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# A Mechanistic Perspective on the Specificity and Extent of COX-2 Inhibition in Pregnancy

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## Abstract

Cyclo-oxygenase (COX)-2-specific inhibitors form one of the most commonly prescribed groups of pain relief drugs. Despite the known reproductive toxicity of NSAIDs, which are nonspecific COX inhibitors, little is known about the differential role between COX-1 and COX-2 inhibition on reproduction.

It has been suggested that COX-2 plays a prominent role in animals at all stages of reproduction, from ovulation to implantation to decidualisation and delivery. Both estrogen and progesterone have been shown to be involved in regulation of COX production in tissues of the reproductive tract. Similar to NSAIDs, warnings on reproduction have been included in the product labelling of marketed COX-2-specific inhibitors. Variations in the level of warnings in these labels are noted, with an order of stringency being celecoxib ≈ etoricoxib > rofecoxib ≈ valdecoxib. The specificity of etoricoxib for COX-2 has been found to be approximately 3-fold greater than that of rofecoxib and valdecoxib and approximately 14-fold more than celecoxib in human whole blood assays.

There is growing evidence to suggest that the inducible COX-2, rather than the COX-1, is the main enzyme responsible for reproduction. It was demonstrated that the change in estrogen and progesterone levels during pregnancy contributes to the dramatic increase in COX-2 expression. This further strengthens the earlier findings that COX-2 activities are necessary to support pregnancy.

It is also worth mentioning that although a definite correlation between the specificity of a COX-2-specific inhibitor and the level of precaution stated in the drug labels in UK was not obtained, a direct relationship between the specificity and the potential to result in teratogenicity has not been excluded. With growing interest of the pharmaceutical industry in developing more COX-2-specific inhibitors and the fact that reproductive toxicity is not tested in pregnant women before marketing, it is important for drug regulators to raise awareness of the potential reproductive adverse effects and provide guidance on the level of caution when using these drugs in pregnancy.

Conventional NSAIDs are one of the most commonly prescribed groups of drugs worldwide. They are highly effective as analgesic, antipyretic and anti-inflammatory agents. However, drugs of this class are responsible for substantial morbidity and mortality as a result of the complications associated with gastroduodenal ulcers.

NSAIDs act largely through nonselective inhibition of cyclo-oxygenase (COX), the enzyme required for the formation of prostaglandins (PGs) from arachidonic acid. The COX enzyme-complex

exists in at least two isoforms, COX-1 and COX-2. COX-1 is constitutively expressed in all tissues. It is responsible for physiological activities of PGs, including protection of the gastrointestinal mucosa. The inhibition of COX-1 is postulated to be the cause of the gastroduodenal adverse effects of NSAIDs. COX-2, whose expression is induced during inflammatory conditions, is responsible for pathological PG that produces pain and high temperature. On the basis of these observations, COX-2-selective inhibitory drugs were developed.

A new class of COX-2-specific inhibitors, generally known as the 'coxibs', includes celecoxib, rofecoxib, valdecoxib and etoricoxib, are highly selective to COX-2.[1] Other compounds, such as meloxicam and nimesulide, might also be described as preferential COX-2 inhibitors as they exhibit concentration-dependent COX-2-selective inhibition.[2,3] Nabumetone and etodolac show some evidence of concentration-dependent selectivity for COX-2 and may also fall into this category but are not generally classified as COX-2 preferential inhibitors.[4] While the advantage of these COX-2-selective inhibitory drugs on gastrointestinal tolerability is still being investigated,[5,6] safety concerns regarding the effect of the COX-2 inhibitors on cardiovascular and renal systems have been called into question[7-11] since the publication of Celecoxib Long Term Arthritis Safety Study (CLASS) and Vioxx Gastrointestinal Outcomes Research (VIGOR) studies conducted on celecoxib[12] and rofecoxib.[13] Much attention was then drawn into further investigation into these safety areas. There has been infrequent research on COX-2-specific inhibitors on reproduction even though COX-2 is known to play a physiological role in reproduction[14] and adverse reproductive toxicity has been known to occur in animals treated with NSAIDs and COX-2 inhibitors.[15]

The pathophysiological significance of COX in various reproduction functions has been studied. However, little is known about the differential role between COX-1 and COX-2 on reproduction. A possible link between COX-2 and teratogenicity was postulated when thalidomide, a drug known for its teratogenicity, was shown to have the ability to modulate the expression of COX-2.[16] No epidemiological studies so far have been published that were designed to systematically evaluate the reproductive safety of selective COX-2 inhibitors in humans. The objective of this review is to have a better understanding of the role of COX-2 on reproduction based on published literature and product labelling on marketed drugs in the UK.

A literature search based on relevant keywords, including reproduction, COX, selectivity, prostaglandins, NSAIDs, tetratogenicity, estrogen and progesterone, pregnancy, was conducted in PubMed up to the year 2003. All papers searched were reviewed and relevant information used as references in this article. The drug labels referenced are the 'Summary of Product Characteristics' (SPC), that contain the product information of the marketed drugs. The SPC was either obtained from the public domain of the European Agency for the Evaluation of Medicinal Products (http://www.emea.eu.int), electronic medicine compendium of UK (http://emc.medicines.org.uk) or requested from the relevant pharmaceutical companies.

## 1. Differential Role of Cyclo-Oxygenase (COX)-1 and COX-2 on Reproduction

Similar to NSAIDs, the use of COX-2 inhibitors can induce infertility and risk in pregnancy in normal mice[17-20] and in women.[12] Mice with deletions of the gene for COX-2 (*COX2*-/-) have been shown to have poor ovulation (in terms of numbers of eggs), low fertilisation rates, failure of implantation, and incomplete decidualisation. These reproductive impairments in the absence of COX-2 activity can be reverted by the administration of PGs.[17] In contrast, mice that are *COX1*-/- are fertile and appear normal; their reproduction was not impaired although an impeded onset of parturition was noted.[21] The use of knock-out mice in these studies has strongly suggested that COX-2 plays a more important role than COX-1 in reproduction. The relative importance of COX-2 to COX-1 has been related to the inducible nature of the COX-2 isoform which correlates well with many of the inducible events that occur during early pregnancy, including ovulation, fertilisation, implantation, decidualisation, remodelling of ductus arteriosus and the production of PGs.

## 2. Differential Expression of COX-1 and COX-2 in Tissues of Reproductive Tract with Gestation Advancement

Reviews of published studies have shown differences between humans and animals in the sites and timing of COX expression in tissues of the reproductive tract. Increases in both COX-1 and COX-2 with the progression of gestation were observed in rat uteri[22,23] and cervices[22] using Western blotting or immunohistochemistry. With the use of ribonuclease protection assays, an increase in COX-2 transcripts was again demonstrated in the uterine tissues of pregnant cows with advancing gestation.[24] In contrast to the results obtained in animals, studies carried out in human fetal membranes[25] and myometrium[26] showed that only COX-2 messenger RNA (mRNA) expression increased significantly with gestation age. COX-1 mRNA expression, which exists at low levels, showed no changes in the level of expression. The common phenomenon that is shared between animals and human is the significant upregulation of COX-2 prior to the onset of labour.

## 3. Regulation of COX-1 and COX-2 Expression by Steroid Hormones

Numerous factors, including steroid hormones, growth factors, cytokines and many others, contribute in the COX-1 and COX-2 regulation process. In females, both estrogen and progesterone are involved, apparently in a tissue- and species-specific manner. During the first trimester of pregnancy, progesterone was shown to decrease the number of COX-2-positive cells with no effect on COX-1-positive cells in cultured human decidual cells;[27] whereas ovarian steroid was shown to have no effect on COX-2 gene expression in the mouse uterus in early pregnancy.[28] In the endometrium and decidua of women, an increase in plasma estrogen levels was associated with an increase in PG production. In the endometrium of prepubertal young cows, pretreatment with progesterone was shown to be necessary for COX-2 transcription. Although estrogen alone had no effect on COX-2, substantial enhancement of COX-2 mRNA expression was observed when used together with progesterone. The ability of progesterone to increase COX-2 transcription was also demonstrated in ovariectomised ewes.[24] In the same experiment, progesterone was found to increase COX-2 transcription several-fold, although it had no effect on COX-1 mRNA transcription. Administration of estradiol, on the other hand, enhanced COX-1 gene expression slightly. Similarly, an association was found with increasing PG production, COX-2 concentration/transcript and estrogen in endometrial cell cultures from uterine tissues of nonpregnant guinea pigs, cows and rats *in vivo*.[17]

## 4. Selectivity of Marketed COX-2 Inhibitors in Human Whole Blood Assay

Reindeau et al.[29] studied the selectivity of etoricoxib, rofecoxib, valdecoxib and celecoxib to COX-2 inhibition using a human whole blood assay. The specificity of etoricoxib was found to be approximately 3-fold greater than that of rofecoxib and valdecoxib and was approximately 14-fold more than celecoxib in the human whole blood assays. The rank of order of potency against COX-2 is etoricoxib > rofecoxib ≈ valdecoxib > celecoxib, with the concentration which produces 50% inhibition for COX-1 to COX-2 ratio being 106, 35, 30 and 7.6, respectively.

## 5. Precautionary Statement in UK Drug Labels for the Use of COX-2 Inhibitors During Pregnancy

For ethical reasons, drugs that are not indicated for use in pregnant women are not tested in pregnant women during clinical trials. It is only based on animal reproductive data that regulators assess whether a drug is safe to be used in human during pregnancy. Animal data submitted to regulatory agencies are under the protection of Proprietary Act and the details are not known to public. The use of a drug during pregnancy has to be under the guidance of drug labelling. The pregnancy section of a drug label is worded according to the extent of reproductive toxicity seen in animal studies. A recent review of the UK drug labels shows valdecoxib and rofecoxib use to be contraindicated in the third trimester of pregnancy. Their use in the first and second trimesters has to be balanced between the potential benefit to the mother and the potential risk

**Table I.** Comparison of warnings regarding pregnancy and breast-feeding in the product labels for celecoxib, rofecoxib, valdecoxib and etoricoxib in the UK[30-33]

| Celecoxib[a] | Rofecoxib[a] | Valdecoxib[b] | Etoricoxib[c] |
|---|---|---|---|
| Contraindicated during breast-feeding | Contraindicated during breast-feeding | Contraindicated during breast-feeding | Contraindicated during breast-feeding |
| Contraindicated in women of childbearing potential unless an effective method of contraception is used | Not recommended for women who are attempting to conceive | Not recommended for women who wish to become pregnant | Not recommended for women who wish to become pregnant |
| Contraindicated in pregnancy | Rofecoxib should not be used during the first and second trimesters of pregnancy unless the potential benefit to the patient outweighs the potential risk to the fetus | Valdecoxib should not be used during the first and second trimesters of pregnancy unless the potential benefit to the patient outweighs the potential risk to the fetus | Contraindicated in all trimesters of pregnancy |
| | Contraindicated in the third trimester of pregnancy | Contraindicated in the third trimester of pregnancy | |

a Indicated for osteoarthritis and rheumatoid arthritis.

b Indicated for osteoarthritis, rheumatoid arthritis and primary dysmenorrhoea.

c Indicated for osteoarthritis, rheumatoid arthritis, pain and acute gouty arthritis.

to the fetus.[30,31] More restricted use in pregnancy was implemented in the other two COX-2-specific inhibitors, etoricoxib and celecoxib. They are contraindicated for use in all stages of pregnancy.[32,33] The order of stringency in their use in pregnancy is considered to be: celecoxib ≈ etoricoxib > rofecoxib ≈ valdecoxib (table I).

## 6. Effect of COX Inhibition on Human Reproduction

Several human case-reports indicate that traditional NSAIDs are implicated in infertility[34] congenital abnormality, low birth weight, preterm birth,[35] and in fetal adverse effects such as ductal constriction and impaired renal function when used in the treatment of preterm labour.[36] Experience with the preferential COX-2 inhibitor nimesulide, when used in preterm labour shows that it is linked with constriction of the ductus arteriosus and oligohydramnios[36] and neonatal chronic kidney failure.[37] In addition, two cohort studies conducted in Denmark[22] and the US[38] independently showed an increased risk of miscarriage when NSAIDs were used in pregnant women.

## 7. Conclusion and Recommendations

It is well accepted that COX is an important regulator for many aspects of the reproductive process from ovulation, fertilisation and pregnancy recognition to labour and parturition. Despite the known adverse effects of NSAIDs on reproduction, the potential of COX-2-specific inhibition to result in reproductive toxicity has not been fully explored. Reviews on published studies showed that COX-2 play a more important role than COX-1 on reproduction, as demonstrated by the significant deleterious reproductive effects in the absence of COX-2 activity. Its significant increase in expression with gestation age also reflects its demands to support pregnancy.

Despite attempts to further understand the regulation of estrogen and progesterone on COX expression in tissues of the reproductive tract at different stages of pregnancy, the conclusiveness of the search is hindered by the tissue- and species-specific manner of the involvement of these hormones in the regulation of COX expression. There is no doubt that the expression of COX is influenced by the change in level of estrogen and progesterone during pregnancy, although the extent of the effect appears to vary at different stages of pregnancy. There are also other factors, such as growth factors and cytokines that contribute in the regulation process of COX, which will affect the overall responsiveness of the target tissue to the hormonal changes. Whether estrogen and progesterone exert a greater effect on COX-2 expression than on COX-1 in tissues of the reproductive tract is yet to be known, but it is

clear that the change in estrogen and progesterone levels during pregnancy contributes to the dramatic increase in COX-2 expression. This further strengthens the earlier findings that COX-2 activities are necessary to support pregnancy.

It is worth mentioning that although a definite correlation between the specificity of a COX-2-specific inhibitor and the level of precaution stated in the drug labels in the UK was not obtained, a direct relationship between the specificity and the potential to result in teratogenicity has not been excluded. The final wording of a drug label is determined by multiple factors. These factors, including the idiosyncratic properties of a drug, the target population that a drug is indicated for, the lack of regulatory experience in dealing with the first member of a new class of drug, and the differences in opinion among regulatory agencies in Europe, will affect the true reflection of teratogenicity potential from the wordings stated in the drug labels.

As mentioned earlier, much of the debate among COX experts was focused on the gastrointestinal, renal and cardiovascular safety of this class of drugs. Unfortunately, unlike the gastrointestinal and cardiovascular events that can be closely monitored in clinical trials, no information on the use of COX-2 inhibitors in pregnant women is available before a drug gets into the market. With growing interest by the pharmaceutical industry in developing more COX-2-specific inhibitors and the fact that risk assessments on reproduction safety rely mostly on animal data before marketing authorisation, it is important for drug regulators to raise awareness of the potential reproductive adverse effects and to provide appropriate guidance on the level of caution when using these drugs in pregnancy.

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