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COMMENTARY

Cyclooxygenase Knockout Mice

MODELS FOR ELUCIDATING ISOFORM-SPECIFIC FUNCTIONS

Robert Langenbach, Charles Loftin, Christopher Lee and Howard Tiano*

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES, RESEARCH TRIANGLE PARK, NC 27709, U.S.A.

ABSTRACT. The development of cyclooxygenase (COX) deficient mice has allowed investigation into the individual physiological roles of the COX-1 and COX-2 isoforms. In the following article, the phenotypes of the two *Ptgs* (genes coding for COX-1 and COX-2) knockouts are summarized, and recent studies to investigate the effects of COX deficiency on cancer susceptibility, inflammatory response, gastric ulceration, and female reproductive processes are discussed. Also, the development and potential uses of mice deficient in both COX isoforms and mice containing only a single copy of one isoform are discussed. Additionally, when the data permit, the effects of genetic ablation of COX activity are compared with those of pharmacological inhibition of COX activity by nonsteroidal anti-inflammatory drugs. The data suggest that prostaglandins derived via the individual COX isoforms have separate as well as common functions. However, for the maintenance of normal physiology, it appears that deficiency of COX-2 has more profound effects than deficiency of COX-1. BIOCHEM PHARMACOL **58**;8:1237–1246, 1999. © 1999 Elsevier Science Inc.

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Two isoforms of cyclooxygenase, COX-1† [1, 2] and COX-2 [3–6], are known. The isoforms are about 60% identical at the protein level, and both enzymes catalyze the first committed step in the synthesis of PGs, i.e. the conversion of AA to PGH_2 [7]. The kinetic constants of both isoforms for this reaction are similar, and the PGH_2 produced by both isoforms then can be metabolized further to the biologically active prostaglandins, PGE_2, PGI_2, $PGF_{2\alpha}$, PGD_2, and thromboxane A_2. Since the discovery of COX-2, elucidating the physiological functions of each COX isoform has been an area of intense study.

Because COX-1 is constitutively expressed in at least some cells of most tissues, it is considered the "housekeeping" isoform [7]. In contrast, COX-2 normally is not detectable in most tissues, but it can be induced by mitogens, cytokines, and certain inflammatory agents and, therefore, has been referred to as the "inducible" isoform [7]. However, this distinction is not entirely accurate, since COX-1 can be induced/up-regulated under certain conditions [8–11], and COX-2 now is known to be expressed constitutively in some cells of tissues such as the brain [12], trachea [13], and kidney [14]. Recent studies have indicated that the two isoforms may have different cellular localizations [15], and that the cytosolic localization of COX-1 may give rise to PGs with autocrine or paracrine activity, whereas the perinuclear localization of COX-2 may lead to PGs with intracrine activity [16]. Indeed, both cell surface membrane and nuclear hormone receptors for PGs have been identified [17–19]. Furthermore, it has been demonstrated in certain cell types that COX-1 and COX-2 utilize AA generated by different phospholipases [20], which further supports the possibility that the isoforms are involved in different signaling pathways.

The observation that COX is the pharmacological target of NSAIDs was reported by Vane [21], and much of our current knowledge about the physiological roles of the COXs, and the PGs they produce, has been obtained from inhibition studies with NSAIDs. With the recent characterization of COX-2, NSAIDs with various degrees of isoform specificity have been developed [22–29]. In addition to their potential therapeutic benefit, isoform-selective inhibitors also are being used to elucidate the physiological functions of the COX isoforms. However, several studies have indicated that NSAIDs have pharmacological mechanisms in addition to, or other than, the inhibition of COX activity [30–37], and, therefore, effects observed with NSAIDs may not always reflect the physiological roles of the COX isoforms.

As an alternative approach to pharmacological inhibition, we have used a genetic approach to develop mouse models to study the roles of the COX isoforms in normal and diseased states. Gene targeting techniques were used to inactivate the *Ptgs* genes and to produce mice genetically deficient in COX-1 or COX-2 [38, 39]. Dinchuk *et al.* [40]

* Corresponding author: Dr. Robert Langenbach, National Institute of Environmental Health Sciences (C4–09), 101 Alexander Drive, Research Triangle Park, NC 27709. Tel. (919) 541-7558; FAX (919) 541-1460; E-mail: langenbach@niehs.nih.gov

† *Abbreviations*: COX, cyclooxygenase or prostaglandin H synthase; *Ptgs-1* and *Ptgs-2*, genes encoding COX-1 and COX-2 RNA and protein; NSAIDs, nonsteroidal anti-inflammatory drugs; AA, arachidonic acid; PGs, prostaglandins; *Apc*, adenomatous polyposis coli; TPA, 12-*O*-tetradecanolylphorbol-13-acetate; *Min*, multiple intestinal neoplasia; NOS, nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; and L-NMMA, N^G- monomethyl-L-arginine.

also have described the development of a COX-2 deficient mouse. While this group used a slightly different targeting strategy, the characteristics of both COX-2 null mouse strains appear to be identical. In the following, recent studies using the *Ptgs-1* and *Ptgs-2* gene-disrupted mice are described. Furthermore, when possible, the physiological effects of genetic inactivation of *Ptgs* are compared with those of pharmacological inhibition of COX activity by NSAIDs.

For additional information about the COX isoforms, NSAIDs, and prostaglandins, the reader is referred to recent reviews [7, 16, 41–47].

GENERAL CHARACTERISTICS OF COX-1 AND COX-2 NULL MICE

The DNA manipulations used to disrupt the genes coding for COX-1 and COX-2 have been reported [38–40]. Both COX-1 and COX-2 deficient mice lacked the respective normal size message and immunoreactive protein. Mice heterozygous for *Ptgs-1* or *Ptgs-2* expressed the respective messages and proteins at about 50% of the levels observed in wild-type mice. In the tissues examined (stomach, colon, kidney, testes), COX-1 null mice did not compensate by up-regulating the expression of COX-2, nor did COX-2 null mice appear to up-regulate the expression of COX-1. Furthermore, in biological responses to exogenous stimuli, the *Ptgs* knockout mice show gene dosage effects, with heterozygous knockouts being intermediate between null and wild-type mice. Whereas we have successfully transferred COX-1 deficiency onto a C57Bl/6 background, the number of viable COX-2 null mice obtained on this background was reduced greatly. Therefore, most studies with the COX deficient mice have been conducted with a mixed strain 129 Ola/C57Bl/6 background.

In heterozygous matings, COX-1 null mice were born in the expected ratios and lived normal lifespans even though their PG levels, in most tissues, were reduced by 99%. Indeed, the health of COX-1 deficient mice was surprising given the "housekeeping" functions attributed to COX-1. The lack of gastric and kidney pathologies was especially noteworthy, as the PGs produced by COX-1 were believed to have key roles in the physiology of these tissues [21, 24, 48–53]. As predicted [54], an impairment of platelet aggregation was observed in the COX-1 null mice. As described in greater detail below, COX-1 null females had difficulties with parturition, whereas COX-1 null males were fertile. Mice heterozygous for COX-1 expression manifested no obvious health effects. The observation that PG levels in COX-1 null mice were reduced by 99%, with minimal health effects, raised questions about the physiological functions of the constitutive level of PG production by COX-1 in wild-type mice.

In contrast, mice lacking COX-2 expression manifested various pathologies. While heterozygous matings on the 129 Ola/C57Bl/6 background produced COX-2 null pups at the expected ratio, only about 60% of the pups survived to weaning, and of those only about 75% survived to one year of age. The causes of death prior to 3 weeks of age are unknown. The deaths of mice after 3 weeks of age were attributed to peritonitis or kidney malfunction [39]. In addition, Dinchuk *et al.* [40] observed that COX-2 null male mice were fertile, but female mice were infertile. These authors also reported that cardiac fibrosis was observed in about 50% of all COX-2 null mice. The health of mice heterozygous for COX-2 appeared to be normal.

Renal abnormalities were present in all adult COX-2 null mice. The kidneys were pale and smaller than those of wild-type mice. In the early stages, the nephropathy was characterized by a reduced number of glomeruli, and the glomeruli present were poorly developed [39]. Renal tubules were dilated and atrophied. With age, the renal pathology became more severe and resulted in end-stage renal disease. As the kidneys of 3-day-old wild-type mice and COX-2 nulls were indistinguishable, the renal pathology was ascribed to postnatal developmental causes. The pathology observed in adult COX-2 null mice did not resemble the toxicity induced by NSAIDs in adult mice or humans [49, 55]. However, the kidney pathology observed in adult COX-2 null mice started during early postnatal development [38, 39]. Therefore, to compare kidney pathologies between COX-2 deficient mice and NSAID-treated mice, neonatal mice treated with NSAIDs should be studied.

The peritonitis observed in COX-2 null mice involved multiple abdominal organs, with neutrophil infiltration and focal bacterial colonies present [39]. We now estimate that 1–5% of the adult COX-2 null mice die of peritonitis. The causes of the increased susceptibility of the COX-2 deficient mice to peritonitis and the source(s) of the bacterial infections are not known.

EFFECTS OF COX DEFICIENCY ON CARCINOGENESIS

One of our original goals for making the COX null mice was to investigate the roles of COX-1 and COX-2 in carcinogenesis. Two types of cancers, colon cancer and skin cancer, had been chosen for study. The rationales for the COXs having roles in colon cancer development are based on both rodent and human epidemologic data. Reddy and colleagues [56 and references therein] had conducted many studies indicating that NSAIDs reduce carcinogen-induced intestinal cancer in rodents. Furthermore, epidemiological studies indicated that aspirin reduces colon cancer mortality in humans [57–59]. A role for the COXs in skin carcinogenesis also was based on observations that NSAIDs could inhibit tumor formation [60–62] and that supplementation with specific PGs restored papilloma formation [61, 62]. Furthermore, recent studies have indicated that COX-2, and not COX-1, was up-regulated in human and rodent intestinal tumors [63–67] and in rodent skin papillomas [62].

Further support for a role of COX-2 in intestinal neoplasia has been presented by Oshima *et al.* [68], who observed

that COX-2 deficiency reduces intestinal polyp formation by 86% in *Apc* knockout mice. The heterozygous *Apc* knockout mouse shows a 100% incidence of intestinal polyps [69]. A *Ptgs-2* gene dosage effect was observed, as *Apc* mice heterozygous for COX-2 showed an intermediate reduction in polyp number. Inhibition of polyp formation in the *Apc* mouse was also observed with an NSAID selective for COX-2. The results of Oshima *et al.* suggested that COX-2 contributed to an early event in the tumorigenesis process, which has been described as self-promotion [42]. However, the experiments of Oshima *et al.* [68] did not address the possibility of a role for COX-1 in intestinal tumorigenesis.

At the time of the report of Oshima *et al.*, we were conducting studies to determine the effects of both COX-1 and COX-2 deficiency on intestinal tumorigenesis [70]. We were using an approach similar to that of Oshima *et al.* [68], but transferred the *Ptgs* knockouts to a C57Bl/6 background and then crossed these mice with the *Min* (multiple intestinal neoplasia) mouse [71, 72] so that the studies were conducted in mice with a homogeneous genetic background. The *Min* mouse has a chemically induced nonsense mutation in the *Apc* gene, which also leads to a 100% incidence of intestinal neoplasia, but develops significantly fewer polyps than the *Apc* knockout mouse. The *Min* mouse is considered a model for the human disease familial adenomatous polyposis. Our results showed that both COX-1 and COX-2 null/*Min* mice had a 70–80% decrease in the number of intestinal polyps [70]. For intestinal tumorigenesis, the fact that deficiency of COX-1, as well as COX-2 [68], decreases intestinal tumorigenesis may not be surprising, as aspirin is a more effective inhibitor of COX-1 than of COX-2 [28], and aspirin has been shown to reduce intestinal tumors in rodents and in humans [57, 58, 73].

Further evidence that the absence of either COX-1 or COX-2 can reduce tumorigenesis comes from studies using a mouse skin initiation/promotion model [74]. In the mouse skin model, we observed that the number of papillomas was reduced by about 75% in mice lacking expression of either COX-1 or COX-2 [75]. Additional support for roles of both COX-1 and COX-2 in skin tumorigenesis comes from studies with resveratrol, a purported COX-1 inhibitor, and SC-58125, a COX-2 selective inhibitor, both of which reduced skin papilloma formation [76, 77].

The data obtained with the *Ptgs* knockout mice indicate that absence of either COX isoform decreases tumorigenesis in carcinogen-treated or genetically predisposed mice. However, to date only decreases in the development of adenomas and papillomas have been observed. Whereas effects on carcinoma development would be expected to be similar, such results have not been reported yet. In addition to studying carcinoma induction directly in the COX-deficient mice, where both host and tumor lack the same isoform, these mice offer models for studying the effects of the COX deficiency in the host on the growth and/or metastatic potential of COX-expressing transplantable tumors.

The mechanisms by which COX-1 or COX-2 deficiency decreases tumorigenesis are unknown. PGs are known to have effects on a number of biological processes involved in carcinogenesis, including angiogenesis, cell proliferation, differentiation, and apoptosis [16, 41, 78–80]. Additionally, the COXs are known to metabolically activate certain classes of chemical carcinogens and/or to produce oxygen and peroxy radicals [41], and both of these events would be reduced in COX deficient mice. Alternatively, it is possible that the individual COX isoforms may lead to different profiles of PGs and/or channel the PGs to different signaling pathways. The COX deficient mice also can be used to determine if the supplementation of specific PGs restores the tumorigenic response, and with this approach, it may be possible to identify the specific PG(s) responsible and the signaling pathways involved.

The above data indicate that both NSAIDs and COX deficiency can impede the development of tumors in two tissues. However, it should be noted that NSAIDs may have anti-tumorigenic activity through pathways additional to, or alternative to, the inhibition of COX activity [33–35, 81] and that COX-dependent and COX-independent modes of antitumor activity by NSAIDs may exist. Therefore, studies with COX deficient mice may represent only one mechanism by which NSAIDs inhibit tumorigenesis.

EFFECTS OF COX DEFICIENCY ON FEMALE REPRODUCTIVE PROCESSES

Prostaglandins are known to have important roles in the female reproductive processes, and understanding of the contributions of the individual COX isoforms in female reproduction has been facilitated by the development of COX deficient mice [82–85].

In initial studies, we observed that COX-1 null female mice produced litters of normal size, but had difficulty with parturition, and most pups were born dead or died shortly after birth [38]. Other aspects of the female reproductive processes in COX-1 null mice appeared normal, and Lim *et al.* [82] recently demonstrated that ovulation and implantation in the COX-1 deficient females did not differ from that in wild-type. Gross *et al.* [86] confirmed our initial findings and observed that the onset of parturition was delayed in COX-1 null mice. Furthermore, the administration of $PGF_{2\alpha}$ to pregnant COX-1 null females resulted in the onset of labor. The up-regulation of COX-1 in the uterus (primarily decidua) in wild-type females indicated COX-1 to be the source of the $PGF_{2\alpha}$. In agreement with this role for $PGF_{2\alpha}$, Sugimoto *et al.* [87] reported that in a $PGF_{2\alpha}$ receptor knockout mouse, parturition was inhibited, while ovulation and implantation occurred normally. Thus, the combined data from these knockouts indicated that $PGF_{2\alpha}$ formed through the COX-1 pathway in the uterus was needed for luteolysis and the normal initiation of parturition.

Whereas deficiency of COX-1 affected only parturition, deficiency of the COX-2 isoform caused impaired ovula-

tion, implantation, and decidualization. Follicular development appeared normal in COX-2 null mice [39, 40], but null female mice were essentially infertile [40]. Lim *et al.* [82] investigated the causes of infertility in COX-2 deficient mice and observed that few eggs were recovered, even after superovulation. Of the few eggs released by COX-2 null females, only about 2% were fertilized compared with those released by wild-type females. Davis *et al.* [85] demonstrated that ovarian PGE_2 was up-regulated by pituitary gonadotropins in wild-type and COX-1, but not COX-2 deficient mice, indicating that COX-2 accounted for increased ovarian PG production following the luteinizing hormone surge. Furthermore, cumulus activation, stigmata formation, and ovulation were abnormal in COX-2 null mice. These processes could be restored by PGE_2 administration. In addition to affecting ovulation, COX-2 deficiency also impeded implantation and decidualization [82]. Lim *et al.* [82] also showed that a COX-2 selective inhibitor, DuP697, but not aspirin, blocked implantation in wild-type and COX-1 null mice. These studies show that the COX null mice can be used to elucidate the role(s) of the individual COX isoforms in reproductive processes and to identify the specific PG involved. Furthermore, there seems to be general agreement between the effects of NSAIDs and of COX deficiency on these processes.

EFFECTS OF COX DEFICIENCY ON THE INFLAMMATORY RESPONSE

Inflammation is a complex biological response modulated by various chemical mediators with which prostaglandins have a synergistic role [45, 88]. Since the recent discovery of COX-2, a significant number of studies have associated this isoform with the inflammatory process, and, therefore, considerable effort has gone into developing NSAIDs that selectively inhibit this isoform [22–29]. To better understand the relative contributions of the COX isoforms in the inflammatory process, we compared the inflammatory responses of wild-type, COX-1, and COX-2 null mice.

In our initial studies, we used AA and the tumor promoter TPA to induce edema in the mouse ear as the measure of inflammation [38, 39]. In COX-1, but not COX-2, deficient mice, edema induced by AA was reduced about 70% compared with wild-type mice [38]. Heterozygous COX-1 mice showed a response to AA intermediate between null and wild-type mice. The ear swelling in COX-1 null mice was maximal 1–2 hr after AA treatment and then, similar to wild-type mice, decreased to near normal by 6 hr. The fact that swelling decreased by 6 hr indicates that the induction of COX-2 is not a significant factor in AA-induced edema. However, the use of AA as an inflammatory agent may be compromised because it is the substrate for both isoforms, and we have shown since that COX-1 is the predominant isoform expressed in the skin. However, the data do suggest that the initial inflammatory response to an agent that causes rapid AA release could be due to COX-1, and could implicate COX-1 in the inflammatory process.

TPA was equally inflammatory in both the COX null and wild-type mice [38, 39]. This observation was unexpected, as COX-2 is induced by TPA and is thought to mediate, at least partially, the inflammatory response to TPA. In these studies, ear swelling was monitored for 48 hr post TPA treatment, and no difference between genotypes was observed at any time point. Dinchuk also reported that COX-2 null and wild-type mice showed similar TPA responses in the ear swelling as well as the carrageenan-induced paw edema model [40]. Since COX-2 null mice still express COX-1, it is possible that COX-1 is contributing sufficient levels of PGs at the treated site for the inflammatory process to occur. However, in these mice the induction of non-PG inflammatory mediators by TPA cannot be excluded. Notwithstanding, the studies with AA, TPA, and carrageenan in the COX deficient mice indicate that COX-1, as well as COX-2, can contribute PGs to the acute phase of the inflammatory response; and the extent to which each isoform contributes PGs may depend on the inflammatory stimuli, the time after insult, and the relative levels of each isoform in the target tissue.

We have continued to study inflammatory responses in the COX null mice using the air pouch model [89–91]. By 6 hr after carrageenan treatment, PG production (PGE_2 and PGI_2) was elevated in the pouch exudate of all genotypes compared with solvent-treated controls (Langenbach R, unpublished observations). In COX-2 null mice the increase in PGs was only about 25% of that of wild-type mice, whereas PG levels in COX-1 null mice were about 75% of wild-type. The COX-2 inhibitor NS-398 [92, 93] reduced PG production in wild-type mice to levels comparable to those seen in COX-2 null mice. Thus, both COX-2 deficiency and pharmacologic COX-2 inhibition indicated that COX-2 is the major pathway for PG production during the early stages of inflammation in this model. However, the data did indicate that COX-1 contributes about 25% of the PGs produced during this early stage. By day 3 following carrageenan treatment, PG levels had declined in all genotypes, but the number of neutrophils and macrophages in the exudates of all genotypes was elevated. However, compared with wild-type mice, macrophage infiltration into the pouch was reduced by about 50% in COX-2 null mice, and was reduced slightly in COX-1 null mice (Langenbach R *et al.*, unpublished results). It is not known if macrophage function as well as recruitment was altered in the COX-2 null mice. By day 7, higher levels of apoptotic neutrophils were present in the pouch fluid of COX-2 null mice, and little resolution of inflammation was apparent compared with wild-type or COX-1 null mice.

Wallace *et al.* [94] investigated inflammatory responses in *Ptgs-2* knockout mice using a carrageenan-induced paw inflammation model. Wild-type and COX-2 null mice responded similarly in their initial inflammatory response. However, indomethacin, but not the COX-2 selective inhibitor NS-398, significantly inhibited carrageenan-in-

duced edema in both wild-type and COX-2 null mice. This observation contributed to the conclusions that significant inhibition of PG synthesis occurred only at NSAID doses that also inhibited COX-1, and that COX-1 contributed to the inflammatory response. Furthermore, the data suggested a role for COX-2 in the resolution phase of the inflammatory response, as lymphocyte infiltration and swelling persisted in the COX-2 null mice compared with wild-type mice. A role for COX-2 in the resolution of gastric ulcers also has been demonstrated with the COX-2 inhibitor NS-398 [95]. These findings with NSAIDs and COX null mice indicate that both COX-1 and COX-2 contribute to PG production at the site of inflammation, and also that COX-2-derived PGs have roles in the resolution or healing phase as well as in the early stages.

Bozza *et al.* [96] have used COX deficient mice to study the effects of COX deficiency and NSAIDs on lipid body formation in leukocytes. Lipid bodies are inducible cellular inclusions associated with inflammation, which contribute to enhanced synthesis of PGs or leukotrienes. Neither the absence of COX-1 nor the absence of COX-2 diminished lipid body formation in mouse leukocytes. However, indomethacin, aspirin, and salicylate, as well as the COX-2 selective inhibitor NS-398, did inhibit lipid body formation. Because macrophages deficient in COX-1, but not COX-2, showed diminished PG production immediately following stimulation, a role for COX-1 in the initial burst of PGs is indicated. These data also suggest anti-inflammatory mechanisms of NSAID action independent of their inhibition of either COX isoform.

EFFECTS OF COX DEFICIENCY ON SPONTANEOUS AND INDUCED GASTRIC ULCERATION

The current hypothesis about the medicinal usage of NSAIDs is that inhibition of COX-2 is responsible for their beneficial effects, whereas the inhibition of COX-1 is responsible for their adverse effects, the most common of which is gastric ulceration. Therefore, it was surprising that COX-1 deficient mice did not spontaneously develop gastric ulcers [38]. Measurement of gastric PG levels in the COX-1 null mice indicated a greater than 99% reduction, and this reduction in gastric PGs was similar to levels observed following an ulcerative dose of indomethacin. It was considered possible that compensatory pathways had been induced in the COX-1 null mice due to the genetic ablation of COX-1. The up-regulation of COX-2 was one mechanism considered; however, gastric COX-2 message levels did not differ from those of wild-type mice [38]. Thus, if compensation was occurring, it did not appear to involve PGs. Other compensatory mechanisms including up-regulation of nitric oxide (NO) levels were considered; however, preliminary studies with the NOS inhibitor L-NAME showed that it was not more ulcerative in COX-1 null mice than in wild-type mice. Whittle *et al.* [97] had reported that non-ulcerative doses of the NOS inhibitor L-NMMA induced acute gastric injury in rats pretreated with non-ulcerative doses of indomethacin. While further studies are needed to determine whether compensation mechanisms exist, the data from the COX-1 deficient mice indicated that elimination of COX-1-derived PGs alone was not sufficient to cause gastric ulcers. Recently, an NSAID with selectivity for COX-1 was described [29], and it will be interesting to determine if this or other COX-1 selective NSAIDs are necessarily ulcerative.

In recent studies, the involvement of both COX-1 and COX-2 in the ulcerative process has been investigated. We determined that the pH of the stomach in COX-1 null mice was about 1.5 compared with about 3.5 in the wild-type and COX-2 null mice. This more acid pH is in agreement with COX-1 having a role in acid and/or bicarbonate secretion [98, 99]. We also observed that aspirin and alcohol, like indomethacin [38], induce fewer ulcers/erosions in the COX-1 deficient compared with wild-type mice. Two recent studies have indicated that COX-2 has a role in ulcer resolution. The COX-2 inhibitor NS-398 has been reported to retard ulcer resolution [95], and Wallace *et al.* [94] reported that ulcerative doses of indomethacin cause death in COX-2 null mice, but not in wild-type mice. In the latter study, the cause of death appeared to be intestinal perforation followed by peritonitis, a spontaneous cause of death we previously reported for some COX-2 null mice [39].

DEVELOPMENT OF MICE DEFICIENT IN BOTH COX ISOFORMS

Because both COX-1 and COX-2 null mice independently showed reasonable survival [38–40], we thought that it might be possible to develop a mouse line deficient in both isoforms, i.e. COX-1(−/−)-COX-2(−/−). A COX-1 and COX-2 double null mouse could offer a model to elucidate the essential physiological functions of PGs. In the course of generating double null mice, COX-1(+/−)-COX-2(+/−), COX-1(+/−)-COX-2(−/−), and COX-1(−/−)-COX-2(+/−) mice also would be produced. As gene dosage effects have been observed in most studies conducted with the COX null mice to date, we hypothesized that the pathologies and/or responses observed in our COX-1 or COX-2 null mice [38, 39] could be exacerbated when the copy number of the remaining gene was reduced to the heterozygous state.

Our previous data with COX-1 null mice indicated that the presence of COX-1 in the embryo or in the mother was not essential for normal development [38], and indicated that if PGs were necessary for embryonic development, they could be produced by fetal and/or maternal COX-2. However, because COX-2 null female mice were infertile, COX-1(+/−)-COX-2(+/−) or COX-1(−/−)-COX-2(+/−) females had to be used for breeding to develop double null mice, and, therefore, it would not be possible to ascertain the necessity for maternal COX-2 during pup development.

TABLE 1. Phenotypes of the COX deficient mice

Phenotype	COX-1 deficient mice	COX-2 deficient mice
Health		
Survival	Normal	Only 60% survive to weaning, 75% of survivors live to 1 year
Gastrointestinal	No spontaneous ulcers	No spontaneous ulcers
	Possible reduced resolution of induced ulcers	Reduced resolution of induced ulcers
	Gastric pH 1.5	Gastric pH 3.5
Kidney	Normal	Progressive renal disease
Male fertility	Normal	Normal
Female	Delayed parturition, otherwise normal	Reduced ovulation, fertilization, implantation, and decidualization
PG levels	1% of normal uninduced levels	Normal uninduced levels
	Inducible	Not inducible
Inflammatory response		
Ear edema	Reduced edema with AA	Normal edema with AA
	TPA induces edema	TPA induces edema
Air pouch	Slightly reduced macrophage recruitment	Macrophage recruitment ~50% of normal
	Slightly reduced resolution	Reduced resolution
Infection	Normal?	Increased peritonitis
Tumorigenic response		
Skin papillomas	Reduced ~75%	Reduced ~75%
Intestinal adenomas	Reduced ~75%	Reduced ~75%

To date all of the double null pups generated died shortly after birth. Developmentally, the pups appear normal, and the cause(s) of death is being investigated (Loftin C and Langenbach R, unpublished observations). Comparison of phenotypes of the COX-1(+/−)-COX-2(−/−) and COX-1(−/−)-COX-2(+/−) are also underway, and such studies should help elucidate the developmental/physiological roles of the individual isoforms. One question concerning the pre/postnatal development of double null pups is the status of the ductus arteriosus. *In utero*, it is believed that maternal PGs keep the ductus patent, and it is a decline in PGs postpartum that causes ductus closure [100]. Furthermore, it is known that administering NSAIDs to the pregnant female will cause ductus closure *in utero* [101, 102]. Recently, it has been reported that the knockout of the EP4 receptor leads to a patent ductus, which causes the death of the pups within 48 hr postpartum [103, 104].

RELEVANCE OF COX-DEFICIENT MICE TO HUMANS

Humans with platelets deficient in COX-1 activity have been identified [105]. Western analyses of three human cases have indicated two distinct types of defects. In two cases, no detectable COX-1 protein was observed, similar to the targeted disruption of the *Ptgs-1* gene in mice [38]. In the second defect type, COX-1 protein was present, but the protein lacked catalytic activity. The characteristics of the three patients were: mild bleeding disorders, reduced platelet aggregation, and reduced serum thromboxane A_2 levels. Two of the patients developed peptic ulcer disease, although it was unclear if COX-1 deficiency was a predisposition factor for ulcer formation. Also, it was not stated if the female patients had parturition difficulties, as would be predicted from the COX-1 null mice. To date, there are no reports of human COX-2 deficiency. The characteristics of humans with a COX-2 deficiency, if the COX-2 null mouse predicts human pathology, would include ovulation and/or other female reproductive disorders, progressive kidney disease, and possible susceptibility to bacterial infection. As more humans with COX-1 or COX-2 deficiency are identified, the *Ptgs* knockout mice may prove to be models for predicting disease prognosis and possibly for developing efficacious therapies.

CONCLUSION

Data obtained with the COX-1 and COX-2 null mice have contributed to a clearer understanding of the physiological roles of the two COX isoforms. A summary of the phenotypes of the COX null mice is shown in Table 1. Based on pathologies observed in the null mice, it appears that deficiency of COX-2 has more severe effects than does deficiency of COX-1. No doubt, additional effects of COX deficiency will be obtained as these mice are studied further.

The mechanisms by which PGs originating from COX-1 or COX-2 carry out different physiological functions are unclear. One possibility is that different cellular localization leads to PG metabolites that differentially activate cell membrane receptors and nuclear receptors/transcription factors. However, our data suggest that COX-2, as well as COX-1, derived PGs may have paracrine and autocrine as

well as intracrine effects. Furthermore, it is possible that COX-1 and COX-2 ultimately lead to the production of quantitatively, if not qualitatively, different spectra of biologically active PGs.

The data obtained to date allow some comparisons of the physiological effects of *Ptgs* gene knockouts with pharmacological inhibition of COX activity by NSAIDs. However, COX-1 and COX-2 deficient mice have a total absence of the respective COX activity, which is a condition probably not achievable even with high doses of NSAIDs. Therefore, the use of mice heterozygous for one or both COX isoforms may provide models that more closely mimic NSAID effects. Furthermore, the COX deficient mice lacked the respective COX expression during both prenatal and postnatal development, and compensation mechanisms may have arisen. Conditional *Ptgs* knockouts [106, 107] may offer an approach to exclude developmental effects for the comparison of genetic ablation with pharmacological inhibition. However, NSAIDs are believed to have effects in addition to inhibition of COX activity, which also can complicate direct comparisons. Notwithstanding the possible limitations of both approaches, most observations made with the COX null mice are in general agreement with the effects predicted by NSAID studies. The major exceptions are gastric ulceration and kidney toxicity. However, whether gastric ulceration was solely an effect of COX-1 inhibition was debated before the development of the COX-1 null mice [98, 108], and the COX-1 null mice support the conclusion that inhibition of COX-1, alone, is not sufficient to cause gastric ulcers. The discrepancy between kidney pathology in the COX-2 null mice and kidney toxicities induced by NSAIDs may be an age-dependent phenomenon. NSAIDs usually are tested for pathological effects in adult animals, whereas COX-2 deficiency appears to cause kidney pathology during postnatal kidney development. Therefore, NSAIDs, including those selective for COX-2, may cause similar pathologies if administered to neonates.

The COX knockout mice have provided useful models for investigating the roles of the COX isoforms in normal physiology and various pathological states. However, the knockout of a gene leads to the decrease (heterozygous) or absence (homozygous null) of COX expression. As several diseases (cancer, arthritis) involve increased expression of COX activity (especially COX-2), the development of mice that overexpress the COXs may lead to mice that are highly susceptible to these diseases. Furthermore, mice that overexpress COX-1 or COX-2, in combination with the COX deficient mice, may facilitate identification of the mechanisms by which the COXs contribute to these diseases.

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