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MINI-REVIEW

Nonsteroidal anti-inflammatory drugs and implications for the cyclooxygenase pathway in embryonic development

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a class of analgesics that inhibit the activity of cyclooxygenase isoenzymes, which drive tissue inflammation pathways. Caution should be exercised when taking these drugs during pregnancy as they increase the risk of developmental defects. Due to the high rates of NSAID use by individuals, possibilities for in utero exposure to NSAIDs are high, and it is vital that we define the potential risks these drugs pose during embryonic development. In this review, we characterize the identified roles of the cyclooxygenase signaling pathway components throughout pregnancy and discuss the effects of cyclooxygenase pathway perturbation on developmental outcomes.

cyclooxygenase; embryo; NSAID; prostaglandin; prostanoid

INTRODUCTION

Current Food and Drug Administration (FDA) guidelines recommend against using nonsteroidal anti-inflammatory drugs (NSAIDs) in the second trimester of pregnancy or later due to contraindications in fetal health. NSAIDs are a class of drugs used for a variety of conditions including relief from pain, fever, and inflammation. A survey of over 20,000 women in the United States of America found that 22.6% of pregnant women report taking NSAIDs during their first trimester of pregnancy (1). Though there is no FDA guidance against NSAID usage during the first trimester, the findings discussed later show that NSAIDs may negatively impact the embryonic processes that occur during the first 12 weeks of pregnancy. These processes include implantation, decidualization, neurulation, neural crest migration and differentiation, cardiogenesis, and nephrogenesis. The potential impacts of developmental exposure are especially concerning as some NSAIDs, including ibuprofen and naproxen, are available over the counter, though most NSAIDs require a prescription. NSAIDs inhibit cyclooxygenase (COX) isoenzymes, thus preventing the synthesis of prostanoids. Though much remains unknown, research suggests that prostanoids play key roles in early development. In this review, we describe the localization and mechanism of the NSAID-inhibited COX pathway factors during embryonic stages. Moreover, we discuss studies investigating the role of the COX pathway and the effects of NSAID exposure on embryonic development.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

NSAIDs are a class of therapeutic medications used to reduce pain, fever, and inflammation. These analgesics

mediate their effects by inhibiting COX isoenzymes. All NSAIDs can cross the placenta, making it important to understand their impact on early development (2). As discussed in subsequent sections, there are two categories of NSAIDs: nonselective NSAIDs and COX-2-specific NSAIDs, also known as Coxibs.

Nonselective NSAIDs

Nonselective NSAIDs include ibuprofen, indomethacin, naproxen, and diclofenac. These NSAIDs work by inhibiting both COX isoenzymes, COX-1 and COX-2. Most NSAIDs compete with arachidonic acid for binding at the active site of COX enzymes (3). The negative charge of carboxylic acids on substrates and NSAIDs binds to the positive charge of Arg-120 in the active site of the COX isoenzymes (4). An exception is aspirin, which covalently modifies COX enzymes by acetylating Ser-530 in COX-1 and Ser-516 in COX-2, thus rendering them permanently inactive (3). NSAIDs are bound by plasma proteins, particularly by albumin (>90%) and their plasma half-life ranges from 0.25 to >70 h depending on the specific drug (5). Orally administered NSAIDs are absorbed primarily by the small intestine in addition to the stomach (5). Most NSAIDs are then metabolized by the liver and excreted in urine or bile (5). Selectivity for COX-1 versus COX-2 varies based on the individual NSAID; we discuss COX-2-specific NSAIDs next.

COX-2-Specific NSAIDs (Coxibs)

COX-2-specific NSAIDs, also known as Coxibs, include celecoxib, rofecoxib, etoricoxib, lumiracoxib, and valdecoxib. Though COX-1 and COX-2 only have ~60% overall identity within a species, the active site identity is higher (~85%),



limiting the ways the two isoenzymes can differ to create selectivity (4). Coxibs take advantage of the extra space in the COX-2 active site to increase the selectivity of these drugs. The active site is 27% larger in COX-2 because a valine residue (Val-523), rather than an isoleucine residue (Ile-523), borders its side pocket (4). Mutation of Val-523 into isoleucine in COX-2 confers resistance to COX-2-specific Coxibs (4), indicating that the extra space in the active site is what grants Coxibs selectivity for COX-2.

CYCLOOXYGENASE PATHWAY COMPONENTS IN EARLY DEVELOPMENT

The COX pathway begins with the substrate arachidonic acid, a polyunsaturated fatty acid found in the membrane of most tissues in the body. The COX isoenzymes convert arachidonic acid into prostaglandin H_2 , and then prostanoid synthases convert prostaglandin H_2 into their corresponding prostanoids. Prostanoids, which include thromboxanes (TXs) and prostaglandins (PGs), are secreted from cells and bind to G protein-coupled receptors. Here, we discuss the COX signaling pathway, from its substrate to the receptors of its metabolites.

Arachidonic Acid

Arachidonic acid is a long-chain polyunsaturated fatty acid biosynthesized from linoleic acid and α -linoleic acid and is found in animal products like meat, eggs, and dairy (6). Arachidonic acid is incorporated into the membranes of all tissues and is freed from membrane phospholipids by the action of phospholipase A_2 (7). Once free from phospholipids, arachidonic acid is metabolized by the cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P-450 monooxygenase pathways (3). Recent studies have identified discrepancies in the concentrations of arachidonic acid in plasma during pregnancy. One study found a significant increase in plasma arachidonic acid concentration from the second to the third trimester of pregnancy (8), while another found an 8% decrease in plasma arachidonic acid from the first to the third trimester of pregnancy (9). Generally, long-chain polyunsaturated fatty acids tend to accumulate in the fetus during the third trimester (7). Studies have yet to show de novo biosynthesis of arachidonic acid in the fetus, but the mother readily supplies arachidonic acid to the fetus because it crosses through the placental barrier (7).

Cyclooxygenases

COX proteins are enzymatic hemeproteins that localize to the membrane of the endoplasmic reticulum lumen (4). There are two COX isoenzymes: COX-1 and COX-2. This family of isoenzymes is responsible for converting arachidonic acid into prostanoids, a class of lipid signaling molecules that includes prostaglandins (PGs) and thromboxanes (TXs). COX isoenzymes oxygenate arachidonic acid to make PGG_2 , then reduce PGG_2 to make PGH_2 , the precursor to all other prostanoids. For this reason, COX isoenzymes are also referred to as prostaglandin G/H synthases (PTGSs). In situ hybridization in zebrafish embryos showed that *cyclooxygenase-1* (*ptgs1*) is expressed ubiquitously during gastrulation, and *cyclooxygenase-2* (*ptgs2a*) is absent during gastrulation

but begins expression in the neuroectoderm at the onset of neurulation (10). At 96 h post-fertilization, in situ hybridization of zebrafish larvae identified expression of *ptgs1* and *ptgs2a* transcripts in the carotid artery and pharyngeal arches, with *ptgs1* also expressed in cranial arteries (11). In humans, analysis of gene and protein expression in the myometrium of the uterus throughout pregnancy showed that *COX-1* expression was not significantly changed, whereas *COX-2* significantly increased at term before labor onset (12).

Prostanoid Synthases

PGH_2 is metabolized by prostanoid synthases to make all other prostanoids. Each prostanoid synthase name corresponds to the prostanoids it produces. Therefore, prostaglandin D synthase (PGDS) makes PGD_2 , prostaglandin E synthase (PGES) makes PGE_2 , prostaglandin F synthase (PGFS) makes $PGF_{2\alpha}$, prostaglandin I synthase (PGIS) makes PGI_2 /prostacyclin, and thromboxane synthase (TXS) makes TXA_2 (Fig. 1) (13). COX-1 preferentially couples with TXS, PGFS, and cytosolic PGES (cPGES), while COX-2 preferentially couples with PGIS and microsomal PGES (mPGES) (Fig. 1) (13). Currently, most research visualizing prostanoid synthases in embryos and adult tissues focuses on PGES. In situ hybridization in zebrafish embryos showed that *PGES* is expressed ubiquitously during gastrulation (10). In situ hybridization and RT-PCR in adult mice showed that *mPGES* is strongly expressed in the implantation site and in decidual cells from day 6 to day 8 of pregnancy (19). Primary culture of amnion cells showed that mPGES colocalized with COX-1 and COX-2 in perinuclear and reticular distributions, whereas cPGES was localized to the cytoplasm (20).

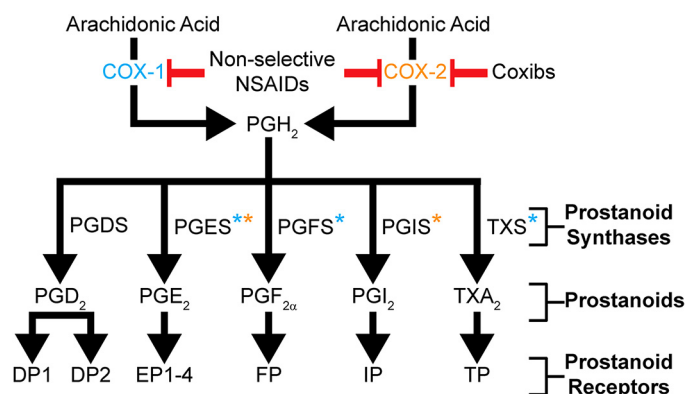


Figure 1. The cyclooxygenase (COX) pathway and roles in early development. COX isoenzymes (COX-1 and COX-2) convert arachidonic acid into PGH_2 , the precursor for all other prostanoids. Nonselective nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX-1 and COX-2, whereas Coxibs selectively inhibit COX-2. Prostanoid synthases convert PGH_2 into their corresponding prostanoids. COX-1 preferentially couples with thromboxane synthase (TXS) to make TXA_2 , prostaglandin F synthase (PGFS) to make $PGF_{2\alpha}$, and cytosolic prostaglandin E synthase (cPGES) to make PGE_2 (13). Meanwhile, COX-2 preferentially couples with prostaglandin I synthase (PGIS) to make PGI_2 and microsomal PGES (mPGES) to make PGE_2 (13). Some of these prostanoids play distinct roles in early developmental processes: PGE_2 in ovulation (14), neural crest differentiation (15), and cardiogenesis (16), PGI_2 in implantation and decidualization (17), and PGD_2 in gonadogenesis (18).

Prostanoids: Prostaglandins and Thromboxanes

Prostaglandins and thromboxanes, collectively called prostanoids, are hormone-like lipids that message through G protein-coupled receptors (GPCRs) (13). Prostaglandins, particularly PGI₂/prostacyclin, are proinflammatory vasodilators, whereas thromboxanes are vasoconstrictors (21). A recently developed technique called nanospray desorption electrospray ionization (nano-DESI) mass spectrometry imaging (MSI) was used for quantitative visualization of prostaglandins in thin tissue sections (22). Use of nano-DESI MSI in conjunction with in situ hybridization of upstream pathway components showed that prostaglandins might not mirror the localization of the COX isoenzymes and synthases that produce them (23). This difference in localization may be attributable to the fact that prostanoids can be secreted from cells, either passively or with the help of transporter proteins, for autocrine and paracrine signaling (24). Each prostanoid has its own G protein-coupled receptor(s) that it activates for downstream signaling transduction within the cell (13) (Fig. 1). More research using nano-DESI MSI to visualize prostaglandins and classical techniques like in situ hybridization and immunohistochemistry to visualize their respective GPCRs is needed to better characterize prostanoid signaling during embryonic development.

CYCLOOXYGENASE PATHWAY SIGNALING IN EARLY DEVELOPMENTAL PROCESSES

More than 20% of pregnant women reported taking NSAIDs during their first trimester of pregnancy (1). Although NSAID use during the first trimester increases the risk of birth defects, much remains unknown about how NSAIDs are implicated in the formation of developmental defects (1). In the following subsections, we discuss studies examining the role of the COX signaling pathway

during vertebrate embryogenesis and propose further avenues for exploration (Fig. 2, Table 1).

Ovulation, Implantation, and Decidualization

Following fertilization, blastocysts hatch from the zona pellucida and penetrate the thickened endometrium of the uterus (48). Epithelial cells then undergo apoptosis and stromal cells proliferate and differentiate into decidual cells at the site of blastocyst entrance. COX-2 is present in the uterine epithelium and stroma at sites of implantation in mink and baboons (49, 50). COX-2 deficiency in mice is linked to impaired ovulation, implantation, and decidualization (34). A possible cause may be that in COX-2 knockout mice, uterine angiogenesis during implantation was reduced due to defective vascular endothelial growth factor (VEGF) signaling (35). However, other studies in mice found that COX-2 was not required for implantation of the embryo (51, 52), suggesting that organism-specific processes or variable genetic backgrounds may lead to differing results. Further work in mice suggests that COX-2 acts through PGE₂ activation of EP₂ to mediate ovulation (14) and through PGI₂ activation of PPAR- δ to mediate implantation and decidualization (17), implicating distinct downstream effectors for each process. Two miRNAs, *mmu-miR-101a* and *mmu-miR-199a*, were expressed simultaneously with COX-2 in the mouse uterus during implantation and they post-transcriptionally regulated COX-2 expression in in vitro experiments (53). Future studies should investigate the role of miRNAs, posttranscriptional modifiers, and posttranslational modifications that may modulate the COX signaling pathway in the processes of ovulation, implantation, and decidualization.

Neurulation: Central Nervous System Formation

The neural tube is an early embryonic structure that ultimately becomes the brain and spinal cord of the central nervous system (CNS). It forms during a process called

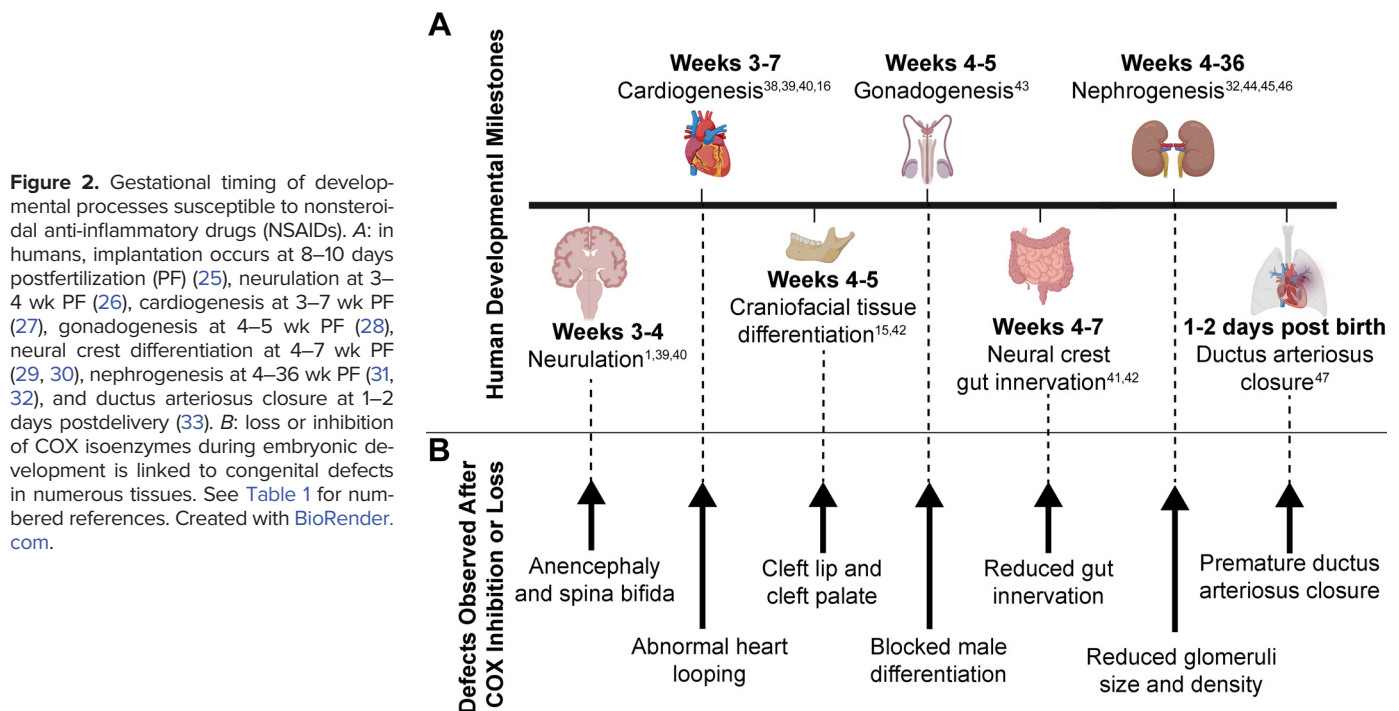


Figure 2. Gestational timing of developmental processes susceptible to nonsteroidal anti-inflammatory drugs (NSAIDs). **A:** in humans, implantation occurs at 8–10 days postfertilization (PF) (25), neurulation at 3–4 wk PF (26), cardiogenesis at 3–7 wk PF (27), gonadogenesis at 4–5 wk PF (28), neural crest differentiation at 4–7 wk PF (29, 30), nephrogenesis at 4–36 wk PF (31, 32), and ductus arteriosus closure at 1–2 days postdelivery (33). **B:** loss or inhibition of COX isoenzymes during embryonic development is linked to congenital defects in numerous tissues. See Table 1 for numbered references. Created with BioRender.com.

Table 1. Developmental processes affected by NSAID exposure and COX knockdown or knockout

Embryonic Process	Adverse Effect	Type of NSAID	References
Ovulation, implantation, and decidualization	Impaired ovulation, implantation, and decidualization	COX-2 knockout	(14, 17, 34, 35)
Embryonic hatching	Delayed hatching	Diclofenac and ibuprofen	(36)
	Delayed hatching	Naproxen	(37)
	Delayed hatching	Diclofenac	(38)
Neurulation	Anencephaly and spina bifida	Ibuprofen, aspirin, naproxen	(1)
	Abnormal brain development	Celecoxib	(39)
	Inhibited pericyte migration and differentiation	Celecoxib	(40)
Cardiogenesis	Increased blood flow	Ibuprofen	(38)
	Abnormal heart looping	Celecoxib	(39)
	Abnormal expression of cardiac genes and heart malformations	Celecoxib	(40)
	Abnormal expression of cardiac genes and heart malformations	Celecoxib	(16)
Neural crest migration and differentiation	Impaired palatal process fusion	Indomethacin	(15)
	Impaired innervation of the bowel	Ibuprofen	(41)
	Reduced migration and lamellipodia formation of enteric cells	Ibuprofen	(41)
	Reduced neural crest migration	Etoricoxib	(42)
Gonadogenesis	Blocked male differentiation	Indomethacin, aspirin	(43)
	Cell death and lowered differentiating glomeruli density	Ibuprofen, aspirin	(44)
Nephrogenesis	Decreased abundance of nephrons	Ibuprofen, indomethacin	(32)
	Reduced nephrogenic zone width	Ibuprofen	(45)
	Thinning of the subcapsular cortical mass, reduced kidney growth, and reduced glomeruli and juxtamedullary glomeruli size	SC-236, SC-560, naproxen, diclofenac, celecoxib, rofecoxib, valdecoxib, etoricoxib, lumiracoxib	(46)
Ductus arteriosus closure	Premature ductus arteriosus closure	Indomethacin, sulindac, celecoxib, nimesulide	(47)

COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs.

neurulation, when the flat neural plate folds and fuses to form a hollow tube. Since the neural tube becomes the CNS, any issues with its closure can lead to brain and spinal cord defects such as anencephaly or spina bifida, respectively. Past work using in vivo pregnant diabetic rats showed that injecting these rats with arachidonic acid during the organogenesis period of their pregnancy significantly reduced the occurrence of defects in neural tube closure and in neural crest-derived tissues (54). Arachidonic acid supplementation also rescued neural tube fusion defects in vitro in hyperglycemic mouse embryo cultures (54). An analysis of data from the National Birth Defects Prevention Study on women who reported taking aspirin, ibuprofen, or naproxen during their first trimester found an increased risk of neural tube defects in NSAID-exposed offspring (1). The adjusted odds ratio showed an increased risk of spina bifida, anencephaly/craniorachischisis, and encephalocele after aspirin and ibuprofen exposure, and less frequently after naproxen exposure (1). Recent work in avians identified that celecoxib exposure during embryogenesis caused dose-dependent defects in brain development (39). Celecoxib exposure also inhibited pericyte migration and differentiation in vitro, which is important for blood vessel formation in the CNS (40, 55). These studies demonstrate that embryonic deficiency of COX-2 or its substrate arachidonic acid leads to defects in CNS formation. However, exposing externally cultured whole mouse embryos to NSAIDs ibuprofen and aspirin did not lead to defects in neural tube closure (56). As these are nonspecific NSAIDs, perhaps COX-2 plays a significant, but not solitary

role in mediating neurulation. Further studies should examine the role of COX-2 in CNS formation.

Cardiogenesis: Cardiovascular System Formation

The cardiovascular system includes the heart, blood vessels, and blood that is circulated throughout the body to deliver oxygen, nutrients, and hormones, among other substances. As the heart develops, it goes through four main stages: tube, loop, chamber formation, and complete septation with coronary circulation (57). At concentrations of 4.3 $\mu\text{g/L}$ and higher in zebrafish embryos, ibuprofen exposure downregulated blood cell density and upregulated blood cell velocity, total blood flow, and the transcription of cardiac-related genes like Natriuretic Peptide A (*Nppa*) and NK2 Homeobox 5 (*Nkx2.5*) (38). Interestingly, exposure to the COX-2-specific NSAID celecoxib showed the opposite trend and downregulated *Nkx2.5* in *Xenopus laevis* (40). However, celecoxib treatment causes consistent morphological defects in cardiovascular development across multiple species, including zebrafish, frog, and chick (16, 39, 40). Celecoxib exposure in zebrafish embryos caused abnormal heart looping, an absence of the heart valve, and atypical expression of heart valve marker genes (16). These defects were rescued by the addition of the COX-2 metabolite PGE_2 , but not TXA_2 , $\text{PGF}_{2\alpha}$, or PGI_2 , thus implicating PGE_2 as a mediator of COX signaling in cardiovascular system development (16). Celecoxib exposure also caused abnormal heart looping in chick embryos (39). In *X. laevis* embryos, celecoxib exposure caused abnormal vasculature

and heart gene expression leading to heart malformations, hemorrhage, and edema (40). Future studies should define the role of the pathway downstream of PGE₂ in cardiovascular system development.

Neural Crest Migration and Differentiation

The neural crest is a population of migratory stem cells that give rise to many derivatives, including the peripheral and enteric nervous systems, craniofacial bone and cartilage, and melanocytes (58). Though in utero NSAID exposure in humans is linked to defects in neural crest-derived tissues such as cleft palate and cleft lip, much remains unknown about how NSAIDs might affect neural crest development (1, 59). Explants of mouse embryo palatal processes exposed to indomethacin developed defects in palatal fusion that were rescued by the addition of PGE₂ (15). In addition, injecting cleft palate-prone rats with arachidonic acid decreased the incidence of cleft palate (54). Neural crest cells also give rise to the enteric nervous system, which innervates the gut (41). In zebrafish embryos, ibuprofen exposure prevented bowel colonization by enteric nervous system precursors (41). Furthermore, ibuprofen reduced migration and lamellipodia formation in vitro in mouse enteric neural crest-derived cells (41). The NSAID etoricoxib also appeared to reduce cranial neural crest migration in chick embryos (42). Although it appears clear that COX signaling is necessary for the formation of neural crest-derived cell types and tissues, additional studies are needed to better understand the mechanistic role of COX-1 and COX-2 in neural crest cell migration and differentiation.

Gonadogenesis: Gonad Formation

At fertilization, mammalian embryos inherit an X or Y chromosome from the father (60). The formation of primary sexual characteristics then begins during gonadogenesis (60). In mammals, the testis-determining protein, Sex-determining region Y (SRY), acts dominantly to drive testis differentiation and prevent ovary maturation (60). Previous work in mice showed that SRY-Box Transcription Factor 9 (SOX9) protein is both necessary and sufficient for testis development, meaning that it is sufficient to rescue male gonadogenesis in the absence of Sry (61). Work in gonadal explants from mouse embryos showed that PGD₂ activates Sox9 transcription to drive differentiation of the male-specific Sertoli cell lineage (18). In fact, exogenous PGD₂ in female gonadal explants increased Sox9 above the level found in male control explants (18). Furthermore, the NSAIDs indomethacin and aspirin were found to block male differentiation in mice but could be reversed by arachidonic acid supplementation (43). Together, these studies demonstrate that the arachidonic acid pathway acts through PGD₂ signals to drive differentiation of male gonads.

Nephrogenesis: Kidney Formation

The formation of the kidney through nephrogenesis starts at *week 4* in human embryos and continues in the fetus through *week 36* (31, 32). During the process of nephrogenesis, metanephric mesenchyme and the ureteric bud form glomerulus-containing nephrons that make up the kidney (31). A recent study in ex vivo human fetal kidneys showed that

COX-1, COX-2, and many downstream prostaglandin synthases and receptors are present during nephrogenesis in the first trimester (44). Exposing human fetal kidney explants aged 7 to 12 developmental weeks to ibuprofen and aspirin increased cell death and lowered differentiating glomeruli density (44). In contrast, a previous study in mouse fetal kidney explants exposed to ibuprofen and indomethacin for 24 h had no effect on the expression of nephrogenesis-promoting genes or ureteric tip development (62). The study in ex vivo human fetal kidneys exposed the explants to NSAIDs for 7 days, first noting cell death after 2 days of exposure (44). Thus, the length and developmental timing of NSAID exposure appear to influence the incidence of nephrogenic defects.

Postnatal NSAID exposure also affects kidney development after birth (32, 45, 46). In newborn mice, the COX-2-specific NSAIDs rofecoxib, etoricoxib, and lumiracoxib induced kidney defects including kidney growth restriction, reduced glomeruli and juxtamedullary glomeruli size, and subcapsular cortical mass thinning (46). Similar changes were caused by exposure to the nonselective NSAIDs diclofenac and naproxen, but interestingly, exposure to celecoxib and valdecoxib caused only minimal changes in renal morphology (46). These results replicate the kidney defects seen in COX-2 knockout mice (34, 46, 52). In prematurely delivered baboon newborns that were exposed to ibuprofen, no differences in kidney weight or glomerular generation number were exhibited, but their nephrogenic zone width was significantly reduced, which may suggest an early termination of nephrogenesis (45). Premature cessation of nephrogenesis could decrease the number of nephrons produced, which was found to be the case in newborn Wistar rats where nephron numbers decreased by 12% in response to ibuprofen treatment (32). Together, these studies suggest that COX-2 plays a key role in kidney formation, both before birth and after delivery in the case of preterm newborns.

Ductus Arteriosus Closure

Before birth, the ductus arteriosus connects the pulmonary artery to the descending aorta (33). Typically, the ductus arteriosus closes 24 to 48 h after delivery, but it often fails to close in preterm newborns (33). Indomethacin and ibuprofen are commonly used in preterm or low birth weight infants for the treatment of patent ductus arteriosus (33, 63–65). However, there is concern that NSAID use before delivery could lead to premature closure of the ductus arteriosus. A meta-analysis of short-term NSAID use during the third trimester of pregnancy found that it significantly increased the risk of premature ductal closure (47). Furthermore, 35% of COX-2-deficient mice die with patent ductus arteriosus within 48 h of birth, whereas COX-1 deficiency in mice had no effect on ductus arteriosus closure (52). Together, these studies suggest that NSAIDs likely drive closure of the ductus arteriosus through COX-2 inhibition, which can cause defects if exposed too early, or could be leveraged for treatment in premature newborns.

Embryonic Hatching in Aquatic Organisms

NSAIDs are an emerging contaminant in the environment and drinking water due to their widespread use and lack of

effective clearance from wastewater (66, 67). This contamination poses a risk not only to organisms living in aquatic ecosystems but also to the humans who consume contaminated water and food from these water sources. NSAID exposure has been studied in the context of embryonic hatching rate in multiple fish species. These studies mainly focus on the ecotoxic and teratogenic effects of NSAIDs as water pollutants in developing fish. In zebrafish embryos, exposure to naproxen, ibuprofen, and diclofenac significantly decreased hatching rate, while celecoxib showed no significant effect on hatching (36–38, 68). Similarly, environmentally relevant concentrations of ibuprofen delayed hatching in Eurasian carp embryos (69). However, these results are not always consistent as another study in zebrafish showed that ibuprofen had no effect on hatching rate (38). Notably, this study used ibuprofen at concentrations between 0 and 22 µg/L, while the study showing hatching delays used concentrations between 0 and 500 µg/L (36, 38). Despite two studies in zebrafish demonstrating delayed hatching after diclofenac exposure (36, 38), diclofenac exposure at similar concentrations showed no effect on hatching rate in brown trout embryos (70). Together, these studies demonstrate that NSAID pollutants may pose a risk to embryonic fish development, however, these risks may be species- and dose-dependent. This work may also have implications in nonfish organisms, though direct comparison between embryonic hatching and live birth is not possible. Nonetheless, delayed hatching indicates a decline in the developmental rate and overall health of the embryo. Further work characterizing the role of COX signaling pathways during early development across species would provide a better understanding of how and why inhibiting those pathways may alter developmental processes.

CONCLUSIONS

Current FDA guidelines recommend against NSAID use starting at 20 wk of pregnancy. However, studies discussed in this review show that NSAID exposure in the first trimester can lead to birth defects in organs and tissues including the brain, heart, kidney, gonads, and enteric nervous system. Continued studies characterizing the mechanistic links between the COX pathway and more established developmental pathways are needed to better understand how NSAID exposure during embryogenesis may cause birth defects. Studies in bone and cancer cells show that COX-2 interacts with common developmental pathways such as the *Wingless/Int* (*Wnt*) (71, 72), bone morphogenetic protein (BMP) (73, 74), and fibroblast growth factor (FGF) pathways (75, 76). Interactions between these well-known developmental pathways and COX signaling pathways should be investigated during early development. New discoveries may not only identify novel developmental pathways but also provide new insight into COX signaling in cancer and regeneration since developmental pathways are often reused in these contexts. In comparing studies on exposure with NSAIDs, care should be taken to note the length of NSAID exposure, the dose and type of NSAID, and the developmental timing of NSAID exposure, as each of these factors could influence whether there is an effect on embryonic development. NSAIDs differ in their affinity for

COX isoenzymes, their mechanism of action, and their recommended dose, which means they act differently on a cellular level in embryos. Defining COX pathway interactions will allow us to construct more accurate gene regulatory networks of key developmental processes during embryogenesis. Moreover, knowing the precise doses and developmental time points at which NSAID exposure can lead to birth defects will allow physicians to better advise patients who are pregnant or plan to become pregnant.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.A.L. and C.D.R. conceived and designed research; prepared figures; drafted manuscript; edited and revised manuscript; approved final version of manuscript.

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