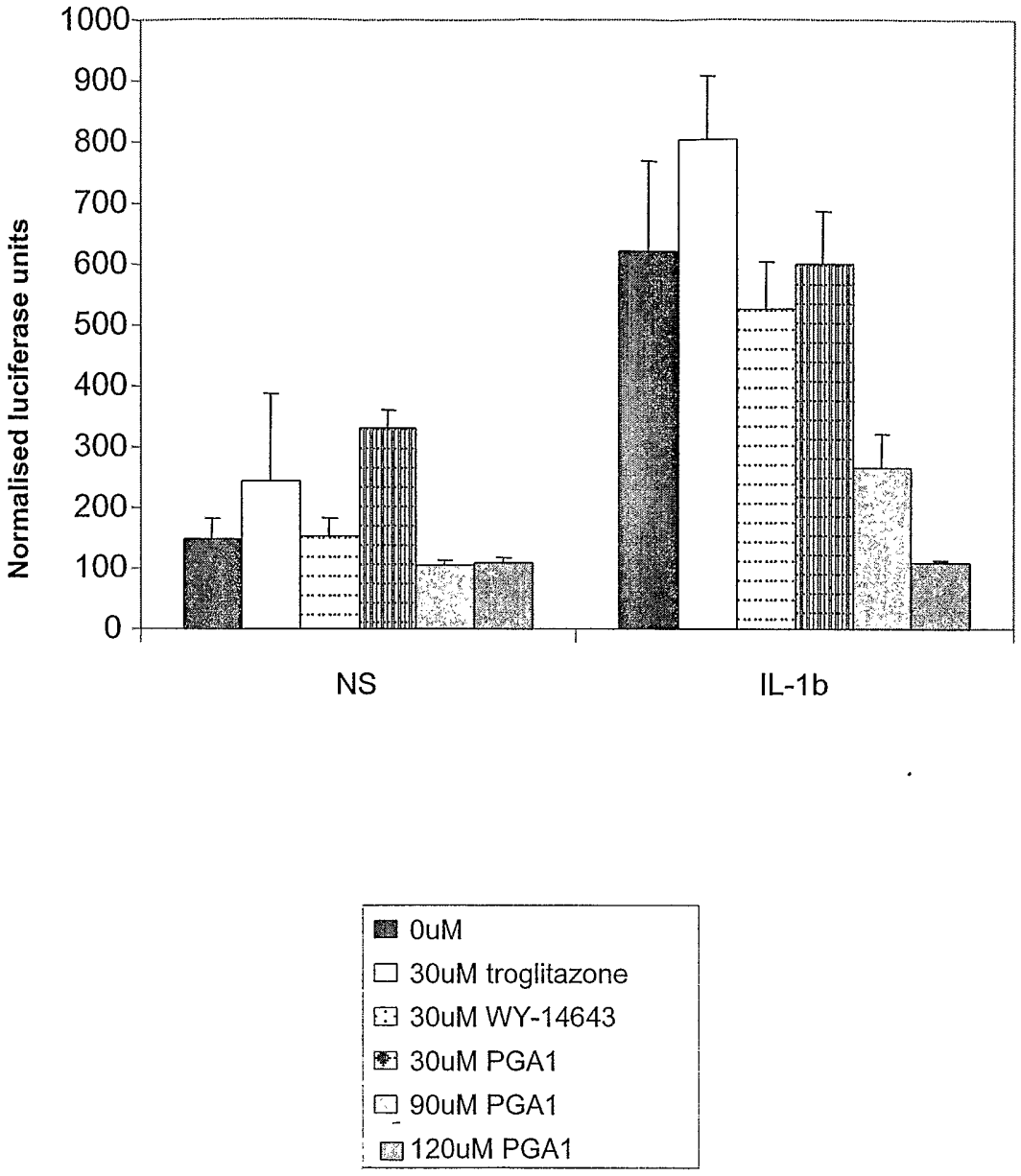

Normalised luciferase units
1000
900
800
700
600
500
400
300
200
100
0
NS
IL-1b
0uM
30uM troglitazone
30uM WY-14643
30uM PGA1
90uM PGA1
120uM PGA1

FIGURE 11

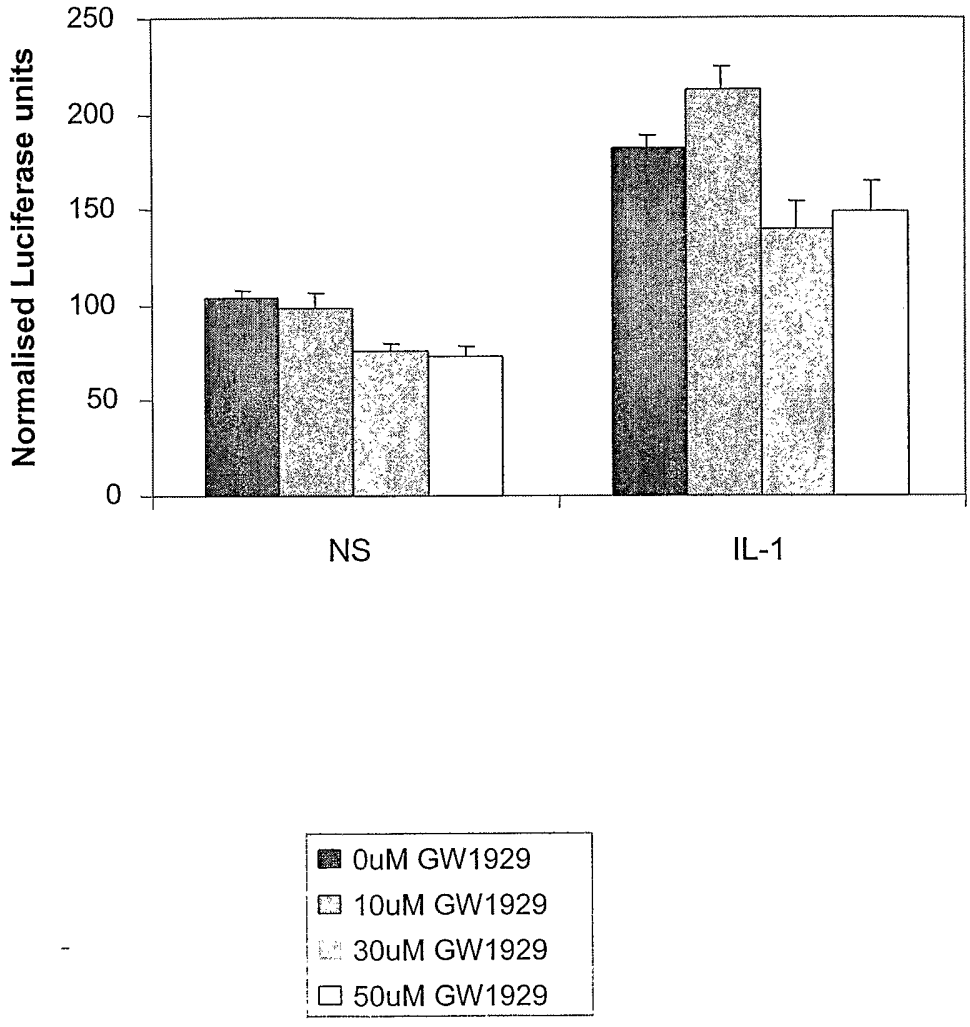
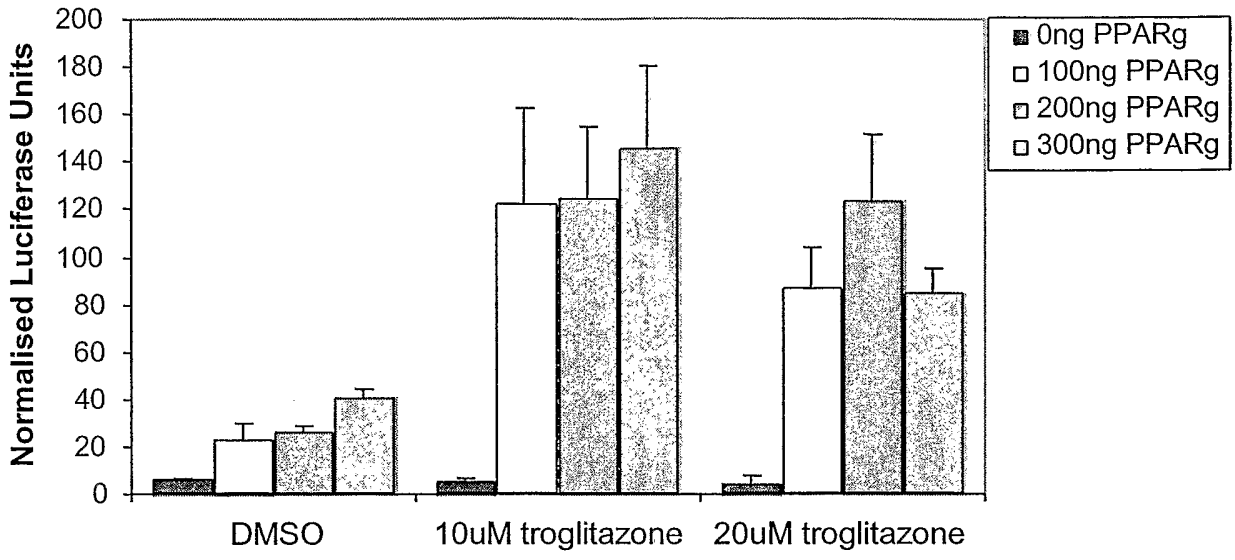
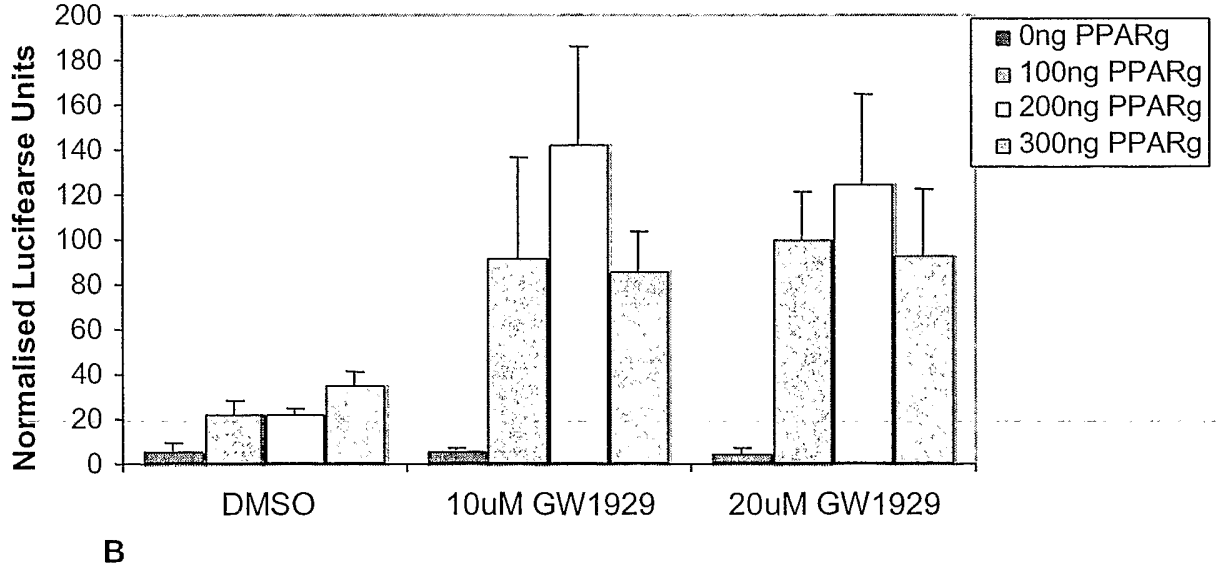

Normalised Luciferase units
250
200
150
100
50
0
NS
IL-1
0uM GW1929
10uM GW1929
30uM GW1929
50uM GW1929

FIGURE 12


Normalised Luciferase Units
200
180
160
140
120
100
80
60
40
20
0
0ng PPARg
100ng PPARg
200ng PPARg
300ng PPARg
DMSO
10uM troglitazone
20uM troglitazone

A


Normalised Lucifearse Units
200
180
160
140
120
100
80
60
40
20
0
0ng PPARg
100ng PPARg
200ng PPARg
300ng PPARg
DMSO
10uM GW1929
20uM GW1929

B

FIGURE 13

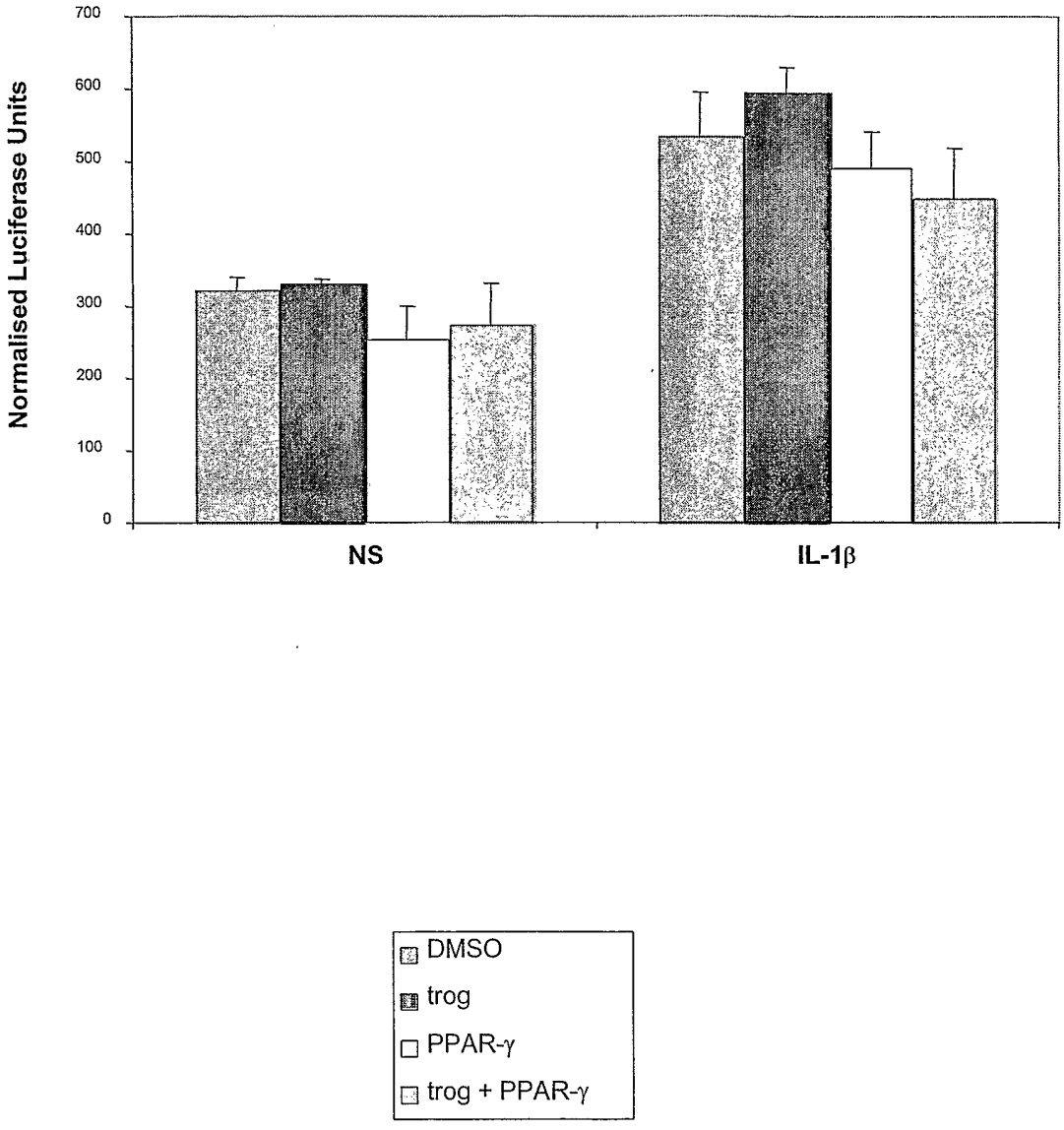

Normalised Luciferase Units
700
600
500
400
300
200
100
0
NS
IL-1β
DMSO
trog
PPAR-γ
trog + PPAR-γ

FIGURE 14

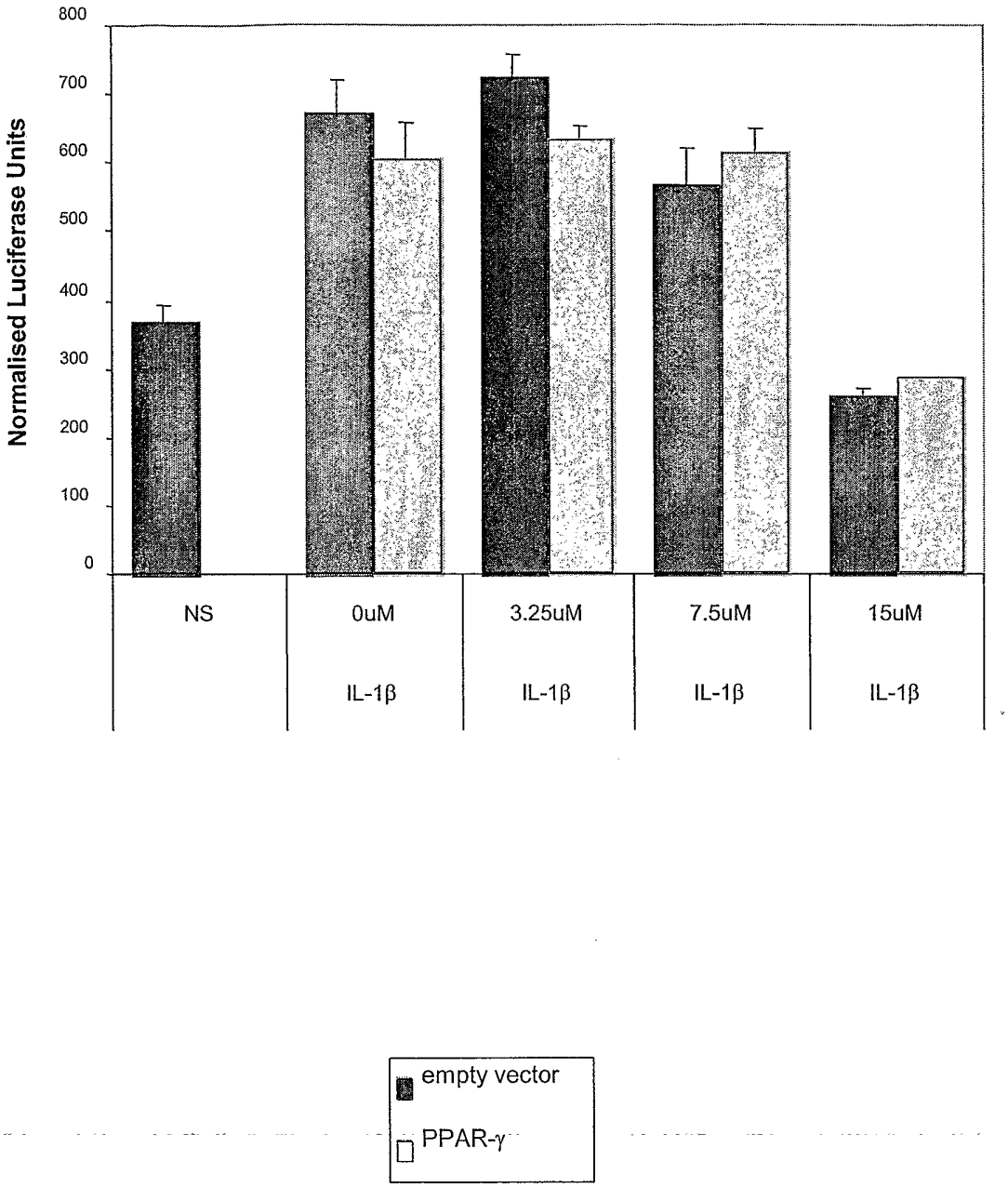
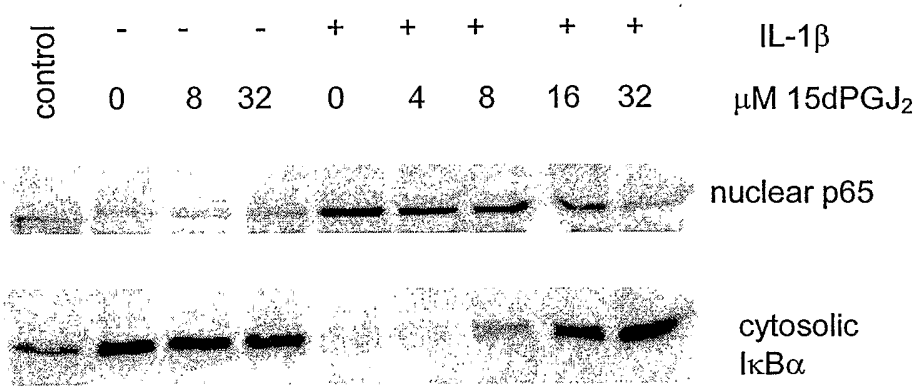

Normalised Luciferase Units
800
700
600
500
400
300
200
100
0
NS
0uM
IL-1β
3.25uM
IL-1β
7.5uM
IL-1β
15uM
IL-1β
empty vector
PPAR-γ

FIGURE 15

A.


control
- - - + + + + +
IL-1β
0 8 32 0 4 8 16 32
μM 15dPGJ2
nuclear p65
cytosolic
IκBα

B.

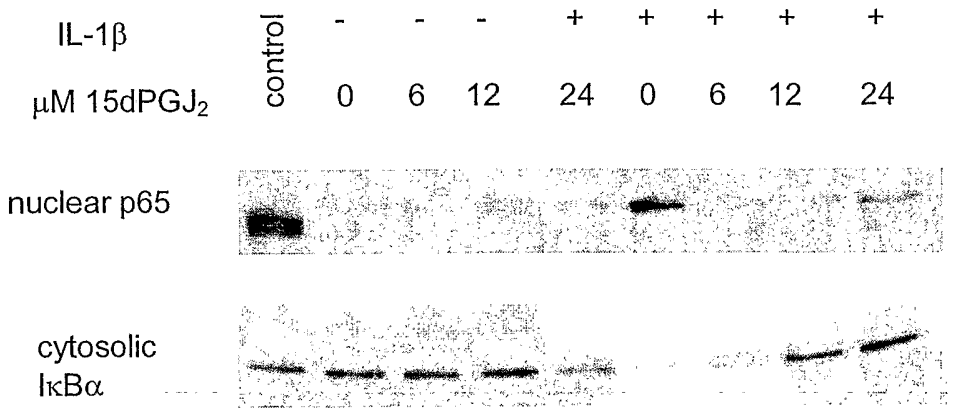
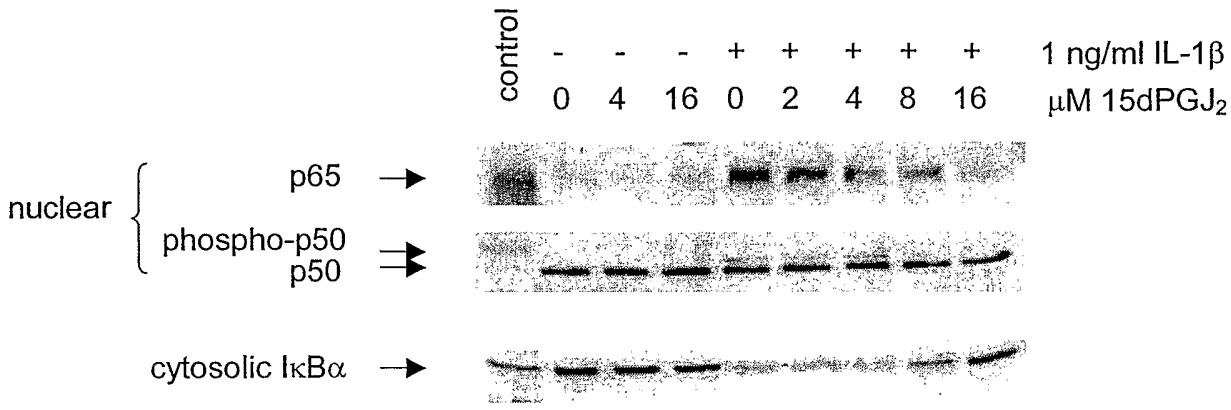

IL-1β
control
- - - + + + + +
μM 15dPGJ2
0 6 12 24 0 6 12 24
nuclear p65
cytosolic
IκBα

FIGURE 15 - *continued*



C.

FIGURE 16

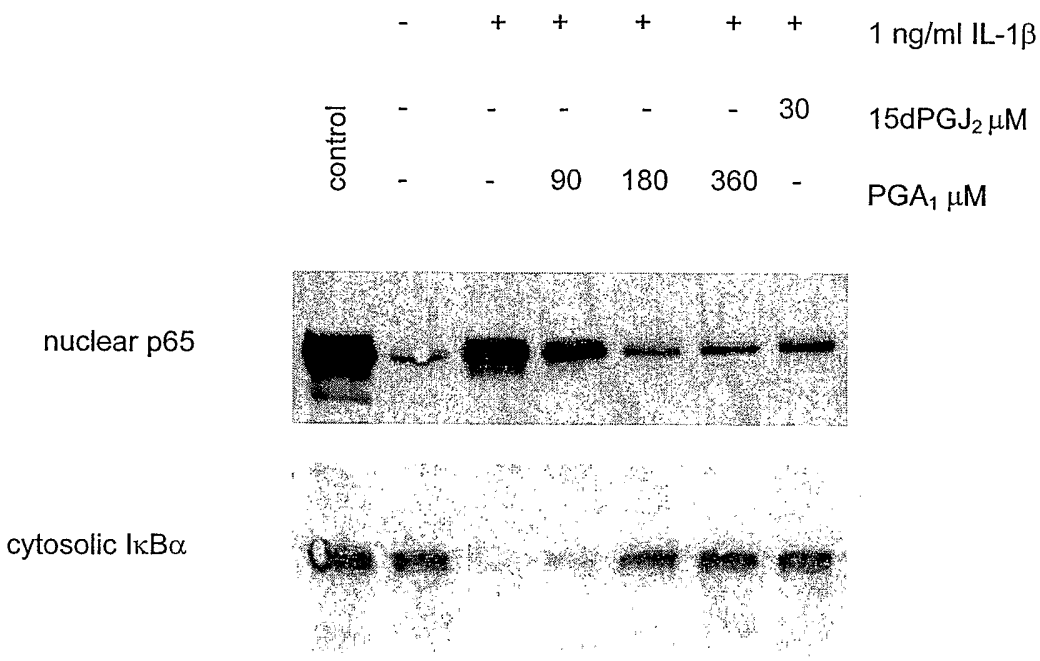
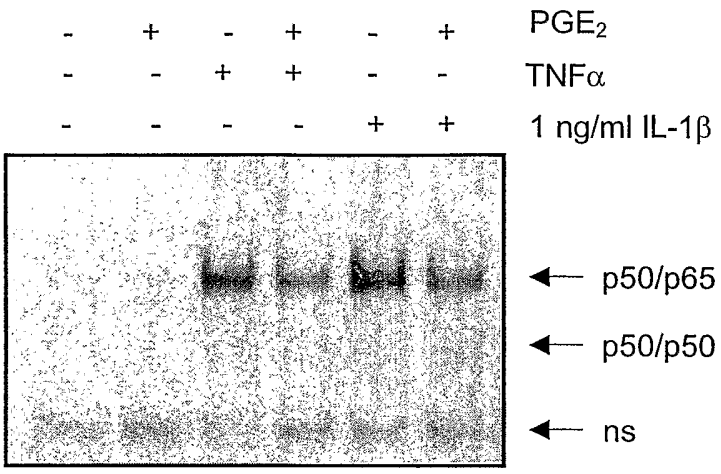
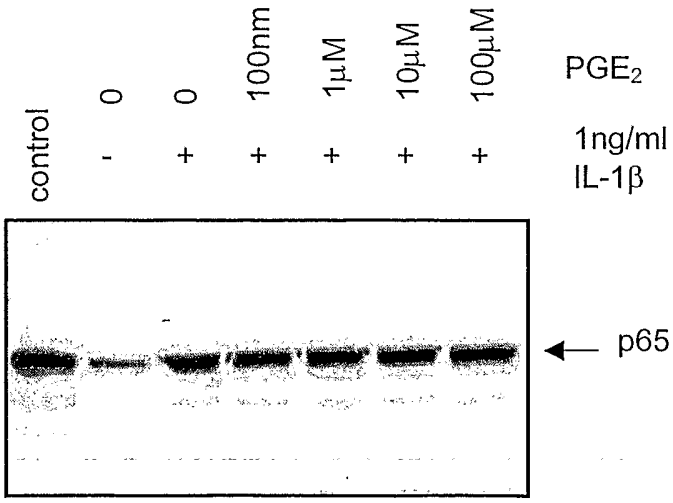

- + + + + + 1 ng/ml IL-1β
control
- - - - - 30 15dPGJ2 μM
- - 90 180 360 - PGA1 μM
nuclear p65
cytosolic IκBα

FIGURE 17


- + - + - + PGE2
- - + + - - TNFα
- - - - + + 1 ng/ml IL-1β
p50/p65
p50/p50
ns

A


control
0
0
100nm
1μM
10μM
100μM
PGE2
- + + + + + 1ng/ml IL-1β
p65

B

FIGURE 18

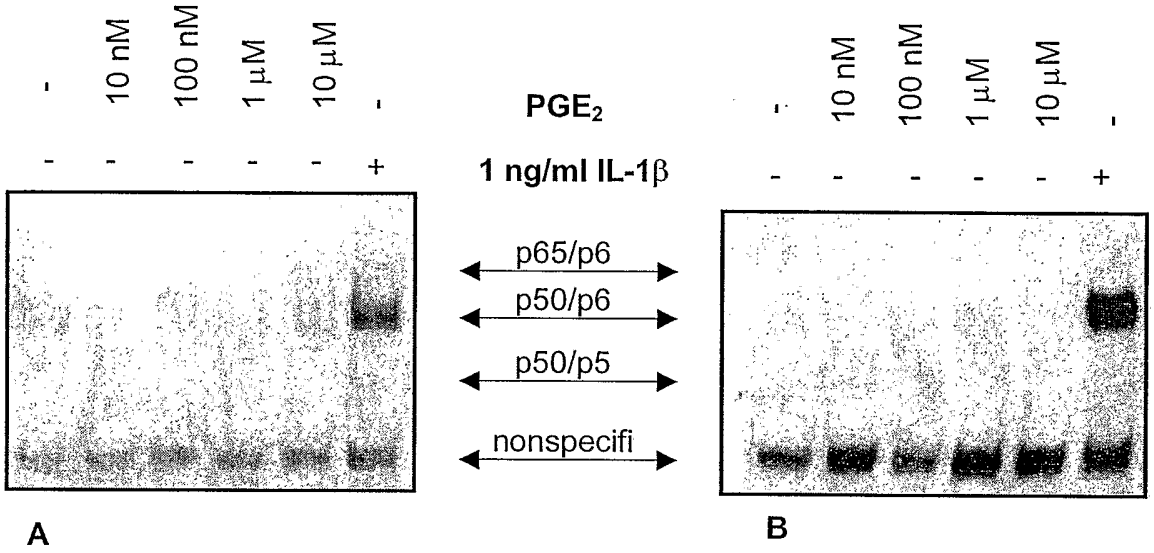
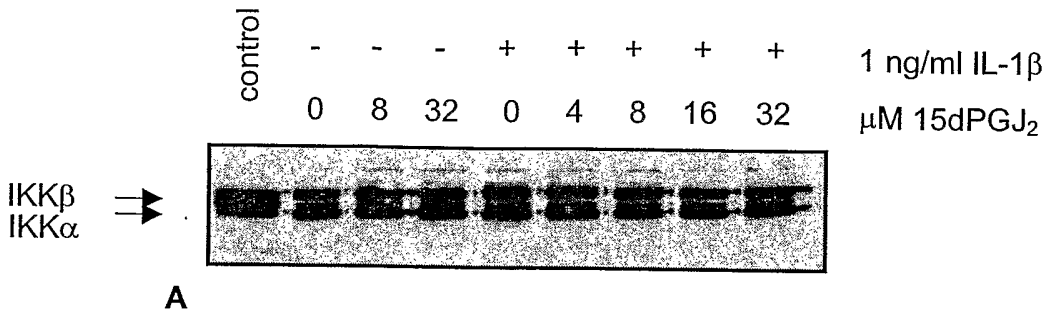

-
10 nM
100 nM
1 μM
10 μM
-
PGE2
-
1 ng/ml IL-1β
+
p65/p6
p50/p6
p50/p5
nonspecifi
A
B

FIGURE 19


control
- - - + + + + +
0 8 32 0 4 8 16 32
1 ng/ml IL-1β
μM 15dPGJ2
IKKβ
IKKα
A

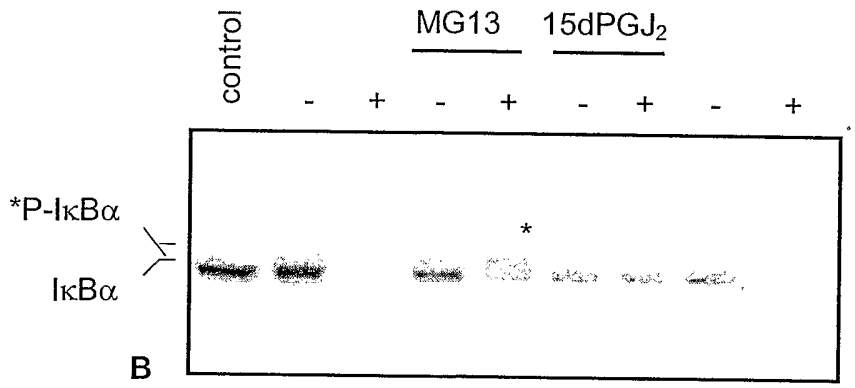

control
MG13
15dPGJ2
- + - + - + - +
*P-IκBα
IκBα
*
B

FIGURE 20

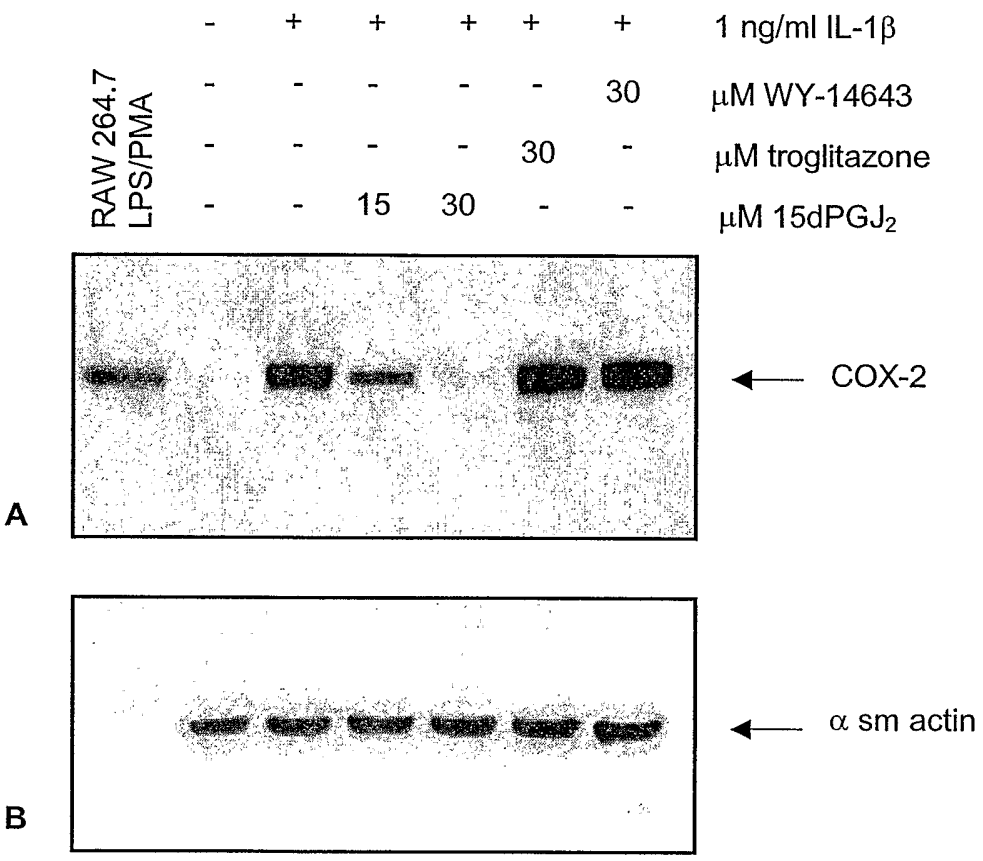
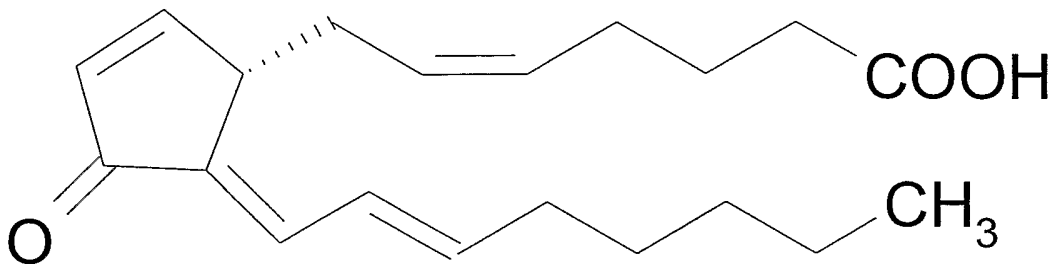

- + + + + + 1 ng/ml IL-1β
RAW 264.7 LPS/PMA
- - - - - 30 μM WY-14643
- - - - 30 - μM troglitazone
- - 15 30 - - μM 15dPGJ2
COX-2
A
α sm actin
B

FIGURE 21

A.



B.

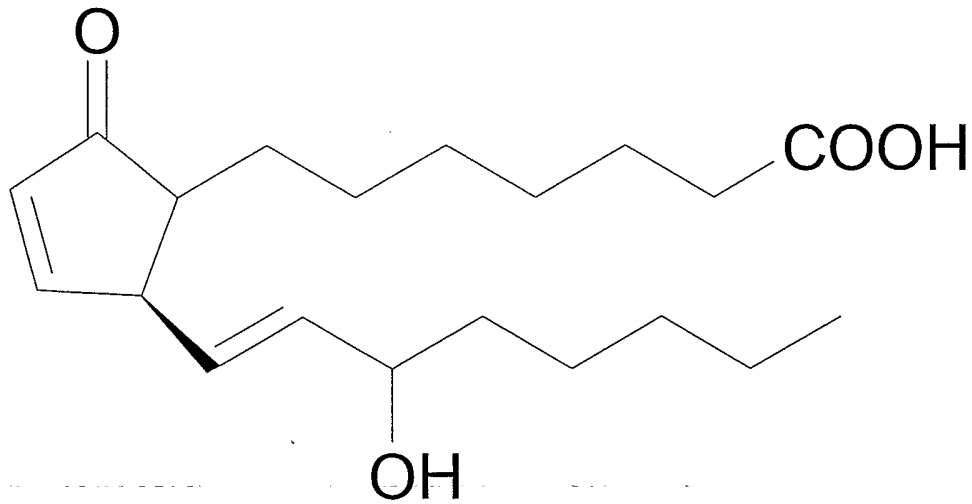


FIGURE 22

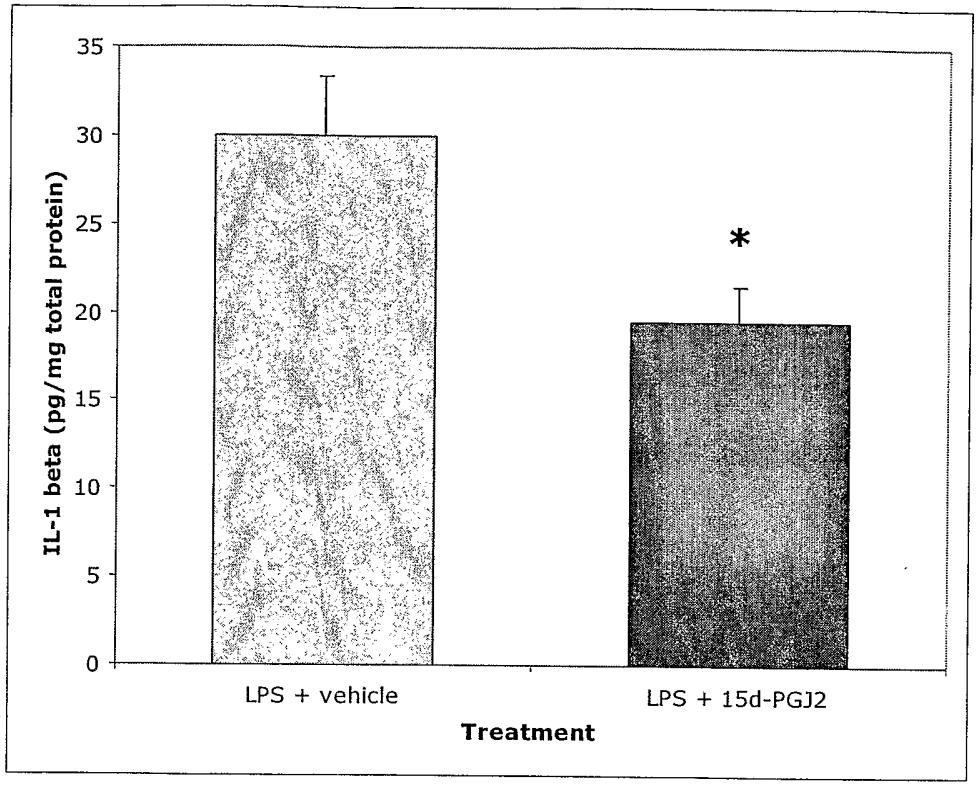
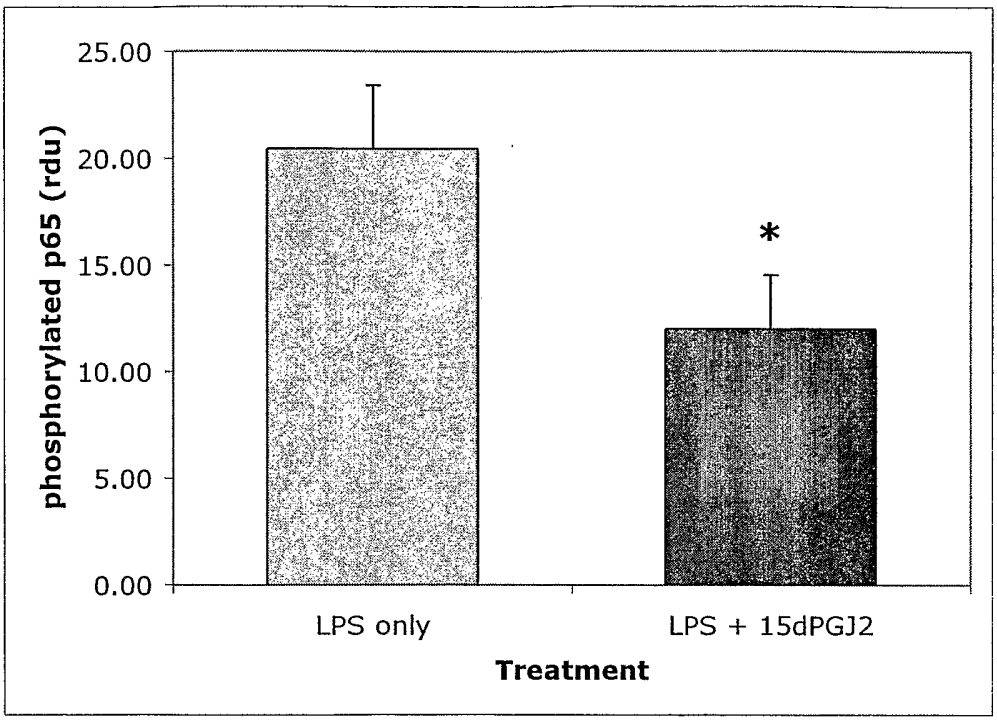

35
30
25
20
15
10
5
0
IL-1 beta (pg/mg total protein)
*
LPS + vehicle
LPS + 15d-PGJ2
Treatment

FIGURE 23


25.00
20.00
15.00
10.00
5.00
0.00
phosphorylated p65 (rdu)
*
LPS only
LPS + 15dPGJ2
Treatment

INTERNATIONAL SEARCH REPORT

PCT/GB2004/001380

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/5575 A61P15/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LAPPAS MARTHA ET AL: "Nuclear factor kappa B regulation of proinflammatory cytokines in human gestational tissues in vitro" BIOLOGY OF REPRODUCTION, vol. 67, no. 2, August 2002 (2002-08), pages 668-673, XP002291976 ISSN: 0006-3363 abstract page 4668, column 1, lines 15-23 page 4670, column 2, line 17 - page 4671, column 1, line 24 page 4672, column 1, lines 20-27 ----- -/--	1-37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 2004	01/09/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Tardi, C

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE EMBASE 'Online! ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL; 2001, STRAUS D S ET AL: "Cyclopentenone prostaglandins: New insights on biological activities and cellular targets" XP002291978 Database accession no. EMB-2001144978 abstract & MEDICINAL RESEARCH REVIEWS 2001 UNITED STATES, vol. 21, no. 3, 2001, pages 185-210, ISSN: 0198-6325	1-37
Y	LAPPAS M ET AL: "Regulation of proinflammatory cytokines in human gestational tissues by peroxisome proliferator-activated receptor-'gamma!: Effect of 15-deoxy-'delta!<12,14>-PGJ2 and troglitazone" JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM 01 OCT 2002 UNITED STATES, vol. 87, no. 10, 1 October 2002 (2002-10-01), pages 4667-4672, XP002291977 ISSN: 0021-972X abstract page 672, column 1, line 25 - column 2, line 33	1-37
A	GROSS G ET AL: "INHIBITION OF CYCLOOXYGENASE-2 PREVENTS INFLAMMATION-MEDIATED PRETERM LABOR IN THE MOUSE" AMERICAN JOURNAL OF PHYSIOLOGY, AMERICAN PHYSIOLOGICAL SOCIETY, BETHESDA, MD, US, vol. 278, no. 6, PART 2, June 2000 (2000-06), pages R1415-R1423, XP008008804 ISSN: 0002-9513 the whole document	
A	GB 2 293 101 A (BRITISH TECH GROUP) 20 March 1996 (1996-03-20) the whole document	
A	WO 99/18942 A (IMP COLLEGE INNOVATIONS LTD ; ALVI SAMIR A (GB)) 22 April 1999 (1999-04-22) the whole document	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/001380

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 37 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB2004/001380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2293101 A	20-03-1996	AU 3394895 A	29-03-1996
		WO 9608254 A1	21-03-1996
		ZA 9507606 A	11-03-1997
WO 9918942 A	22-04-1999	AU 9449398 A	03-05-1999
		CA 2316296 A1	22-04-1999
		EP 1021173 A1	26-07-2000
		WO 9918942 A1	22-04-1999
		JP 2001519381 T	23-10-2001