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BRIEFING PACKAGE

EVALUATION OF THE ROLE OF COX-2 IN ANIMALS AND MAN: FOCUS ON THE POTENTIAL IMPACT OF SELECTIVE COX-2 INHIBITION

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EXECUTIVE SUMMARY: OVERVIEW OF THE ROLES AND DISTRIBUTIONS OF COX-1 AND COX-2 IN ANIMALS AND MAN AND POTENTIAL IMPLICATIONS FOR SELECTIVE COX-2 INHIBITION

EXECUTIVE SUMMARY: OVERVIEW OF THE ROLES AND DISTRIBUTIONS OF COX-1 AND COX-2 IN ANIMALS AND MAN AND POTENTIAL IMPLICATIONS FOR SELECTIVE COX-2 INHIBITION

When aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) initially were developed, it was thought that all NSAIDs produced anti-inflammatory effects by inhibiting a single cyclooxygenase enzyme. NSAID-mediated inhibition of prostaglandin production led to a variety of promising anti-inflammatory, antiphlogistic, and analgesic effects, although it soon became apparent that not all NSAID-mediated effects were beneficial. Inhibition of prostaglandin synthesis in the gastrointestinal (GI) tract had deleterious effects on gastric mucosal protection and, in the kidney, led to decreased renal blood flow under certain conditions (see review by Donnelly and Hawkey, 1997). (It is recognized, however, that there are a number of other factors that may contribute to the deleterious effects of NSAIDs.)

Since the discovery of two isoforms of cyclooxygenase (COX), COX-1 generally has been considered to be expressed constitutively and is responsible for production of prostaglandins that participate in normal physiologic processes and protective functions (eg, maintaining integrity of GI mucosa, mediating normal platelet function, regulating renal blood flow and sodium resorption). In contrast, COX-2 was considered to be rapidly inducible in response to inflammation (Crofford, 1997; DeWitt et al, 1993; Donnelly and Hawkey, 1997; Jouzeau et al, 1997; Smith and DeWitt, 1995) and to produce prostaglandins involved in inflammation. It was hypothesized that inhibition of the "housekeeping" COX-1 enzyme resulted in many of the adverse effects associated with the use of NSAIDs and that COX-2 inhibition led to anti-inflammatory and analgesic effects.

This simplified view now appears to be misleading based on the following information:

- COX-1 is not just constitutive, but also is inducible.

- COX-1 has a role in certain types of inflammation.
  - Data from COX-1 and COX-2 knockout mice suggest that COX-1 plays a role in inflammation, and that COX-2 is not obligatory for an inflammatory effect. The relative importance of COX-1 and COX-2 in inflammation may depend, in part, on whether the inflammation is acute or chronic.

- COX-1 is not essential in preserving GI integrity and may not necessarily be involved in the development of GI toxicity associated with NSAIDs.
  - The role of COX-1 in homeostasis may have been overstated because COX-1 knockout mice do not exhibit excessive GI damage and remain susceptible to gastric damage with indomethacin (Langenbach et al, 1995; Mahmud et al, 1996). There are effects of NSAIDs unrelated to cyclooxygenase inhibition (eg, effects on mitochondria) that may be important (Chavez et al, 1993; Somasundaram et al, 1997).

- COX-2 is expressed constitutively in several tissues.
  - Although COX-2 is expressed in response to inflammatory stimuli, such as lipopolysaccharide and interleukins (Donnelly and Hawkey, 1997), it also can be detected in a variety of normal animal and human tissues.

- COX-2 has important physiologic activities, in addition to its role in inflammation.
  - COX-2 appears to be involved in a variety of physiologic or homeostatic functions, including:
    - Gastric cytoprotection
    - Ulcer healing and repair of mucosal injury
    - GI epithelial integrity and resistance to infection (peritonitis)
    - Renal salt and water homeostasis
    - Cardiovascular repair following injury
    - Pulmonary repair following injury
    - Central nervous system function
    - Reproduction (ovulation, fertilization, implantation, maintenance of pregnancy, parturition), and
    - Normal organogenesis in the fetus.

A more specific summary of the expression and roles of COX-1 and COX-2 in various body systems follows.

ROLE OF COX-1 AND COX-2 IN INFLAMMATION

During inflammation, COX-2 clearly is upregulated (Anderson et al, 1996; Crofford, 1997; Sano et al, 1992). Although COX-1 was not thought to be directly involved in inflammation, recent studies have demonstrated that both cyclooxygenase isoforms are detected in synovial cells from inflammatory joints (Crofford et al, 1994; Gilroy et al, 1998; Iñiguez et al, 1998). In vitro analyses of cultured human synovial tissues show that macrophage, fibroblast, endothelial, and mononuclear inflammatory cells express both COX-1 and COX-2. In addition, in studies of COX-2 knockout mice, experimental challenges with inflammatory agents resulted in inflammatory responses that did not differ significantly from those in wild-type animals (Dinchuk et al, 1995; Morham et al, 1995). Thus, the presence of the

COX-2 enzyme is not essential for inflammation to occur. In contrast to findings in COX-2-deficient mice, inflammatory challenge in COX-1 knockout mice resulted in reduced inflammatory response (Langenbach et al, 1995). These findings are contrary to established views that COX-1 is a constitutive, housekeeping enzyme responsible only for maintaining normal cell function and that COX-2 is inducible and solely responsible for inflammatory response.

GASTROINTESTINAL TRACT

In the GI tract, COX-1 is expressed constitutively in almost all tissues (Kargman et al, 1996). However, COX-2 is also expressed constitutively (to a much lesser extent), with highest concentrations found in the cecum and distal intestine in rats (Kargman et al, 1996). In rats, COX-2 is expressed in gastric epithelial cells (Sasaki et al, 1998) and is necessary for proliferation (Sawaoka et al, 1997).

In humans, constitutive COX-2 was found in intestinal mucosa (Mahida et al, 1997). In patients with ulcerative colitis, COX-2 is upregulated in colonic apical epithelial cells (Singer et al, 1998).

COX-2 appears to have an important role, which was previously attributed only to COX-1, in preventing or repairing GI mucosal damage. In COX-1 knockout mice, the absence of COX-1, which is purported to provide the majority of cytoprotective prostaglandins, did not cause spontaneous GI ulceration (Langenbach et al, 1995), indicating that compensatory protective mechanisms (possibly COX-2-mediated) must be involved. In addition, these mice remained susceptible to NSAID-induced damage, which could not have been COX-1-mediated. (Other explanations, unrelated to cyclooxygenase inhibition, for NSAID-induced damage to the GI tract have been proposed, including local, physicochemical effects [Lichtenberger et al, 1995] and effects on mitochondrial function [Chavez et al, 1993; Somasundaram et al, 1997]). Selective COX-2 inhibitors increase ischemia/reperfusion injury (Maricic

et al, 1998) and decrease adaptive cytoprotection (Brzozowski et al, 1998; Ehrlich et al, 1997; Gretzer et al, 1998a). Studies in rats and mice indicate that specific inhibition of COX-2 may delay ulcer healing (Mizuno et al, 1997; Schmassmann et al, 1998; Shigeta et al, 1998; Tsuji et al, 1997).

In the colon, COX-2 may protect against invasive bacteria because COX-2 knockout mice develop peritonitis (Morham et al, 1995; Morteau et al, 1997a). In addition, selective COX-2 inhibitors exacerbate experimental colitis in rats, with resultant septicemia (Reuter et al, 1996), and COX-2 stimulates fluid secretion by colonic epithelial cells (Blume et al, 1998; Eckmann et al, 1997).

Inhibition of COX-2 enhances apoptosis, which may inhibit tissue repair (Von Knethen and Brüne, 1997). This effect may be advantageous in preventing colonic polyps but may be disadvantageous in normal GI mucosa that is subject to repeated toxic insult and is characterized by one of the highest turnover rates of all body tissues.

KIDNEY

In the kidney, COX-1 activity occurs primarily in medullary collecting ducts and interstitial cells. However, COX-2 also is expressed constitutively in the kidney and has an important role in renal homeostasis (Harris et al, 1994). In the human kidney, COX-2 is present in endothelial cells, smooth muscle cells, and glomerular podocytes (Kömhoff et al, 1997). Under basal conditions, COX-2 has been located in the renal cortex (Seibert et al, 1994). The presence of COX-2 in the macula densa of the juxtaglomerular apparatus and ascending limb of Henle suggests that COX-2 expression may correlate with volume expansion or contraction. Chronic volume depletion increases renal COX-2 expression in rats (Harris et al, 1994).

A recent study confirms that selective COX-2 inhibitors affect renal function in man (Rossat et al, 1998). The role of COX-2 in human renal homeostasis, and its importance relative to COX-1 inhibition, will need to be delineated by studies on the effects of specific COX-2 inhibitors in patients with conditions in which COX-2 is upregulated.

REPRODUCTIVE TRACT

In the reproductive tract, both cyclooxygenase isoenzymes are expressed at various times during pregnancy (Vane et al, 1998). During early pregnancy, expression of COX-2 is necessary for ovulation, fertilization, implantation, and decidualization. COX-2 appears to be necessary for delivery of the fetus, and COX-2 levels increase significantly before and after labor (Gibb and Sun, 1996). Studies in COX-2 knockout mice indicate that females lacking COX-2 have reproductive defects and are infertile (Dinchuk et al, 1995; Lim et al, 1997). Although ovarian follicular development was normal, ovaries were small because of absence of corpora lutea (Dinchuk et al, 1995). COX-2 knockout mice also have impaired oocyte maturation, defective implantation of blastocysts in the uterus, and failed decidualization of the uterus (Lim et al, 1997). In contrast, both male and female COX-1 knockout mice remained fertile in the absence of COX-1, although the newborns were not always viable (Langenbach et al, 1995).

Development of luteinized unruptured follicles associated with infertility has been reported in women taking NSAIDs and could be associated with COX-2 inhibition (Smith et al, 1996). These data suggest that constitutive COX-2 is absolutely necessary for maintaining fertility. The potential long-term consequences of inhibiting normal luteal function, with consequent hyperestrogenemia, on endocrine-sensitive tissues need to be explored.

CENTRAL NERVOUS SYSTEM

The human brain contains equal amounts of messenger ribonucleic acid (mRNA) for COX-1 and COX-2 (O'Neill and Ford-Hutchinson, 1993). Although the exact function of COX-1 and COX-2 in the brain remains to be determined, it is important to note that both isoenzymes are expressed constitutively.

CARDIOVASCULAR AND PULMONARY SYSTEMS

In cardiovascular tissue, COX-2 knockout mice develop diffuse myocardial fibrosis (Dinchuk et al, 1995), and COX-2 is found in fibrotic cardiac tissue of patients with dilated cardiomyopathy (Wong et al, 1998). In pulmonary tissue, COX-2 also is expressed constitutively in rat lung (Brannon et al, 1998; Charette et al, 1995; Ermert et al, 1998) and is responsible for maintaining intrinsic tone in the guinea pig trachea (Charette et al, 1995); inhibition of tone may be greater with a selective COX-2 inhibitor than with a nonselective COX-1/COX-2 inhibitor (Charette et al, 1995). Patients with idiopathic pulmonary fibrosis are unable to induce COX-2 (Wilborn et al, 1995), suggesting that COX-2-mediated prostaglandin formation may be important in healing lesions and preventing fibrosis from occurring. This may be particularly relevant when selective COX-2 inhibitors are used to treat patients with rheumatoid arthritis, in whom the incidence of interstitial fibrosis is already increased (Anderson, 1993).

SUMMARY AND CONCLUSIONS

The view that COX-1 is purely a constitutive enzyme functioning in housekeeping roles (such that inhibition of COX-1 is necessarily bad), whereas COX-2 is purely an isoenzyme induced during inflammation (such that inhibiting COX-2 only suppresses inflammation and is therefore necessarily good) is, clearly, an

oversimplification. COX-2 has important physiologic functions, and the potential impact of inhibiting these functions should be considered carefully.

A common theme surrounding the known roles of COX-2 and the known effects of selective COX-2 inhibitors is that COX-2-mediated prostaglandins participate in cellular proliferation in inflammatory cells, angiogenesis, tissue repair (in the GI, cardiovascular, and respiratory systems), neoplasia, reproduction, and osteogenesis (Majerus, 1998; Majima et al, 1997; Onoe et al, 1996; Sarrazin and de Brum-Fernandes, 1998; Sato et al, 1997; Stenson, 1997; Tsuji and DuBois, 1995; Vane et al, 1998). As stated by William Stenson, MD, in a recent *Gastroenterology* editorial (1997), it may well be that "...inflammation and wound healing form a seamless continuum; drugs that inhibit inflammation may also retard healing."

It should be noted that the effects of inhibiting COX-2 are not necessarily restricted to selective COX-2 inhibitors. Indeed, many typical adverse effects associated with nonselective NSAIDs (such as impairment of ulcer healing, effects on renal function, effects on fertility) may be due to inhibition of COX-2. Therefore, selective COX-2 inhibitors should be considered similar to nonselective NSAIDs in sharing these typical adverse effects.

In contrast, it is not self-evident that sparing COX-1 will have no effects other than obviating toxicity mediated by COX-1. Selective COX-2 inhibitors may exhibit diminished therapeutic activity for certain applications because they lack associated COX-1 inhibition. Furthermore, the complex relationship between COX-1 and COX-2, and connections between the cyclooxygenase system and inducible nitric oxide synthase or other intracellular pathways, make it difficult or impossible to predict what effects will be associated with unopposed suppression of COX-2.

Finally, not all of the beneficial or detrimental effects of NSAIDs are necessarily associated with cyclooxygenase inhibition. NSAIDs, through effects on divalent cation translocation, have important effects on mitochondrial function that are independent of any effect on cyclooxygenase function (Chavez et al, 1993; Somasundaram et al, 1997); and this may be more important than inhibition of cyclooxygenase in GI toxicity. Another physicochemical effect of NSAIDs, unrelated to cyclooxygenase inhibition, involves alterations in hydrophobicity of the phospholipid barrier in the gastric mucosa (Lichtenberger et al, 1995; Lugea et al, 1997). NSAIDs (both nonselective and COX-2-specific) may have important effects on apoptosis and polyp regression that are independent of effects on cyclooxygenase (Piazza et al, 1997) or on prostaglandin formation (Chan et al, 1998).

In view of the evolving science in this area, the following points need to be considered when evaluating more highly selective COX-2 inhibitors:

- COX-1 and COX-2 have overlapping functions. It is unlikely that the pharmacodynamic profile of a drug can be predicted by knowledge of its effects on these isoenzymes.

- Because COX-1, as well as COX-2, is involved in inflammation, it is not immediately apparent that inhibition of COX-2 alone will provide optimal anti-inflammatory activity. However, because of the variability inherent in clinical trials designed to compare active agents, definitive differences in relative efficacy will be difficult to demonstrate.

- Because the functions of the cyclooxygenase isoenzymes are interrelated with each other, and with other intracellular pathways, the effects of isolated inhibition of one of the isoenzymes (ie, COX-2) cannot easily be predicted. It cannot be assumed that an agent that does not inhibit COX-1 is either completely safe or has a safety profile that can be predicted.

- In addition to any effects or lack of effects on prostaglandins produced by COX-1 or COX-2, NSAIDs have other pharmacologic properties that may affect their safety profile.

- Because inhibition of COX-2 by both conventional NSAIDs and selective COX-2 inhibitors contributes to their efficacy and side-effect profiles, selective COX-2 inhibitors should be considered NSAIDs. As with other NSAIDs, the individual safety and efficacy profiles of selective COX-2 inhibitors should be determined by clinical trials and clinical experience.

APPEARS THIS WAY ON ORIGINAL

INTRODUCTION

INTRODUCTION

Over the past decade, significant research has been conducted regarding the physiologic role and expression of cyclooxygenase isoforms 1 and 2 and knowledge in this area is continually evolving. The majority of available data are from animal models, and therefore may not be readily translatable to humans. Early in the 1990s, when COX-2 was first identified, it became apparent that COX-2 was primarily inducible as a component of inflammatory reactions, whereas COX-1 was primarily constitutive and involved in "housekeeping" functions. Since then, research has shown that this theory is truly oversimplified and that the separation of roles and functions of these isoenzymes is far from clear. Because the specific roles of COX-1 and COX-2 are still incompletely understood, cyclooxygenase selectivity profiles are of unknown value in predicting the effects of individual agents during clinical use and, in particular, in suggesting clinical superiority or absence of effect on clinical parameters such as renal function or ulcer formation.

Although it cannot be concluded that cyclooxygenase selectivity has no role in the activity or adverse-event profile of a particular agent, COX-2 selectivity does not guarantee clinical efficacy or safety. This document provides the Agency and the Arthritis Advisory Committee with a resource that reviews available literature and provides additional perspectives that may assist in regulatory review of selective COX-2 inhibitors. The data contained herein support the conclusion that the original COX-1/COX-2 hypothesis is oversimplified and that clinical data are still necessary to assess the adverse-event profile of any given agent. Further, the following summary provides preclinical evidence that the Agency may wish to pursue with regard to the potential safety of agents that specifically inhibit COX-2.

ROLE OF COX-1 AND COX-2

IN INFLAMMATION

ROLE OF COX-1 AND COX-2 IN INFLAMMATION

During inflammation, COX-2 clearly is upregulated (Crofford, 1997). Animal models demonstrate expression of COX-2 during acute and chronic inflammation (Anderson et al, 1996; Sano et al, 1992). Treatment with glucocorticoids and specific COX-2 inhibitors decreased COX-2 expression and reduced paw swelling in rodent inflammatory models (Anderson et al, 1996). In murine osteoblasts, two anti-inflammatory cytokines, interleukin-4 and interleukin-13, inhibited COX-2 expression (Onoe et al, 1996). Analysis of human synovial tissue from patients with rheumatoid arthritis or traumatic injury showed increased COX-2 expression (Crofford et al, 1994).

Although COX-1 was not thought to be directly involved in inflammation, recent studies have demonstrated that both cyclooxygenase isoforms are detected in synovial cells from inflammatory joints (Crofford et al, 1994; Gilroy et al, 1998; Inigüez et al, 1998). In vitro analyses of cultured human synovial tissues show that macrophage, fibroblast, endothelial, and mononuclear inflammatory cells express both COX-1 and COX-2. In addition, in studies of COX-2 knockout mice, experimental challenges with inflammatory agents resulted in inflammatory responses that did not differ significantly from those in wild-type animals (Dinchuk et al, 1995; Morham et al, 1995). Thus, the presence of the COX-2 enzyme is not essential for inflammation to occur.

In contrast to findings in COX-2-deficient mice, inflammatory challenge in COX-1 knockout mice resulted in reduced inflammatory response (Langenbach et al, 1995), indicating that COX-2 is not the sole source of inflammatory prostanoids. These findings are contrary to established views that COX-1 is a constitutive, housekeeping enzyme responsible only for maintaining normal cell function and that COX-2 is inducible and solely responsible for inflammatory response. Rather,

the results of studies in cyclooxygenase knockout animals indicate that the physiologic roles of COX-1 and COX-2 require reassessment. Furthermore, if the two cyclooxygenase isoforms are involved in expressing inflammatory prostaglandins, agents that target both COX-1 and COX-2 may be more appropriate for anti-inflammatory therapy.

APPEARS THIS WAY ON ORIGINAL

ROLE OF COX-2 IN THE GASTROINTESTINAL TRACT

ROLE OF COX-2 IN THE GASTROINTESTINAL TRACT

It is well established that many NSAIDs cause acute and chronic damage to the gastric mucosa (Wallace, 1997) and it has been postulated that this activity is related to cyclooxygenase inhibition. NSAID toxicity also extends to other areas of the GI tract, including the esophagus, small bowel, and colon (Gibson et al, 1992; Kaufman et al, 1996; Stamm et al, 1994). The pathogenesis of these other lesions is poorly understood, but presumably involves inhibition of prostaglandin synthesis by one or both cyclooxygenase enzymes. The effects of NSAIDs on the GI tract can be discussed, for convenience, under the categories of Gastric Lesions, Other Gastrointestinal Lesions, Healing of Lesions, and Effects on Epithelial Integrity.

GASTRIC LESIONS

With the identification of COX-1 and COX-2 isoenzymes, it was proposed that COX-1 was the isoform responsible for mediating gastric mucosal defense because inhibition of COX-1 led to gastric damage that was ameliorated by prostaglandin replacement. It was further proposed that COX-2 was not involved in gastric mucosal protection, and selective inhibition of COX-2 would spare gastric prostaglandin synthesis, reducing the likelihood for ulcerogenic effects. It is, of course, recognized that the cause of gastric lesion development with currently available NSAIDs is multifactorial and not completely understood.

Several recent studies have focused on the role of adaptive cytoprotection as a mechanism for preventing gastric injury. Chronic, low-grade injury of various kinds protects against acute injury. This adaptive cytoprotection may result from a local increase in protective prostaglandins.

OTHER GASTROINTESTINAL LESIONS

The cause of NSAID-induced ulceration in other areas of the GI tract is not well established. Prostaglandin replacement using misoprostol is not as effective in preventing (or treating) duodenal lesions as it is in preventing gastric lesions (Hawkey et al, 1998), suggesting that prostaglandin deficiency may not be as important in the initiation of duodenal ulcers. An important component related to development of duodenal ulcers in patients taking NSAIDs may be underlying *Helicobacter pylori* infection, with NSAIDs leading to an increased ulcer prevalence more by inhibiting healing than by causing ulcers de novo (see below). Finally, NSAIDs have been associated with strictures of the esophagus and colon (Davies, 1995; Eis et al, 1998), which could represent fibrosis secondary to delayed healing of lesions.

HEALING OF LESIONS

NSAIDs that nonselectively inhibit COX-1 and COX-2 have been shown to delay ulcer healing in animal models (Schmassmann, 1998; Schmassmann et al, 1995), and in man (Lancaster-Smith et al, 1991; Walan et al, 1989), and relevant effects on growth factor release have been demonstrated in cultured human gastric fibroblasts (Bamba et al, 1998). This is not surprising, in view of the close relationship between inflammation and wound healing (Stenson, 1997). COX-2 is intimately involved in wound healing, in the GI tract as well as in other tissues. However, it is not known to what extent the effect of nonselective NSAIDs on wound healing is mediated through inhibition of COX-2.

EFFECTS ON EPITHELIAL INTEGRITY

The GI tract constitutes an effective barrier against invasion by the large intestinal flora. The nature of the barrier is poorly understood, but it may involve fluid secretion, epithelial restitution, and other mechanisms. There is evidence to suggest involvement of prostaglandins in the function of this barrier. In addition, NSAIDs have been associated with disruption of the barrier and deleterious effects in patients with inflammatory bowel disease (Evans et al, 1997).

COX-2 EXPRESSION

The majority of cytoprotective prostaglandins in the GI tract are synthesized by constitutively expressed COX-1, which is found in essentially all GI tissues and is highly concentrated in the stomach, cecum, and colon (Kargman et al, 1996). However, a simplified theory whereby only COX-1 is responsible for production of cytoprotective prostaglandins can be called into question because COX-1 knockout mice did not demonstrate any abnormal GI physiology nor did they spontaneously develop gastric lesions, but did develop lesions when given oral indomethacin, albeit fewer than wild-type mice (Langenbach et al, 1995). This shows conclusively that inhibition of COX-1 is not necessary for NSAID-induced injury, and that other mechanisms must be involved. These could involve local effects (Somasundaram et al, 1997) or effects on COX-2. Although COX-2 in gastric mucosa is generally inducible, studies have shown that it also is constitutively expressed in the GI tract to some extent (Kargman et al, 1996). In rats, the highest concentration of constitutive COX-2 was found in the cecum and distal intestine. Furthermore, COX-2 has been shown to be constitutively expressed in human intestinal mucosa (Mahida et al, 1997).

Preclinical studies in mice and rats demonstrate a key role for COX-2 in maintaining mucosal integrity. In rats, COX-2 is expressed in gastric epithelial cells (Sasaki et al, 1998) and is important for proliferation (Sawaoka et al, 1997). In rat and mouse models of gastric mucosal lesion, COX-2 is expressed during acute stages of gastric mucosal injury (erosion, ulceration) and during ulcer healing (Kishimoto et al, 1998, 1997; Mizuno et al, 1997; Schmassmann et al, 1998). In one recent study in rats, COX-2 mRNA expression increased dramatically during the early phases of ischemia/reperfusion injury, then decreased as discrete ulceration occurred and was low during the ulcer healing stage (Kishimoto et al, 1998). However, because whole stomachs were used for the analysis, major elevations of COX-2 could have been present in the ulcer margins or ulcer base during the healing process.

Preclinical studies have shown that cytoprotection of the gastric mucosa in some situations (ethanol-induced damage after long-term endotoxin administration in rats; radiation injury) may relate, in part, to an inducible form of COX-1 (Cohn et al, 1997; Ferraz et al, 1997).

In one study, COX-2 was expressed in normal human gastric mucosa and human endothelial cells, and was significantly increased in the gastric ulcer margin (Tarnawski et al, 1997).

Spontaneous GI inflammation did not occur in COX-1 or COX-2 knockout mice, leading the authors of one study to question the proposed role of COX-1 in GI homeostasis (Morteau et al, 1997a). However, some COX-2 knockout mice developed peritonitis (Morham et al, 1995; Morteau et al, 1997a). In one recent study, indomethacin caused intestinal injury in COX-2 knockout mice, but not in normal controls (Wallace et al, 1998). The authors suggest that indomethacin administration may have led to dysregulation of inflammation in the COX-2 knockout mice, leading to tissue injury, whereas in normal mice the inflammation

would have resolved and any tissue damage would have healed. Furthermore, a study in rats demonstrated an elevation of COX-2 during inflammatory conditions, such as colitis, in the GI tract (Reuter et al, 1996). Increased susceptibility to experimentally induced colitis was seen in COX-2 knockout mice, but not in COX-1 knockout mice, suggesting that COX-2 may have more of a protective than inflammatory role in colitis (Morteau et al, 1997b).

In patients with ulcerative colitis, COX-2 has been shown to be upregulated in colonic apical epithelial cells (Singer et al, 1998). Biopsies from patients with active inflammatory bowel disease (ulcerative colitis or Crohn's disease) had a significantly ($P<.005$) higher expression of COX-2 than controls or patients with inactive disease; no difference in COX-1 expression was found (Hendel and Nielsen, 1997). In addition, COX-2 was shown to be upregulated in studies with human intestinal epithelial cells experimentally infected by invasive bacteria, as would occur during enteric infection, causing rapidly increased intestinal fluid secretion as a protective mucosal mechanism (Eckmann et al, 1997). COX-1 expression was not affected by bacterial infection. The authors suggest that mucosal injury after infection with invasive bacteria could be exacerbated by selective inhibition of COX-2. Another recent study describes the role of endothelial-derived prostaglandin production produced by COX-2 in mediating secretion of fluid by intestinal epithelial cells (Blume et al, 1998), which contributes to questions regarding the protective role of COX-2 in prevention of mucosal injury.

USE OF SELECTIVE COX-2 INHIBITORS IN ANIMAL MODELS

Currently available preclinical data on COX-2 expression suggest that it is involved in mucosal repair. In animal models of chronic gastric erosion and ulceration, a highly selective COX-2 inhibitor impaired or delayed ulcer healing in mice (Mizuno et al, 1997) and in rats (Schmassmann et al, 1998; Shigeta et al, 1998; Tsuji et al, 1997). Stress-induced gastric ulcers were significantly increased with the use of a

selective COX-2 inhibitor in rats (San Miguel et al, 1998) and healing of stress-induced ulcers was significantly delayed in mice treated with selective COX-2 inhibitors (Ukawa et al, 1997).

Adaptive cytoprotection, thought to have a role in maintaining mucosal integrity, can be impaired by nonselectively inhibiting both COX-1 and COX-2 with indomethacin or by selectively inhibiting COX-2 (Brzozowski et al, 1998; Gretzer et al, 1998a). A selective COX-2 inhibitor also inhibits the protective effect of peptone in rat gastric mucosa and does not cause different effects than those seen with indomethacin (Ehrlich et al, 1997). In a rat model of mucosal resistance to ischemia/reperfusion injury, a selective COX-2 inhibitor significantly increased mucosal injury (Maricic et al, 1998). Other forms of adaptive cytoprotection do appear to be COX-1 mediated (Cohn, 1997). These findings suggest that the COX-2 isoenzyme is involved in generating prostaglandins necessary for gastric mucosal defense.

Highly selective COX-2 inhibitors have been shown to exacerbate experimental colitis in rats, leading to death caused by colonic perforation in many of the animals (Reuter et al, 1996).

Interestingly, results of a recent study demonstrate that the lowest doses of two selective COX-2 inhibitors necessary for statistically significant reduction of carrageenan-induced rat paw edema were high enough to also significantly inhibit COX-1 activity (Wallace et al, 1998). At the doses necessary to significantly reduce prostaglandin synthesis in the rat paw, gastric prostaglandin synthesis was also significantly inhibited and the selective COX-2 inhibitors caused hemorrhagic erosions ($P<.05$ versus vehicle). These results were confirmed by other researchers who determined that doses of a selective COX-2 inhibitor necessary to inhibit prostanoid production in human bursal tissue also inhibit COX-1 (Gretzer et al, 1998b).

USE OF SELECTIVE COX-2 INHIBITORS IN MAN

Limited human data are available to address potential concerns based on animal studies with selective COX-2 inhibitors. Studies are available demonstrating that a selective COX-2 inhibitor (MK-0966) did not increase intestinal permeability (Bjarnason et al, 1998) or fecal blood loss (Hunt et al, 1998) to any greater degree than placebo, although the clinical relevance of these data are unclear. Short-term (1- to 2-week) endoscopic studies in healthy volunteers have shown no or minimal GI damage based on endoscopic score or erosion/ulcer development with MK-0966 (Lanza et al, 1997a), celecoxib (Lanza et al, 1997b), or nimesulide (Shah et al, 1998) that was similar to placebo and significantly less than with ibuprofen or naproxen. However, short-term endoscopic data have not been shown to be a reliable indicator of the development of potentially serious GI complications. Nimesulide was evaluated endoscopically for 1 week in 30 patients with dyspepsia; patients with a history of ulcer, complication, or severe dyspepsia were excluded (Marini and Spotti, 1993). Based on endoscopic score, the effects of nimesulide were shown to be similar to those of placebo.

HYPOTHETICAL APPLICATION TO HUMANS OF PRECLINICAL RESULTS IN THE GASTROINTESTINAL TRACT

In the GI tract, it is apparent that COX-2 has a more expanded role than originally believed and is important for several normal mucosal-protective mechanisms. Adaptive cytoprotection may prove to be a very important phenomenon in the stomach, particularly if it results from chronic *H pylori* infection. The extent to which it is dependent on COX-1 or COX-2 needs further elucidation. However, if COX-2 is shown to have an important role, the possible effects of COX-2 inhibition by highly selective inhibitors will need further evaluation.

Although highly selective COX-2 inhibitors may be less likely than other NSAIDs to *cause* initial gastric mucosal lesions, it is possible that they will delay or impair ulcer healing, either in the stomach or elsewhere. This is important because most NSAID-induced ulcers heal spontaneously, and it is the failure to heal, with progression to penetration through the muscularis mucosae, that leads to most complications.

Finally, delayed or dysregulated healing of lesions in other parts of the GI tract might be responsible for NSAID-induced fibrosis and stricture formation (eg, in the esophagus or colon). (Refer also to discussions of fibrosis in the cardiac and pulmonary sections of this document.) NSAIDs, through effects on healing or other mechanisms, could compromise the colonic barrier to infection, with consequent sepsis or peritonitis; this might be most consequential in patients suffering from inflammatory bowel disease or in patients with ascites. If these effects of NSAIDs are mediated preferentially by inhibition of COX-2, they may constitute important concerns about the use of highly selective COX-2 inhibitors. Some of these questions regarding the GI safety of selective COX-2 inhibitors have been raised previously (Stenson, 1997; Yeomans et al, 1998).

APPEARS THIS WAY ON ORIGINAL

ROLE OF COX-2 IN

THE KIDNEY

ROLE OF COX-2 IN THE KIDNEY

The kidney is a significant source of cyclooxygenase-mediated prostaglandin synthesis as well as a target organ for prostaglandin effects, particularly with regard to maintenance of renal blood flow in the compromised kidney. The use of NSAIDs in normal, healthy individuals is not associated with an untoward risk of adverse renal effects. However, patients with volume-depleted states, congestive heart failure, advanced age, or conditions associated with preexisting renal insufficiency are at risk for NSAID-induced adverse renal events, such as edema, hyperkalemia, acute renal failure, or nephrotic syndrome with interstitial nephritis (Whelton and Hamilton, 1991). Acute deterioration of renal function in high-risk patients is most concerning and occurs with disruption of the delicate balance between pressor mechanisms and prostaglandin-associated vasodilation. Inhibition of prostaglandins by NSAIDs allows for unopposed vasoconstriction, potentially leading to serious renal sequelae. Chronic, long-term use of NSAIDs has been associated with papillary necrosis in some rare situations. The effects of NSAIDs on salt and water homeostasis also may be responsible for the increased risk of hospitalization due to congestive heart failure (Heerdink et al, 1998) and interactions with antihypertensive medications (Houston et al, 1995; Johnson et al, 1993; Pope et al, 1993).

Historically, it was believed that COX-1 was expressed constitutively in the kidney for purposes of maintaining renal homeostasis. COX-2 was thought to be an inducible isoform that was normally absent, but would rapidly be expressed to meet a specific physiologic challenge, such as inflammation or compromised renal blood flow (Morham et al, 1995; Vane et al, 1998). However, emerging data demonstrate the role of COX-2 in normal renal development, the constitutive expression of COX-2 in the kidney, and the renal effects of COX-2 inhibition (Schneider and Stahl, 1998), all of which may have implications for the use of selective COX-2 inhibitors

in patients with compromised renal function, those taking antihypertensive medication, or in those with borderline cardiovascular compensation.

COX-2 EXPRESSION

COX-2 plays a role in the normal development of the kidney. High levels of COX-2 have been found in the developing nephrons and bladders of neonatal mice, underscoring the involvement of COX-2 in nephrogenesis (Park et al, 1997; Zhang et al, 1997). Several studies have demonstrated that COX-2 knockout mice exhibit severe congenital renal abnormalities (Dinchuk et al, 1995; Morham et al, 1995; Norwood, 1998). Kidneys of neonatal COX-2 knockout mice are significantly underdeveloped, with a disproportionately small number of functional nephrons and a large quantity of undeveloped mesenchymal tissue. In contrast, neonatal COX-1 knockout mice did not exhibit renal abnormalities, indicating that this isoform is not essential to normal renal growth and development (Langenbach et al, 1995). Adult COX-2 knockout mice exhibit reduced numbers of functioning nephrons and severe nephropathy, including glomerular sclerosis and tubulointerstitial injury (Dinchuk et al, 1995; Morham et al, 1995).

The constitutive expression of both COX-1 and COX-2 has been identified in renal tissues of animal models and man. In normal, unchallenged rats, COX-2 is expressed constitutively in the macula densa of the juxtaglomerular apparatus, in bordering epithelial cells of the cortical thick ascending limb of Henle, and in papillary interstitial cells. COX-2 is not found in renal arterioles, glomeruli, or collecting ducts in the rat. COX-1 is also expressed constitutively, but in distinctly different locations, namely the medullary collecting ducts and medullary interstitial cells (Harris et al, 1994). In rabbits, COX-2 is expressed constitutively in the bladder and in the outer medullary interstitial cells and cortical macula densa of the kidney. COX-1 is not found in the medullary interstitial cells of rabbits (Guan et al, 1997).

COX-2 is also expressed constitutively in adult and fetal human kidneys. In a study of adult human kidneys, COX-2 was located in glomerular podocytes and in endothelial and smooth muscle cells of renal arteries and veins, but not in glomerular endothelia. COX-1 was located in collecting duct cells, cortical and medullary interstitial cells, and endothelial cells of the afferent arteriole. In human fetal kidneys, COX-2 was found in arterial and venous endothelia and smooth muscles and in mature glomeruli. COX-1 was found in the collecting duct cells and podocytes of fetal human kidneys, suggesting a role in nephrogenesis (Kömhoff et al, 1997). The authors of this study conclude that the location of COX-2 suggests this isoform is involved in maintenance of renal hemodynamics and that development of a completely renal-sparing COX-2 inhibitor is unlikely.

Preclinical data demonstrate the role of COX-2 in maintaining normal sodium and water balance. Certain experimental, physiologic challenges mimicking clinical disease (eg, intravascular volume expansion or contraction) result in increased production of COX-2 in the kidney (Harris et al, 1994; Yang et al, 1998). For example, mice fed a low-sodium diet (ie, volume-contraction) exhibit markedly increased expression of COX-2 that is threefold greater than basal levels (Harris et al, 1994). Chronic volume expansion had no effect on basal expression of COX-2 in one murine study (Harris et al, 1994), but resulted in increased levels in another (Yang et al, 1998). Surgically induced renovascular hypertension in mice increases levels of COX-2 in the macula densa that parallel increases in renin levels, indicating a role for COX-2 in renin release associated with altered renal perfusion (Hartner et al, 1998).

The location of COX-2 in the kidney may dictate specific functions. COX-2 found in the renal medulla has been implicated in the control of sodium and water excretion in volume-overload states. In addition, it has been suggested that cortical COX-2 mediates glomerular circulation in volume-depleted states (Yang et al, 1998).

USE OF SELECTIVE COX-2 INHIBITORS IN ANIMAL MODELS

In one study, dogs received intravenous doses of indomethacin, 6-MNA (the active metabolite of nabumetone), or a selective COX-2 inhibitor (Brooks et al, 1998). The selective COX-2 inhibitor and indomethacin caused significant, dose-related reductions in urine flow, sodium excretion, renal plasma flow, and glomerular filtration rate. 6-MNA had no measurable effect on these or other parameters of renal function. In another study in mice, the normal renal response in animals fed a low-sodium diet (ie, increased renal renin release) was blocked by a selective COX-2 inhibitor (Harding et al, 1997), suggesting that COX-2 is a requirement for renin release by the kidney.

USE OF SELECTIVE COX-2 INHIBITORS IN MAN

The renal effects of two selective COX-2 inhibitors, nimesulide and flosulide, have been measured in short-term studies in young, healthy subjects (Brunel et al, 1995; Steinhäuslin et al, 1993) and the renal effects of celecoxib were recently evaluated in healthy, salt-depleted subjects in a short-term study (Rossat et al, 1998). Nimesulide has been withdrawn from the market, and clinical testing of flosulide has been halted (Donnelly and Hawkey, 1997). Administration of flosulide (25 mg twice daily for 9 days) or nimesulide (200 mg twice daily for 10 days) resulted in significant blunting of compensatory increases in plasma renin and aldosterone concentrations compared with placebo during orthostatic challenge and sodium depletion with furosemide (Brunel et al, 1995; Steinhäuslin et al, 1993). These observations, made during short-term administration to young, healthy volunteers, support the notion that selective inhibition of COX-2 is not sufficient to protect against renal toxicity during NSAID use, particularly in patients at risk for renal dysfunction.

A recent study evaluated the renal effects of a selective COX-2 inhibitor, celecoxib (200 or 400 mg/d), compared with placebo or naproxen (500 mg twice daily), for 8 days in 40 healthy volunteers on a low-sodium diet (Rossat et al, 1998). Peak changes in renal hemodynamics occurred at 1 hour on day 1, when 400 mg/d of celecoxib significantly ($P<.05$) decreased glomerular filtration rate and renal plasma flow when compared with baseline. Significant reductions in sodium excretion also occurred at 2 hours on day 1 with either dose of celecoxib versus placebo ($P<.01$) and in potassium and lithium excretion with the higher celecoxib dose ($P<.01$ versus placebo). The authors indicate in their conclusions that COX-2 has a major role in maintaining sodium balance and renal vascular tone in salt-restricted individuals.

HYPOTHETICAL APPLICATION TO HUMANS OF PRECLINICAL RESULTS IN THE KIDNEY

COX-2, like COX-1, is expressed constitutively in the kidney and mediates sodium and water balance, intravascular volume, and blood pressure. Because many NSAIDs inhibit both isozymes, it is not clear whether the known effects of NSAIDs on renal function are due to inhibition of COX-1, inhibition of COX-2, or a combination. COX-2 appears to be involved in renal homeostasis to a greater degree than previously believed, therefore, the concept that selective COX-2 inhibitors are renal-sparing requires reconsideration. The effects of these agents will need to be assessed in patients in whom COX-2 is upregulated, including patients with compromised renal function, patients taking an angiotensin-converting enzyme (ACE) inhibitor and diuretics for hypertension, and patients with volume overload and borderline cardiac decompensation.

APPEARS THIS WAY ON ORIGINAL

ROLE OF COX-2 IN FERTILITY AND REPRODUCTION

ROLE OF COX-2 IN FERTILITY AND REPRODUCTION

Prostaglandins play a key role in ovulation and pregnancy, and inhibition of prostaglandin synthesis blocks ovulation in several species. Following fertilization, development of the embryo during the blastocyst stage and synchronized differentiation of the uterus depend on the coordinated effects of estrogen, progesterone, and vasoactive prostaglandins. COX-1 and COX-2 are expressed in uterine tissues at various times during pregnancy (Vane et al, 1998). Because the presence of COX-2 in the uterus has only recently been established, the roles of COX-1 and COX-2 in reproductive functions remain to be determined. Many prostaglandin effects initially thought to be associated with constitutively expressed COX-1 may actually be attributed to COX-2. Indeed, one author suggested that ovulation and implantation are processes that may be considered analogous to a proinflammatory response (Lim et al, 1997).

COX-2 EXPRESSION

During pregnancy, COX-2 is highly involved in a number of crucial events. Animal studies indicate that COX-2 expression is essential for ovulation, fertilization, implantation, and decidualization (Lim et al, 1997; Song et al, 1998). Remarkably, COX-2 expression appears to be precisely timed with respect to ovulation, although timing varies from species to species (Boerboom and Sirois, 1998; Liu et al, 1997; Richards, 1997; Sirois, 1994; Sirois and Doré, 1997). COX-2 is induced in rat preovulatory follicles by luteinizing hormone and human chorionic gonadotropin (Sirois and Richards, 1992). Studies in bovine endometrial cells showed that oxytocin stimulated prostaglandin $F_{2\alpha}$ production, and that this correlated with upregulation of COX-2 gene expression during luteolysis (Asselin et al, 1997; Xiao et al, 1998). COX-2 levels increase significantly before and after labor, and prostaglandins produced by COX-2 may be necessary for delivery of the fetus (Gibb

and Sun, 1996; Slater et al, 1998). In bovine uterine tissues, COX-2 is the predominant isoform and concentrations increase during late pregnancy (Fuchs et al, 1998). In cultured cells, significant levels of COX-1 and COX-2 also are expressed in human umbilical endothelial cells and smooth muscle cells, although minute amounts of the two isoenzymes are found in biopsies from human umbilical arteries and veins (Öst et al, 1998).

Reproductive data elucidated from COX-2 knockout mice are particularly interesting and suggest that COX-2 has an essential role in maintaining fertility and is necessary during each stage of pregnancy (Lim et al, 1997; Majerus, 1998). In COX-2 knockout mice, females lacking COX-2 are infertile, having defective ovulation and failure of fertilization (Dinchuk et al, 1995; Lim et al, 1997). Although ovarian follicular development appears normal in COX-2 knockout mice, ovaries are small because of the absence of corpora lutea (Dinchuk et al, 1995). Female COX-2 knockout mice produce half as many ova per cycle as wild-type mice, and none of the ova was fertilized during mating attempts (Lim et al, 1997). COX-1 knockout mice are fertile, although the homozygous matings tend to result in stillborn normal-size litters. The cause for litter death in COX-1 knockout mice remains undetermined (Langenbach et al, 1995).

In addition to impaired oocyte maturation, COX-2 knockout mice have failed decidualization of the uterus and defective implantation of blastocysts (Lim et al, 1997; Majerus, 1998). Blastocyst implantation was noted in only 1% of COX-2 knockout mice compared with 50% of wild-type mice (Lim et al, 1997). During the implantation period, COX-2 also is highly expressed in the embryo. In ovine embryos, COX-1 is absent, whereas COX-2 is expressed during days 8 to 17 following fertilization, suggesting that COX-2 has an early role in embryonic development and interaction with the uterus (Charpigny et al, 1997).

Decidualization of the uterus also is impaired in COX-2 knockout mice. In endometrial stromal cells isolated from rats that have been sensitized for decidualization, both COX-1 and COX-2 are produced in response to epidermal growth factor (Bany and Kennedy, 1997). However, COX-2 knockout mice that are artificially induced to a pseudopregnant stage via oil infusion into the uterus fail to develop increased uterine weight, indicating that decidualization does not occur in these mice (Lim et al, 1997). Thus, implantation and decidualization are impaired in the absence of COX-2.

Prostaglandins produced in response to COX-2 also play an important role in parturition. In human amnion at term, COX-2 expression increases after the onset of labor, and expression of COX-2 mRNA is 100-fold greater than that of COX-1 mRNA (Slater et al, 1995, 1994). Additionally, COX-2 expression in the amnion increases twofold in laboring women compared with nonlaboring women who deliver via cesarean section, suggesting that COX-2 is necessary for spontaneous labor (Fuentes et al, 1996). COX-2 expression also increases in chorion-decidual cells with the onset of labor (Slater et al, 1998). Both COX-1 and COX-2 are expressed in the myometria during pregnancy. During term pregnancy, COX-2 expression is greater than COX-1 expression in the myometrium (Zuo et al, 1994). These findings suggest that COX-2 is responsible for prostaglandin production during labor and that selective inhibition of the COX-2 isoenzyme may delay spontaneous labor in pregnant women.

In the human placenta at term, COX-2 is the predominant cyclooxygenase isoform and COX-1 is detected only in negligible quantities (Macchia et al, 1997). Using a rat model for preterm labor, COX-2 expression was rapidly stimulated in the placenta in response to lipopolysaccharide (Swaisgood et al, 1997). Thus, it has been suggested that COX-2 is responsible for placental production of prostaglandin E_2, which is essential for maturation and maintenance of the placenta. This may correlate with the antiapoptotic and growth-promoting properties of prostaglandins

produced by the COX-2 isoenzyme. In the human placental bed, COX-1 expression is increased in preeclampsia, whereas COX-2 expression remains the same as in normal pregnancies (Wetzka et al, 1997). Both cyclooxygenase isoenzymes are increased in preeclamptic umbilical cord vessels (Beharry et al, 1998).

The role of prostaglandins in male reproductive functions remains largely unknown. There is evidence that COX-2-derived prostaglandins may be involved in erection. In rats, constitutively expressed COX-2 is the predominant isoform in the male reproductive system and is primarily located in the epithelium of the distal vas deferens (McKanna et al, 1998). It has been postulated that prostaglandins from the vas deferens are largely responsible for erectile function.

USE OF SELECTIVE COX-2 INHIBITORS IN ANIMAL MODELS

COX-1 knockout mice have normal blastocyst implantation. However, treatment with high doses of a selective COX-2 inhibitor resulted in failure of implantation (Lim et al, 1997). Similar effects were noted when wild-type mice were treated with high-dose, selective COX-2 inhibitors.

USE OF SELECTIVE COX-2 INHIBITORS IN MAN

Although data on potential reproductive effects of selective COX-2 inhibitors have not been published, there is a report of infertility, associated with luteinized, unruptured follicles, in three women who received NSAIDs for arthritis (Smith et al, 1996). Ovulation occurred in each patient when the NSAIDs were discontinued. In view of the important role of COX-2 in ovulation, this occurrence may have been related to inhibition of COX-2.

HYPOTHETICAL APPLICATION TO HUMANS OF PRECLINICAL RESULTS IN REPRODUCTION AND FERTILITY

Preclinical data from COX-2 knockout mice and studies with selective COX-2 inhibitors in mice suggest that expression of COX-2 is an essential feature of ovulation and pregnancy and that complete inhibition of COX-2 may prevent normal ovulation. Furthermore, these findings suggest that the case reports of infertility in humans may be related to COX-2 inhibition. Thus, it may be speculated that selective COX-2 inhibition may produce unwanted effects on fertility.

In addition, data from COX-2 knockout mice show that COX-2 expression is necessary for each step of pregnancy (eg, ovulation, fertilization, implantation, and decidualization). Prostaglandins generated by COX-2 are necessary for maintaining pregnancy in several species. Therefore, selective inhibition of COX-2 may interfere with pregnancy in several ways (Majerus, 1998). Agents that selectively inhibit COX-2 probably will require the same precautions as other NSAIDs with respect to use during pregnancy.

Finally, irrespective of effects on pregnancy, the effects of selective COX-2 inhibition on corpus luteum development in nonpregnant women may result in long-term hormonal changes that may affect estrogen-sensitive tissues, such as uterus, breast, and bone.

APPEARS THIS WAY ON ORIGINAL

ROLE OF COX-2 IN THE CENTRAL NERVOUS SYSTEM

ROLE OF COX-2 IN THE CENTRAL NERVOUS SYSTEM

It is unclear whether NSAID-induced central nervous system effects, such as headache, dizziness, drowsiness, and confusion (Insel, 1990; Kaplan and Swain, 1993) are directly related to cyclooxygenase inhibition. Although the cellular source of prostaglandins in the brain is uncertain, prostaglandins are implicated in several brain functions, including control of sleep-wake cycles, temperature regulation, and pituitary hormone production (Caggiano et al, 1996; Kaufmann et al, 1997). In certain regions of the brain, COX-2 is expressed by neurons, and expression increases during neuronal activity. The cyclooxygenase isoenzymes may have a role in neurologic disorders, cerebrovascular disease, migraines, neuronal death following ischemia, and in Alzheimer's disease, although the precise roles of the two isoenzymes remain to be determined (Kaufmann et al, 1997; Osuka et al, 1998; Tocco et al, 1997).

COX-2 EXPRESSION

The animal brain, and probably the human brain as well, contains high concentrations of prostaglandins D_2 and E_2, which may be involved in sleep regulation (Hayaishi, 1991). In animal models, COX-1 is found mainly in the forebrain, where prostaglandins may be involved in sensory processing (Yamagata et al, 1993). The human brain contains equal amounts of mRNA for COX-1 and COX-2, and both isoenzymes are expressed constitutively in the brain and spinal cord (O'Neill and Ford-Hutchinson, 1993; Resnick et al, 1998; Vane et al, 1998).

Although COX-1 is constitutively expressed in most tissues, there is evidence that COX-1 expression may be induced in rat cerebral cortex following ischemia (Holtz et al, 1996). COX-2 also is induced following cerebral ischemia and is associated with a significant increase in prostaglandin E_2 production (Collaco-Moraes et al, 1996;

Nogawa et al, 1997). Data from animal studies suggest that COX-2 may be involved in the mechanisms of delayed neuronal death following a cerebral infarct (Iadecola and Ross, 1998; Nogawa et al, 1997; Osuka et al, 1998), and similar findings have been observed in the infarcted human brain (Sairanen et al, 1998). COX-2 also is induced in the hippocampus during intense nerve stimulation (eg, seizures) and in the cerebral cortex during acute stress, suggesting that COX-2 is upregulated during neuronal activity (Marcheselli and Bazan, 1996; McCown et al, 1997; Yamagata et al, 1993). Following brief, noninjurious electrical stimulation in rat brains, COX-2 levels remained elevated for weeks and may have sustained effects on brain function (Caggiano et al, 1996).

COX-2 expression is induced in the brain by the presence of pyrogenic substances, such as interleukin-1 and tumor necrosis factor (Cao et al, 1998, 1996). In rodents, COX-2 induced by interleukin-1 is responsible for the production of fever-producing prostaglandins (Cao et al, 1996). COX-2 clearly is involved in the febrile response (Cao et al, 1998; Matsumura et al, 1997). Using a murine model of infection, systemic injection of lipopolysaccharide led to robust expression of COX-2 in perivascular cells and the choroid plexus (Breder, 1997).

Aside from pathologic expression, low levels of COX-2 mRNA are detected in the animal brain under basal conditions, particularly in neonates (Breder et al, 1995; Parfenova et al, 1997; Yamagata et al, 1993). In the newborn pig, both cyclooxygenase isoenzymes are constitutively expressed in cerebral microvessels and microvascular endothelium (Parfenova et al, 1997). In the rat brain, COX-2 is constitutively expressed in the cortex, hippocampus, hypothalamus, and spinal cord (Breder et al, 1995; Breder and Saper, 1996). In rat cerebral cortex, COX-2 is located in the discrete dendritic branches and dendritic spines of excitatory pyramidal neurons. These findings suggest that COX-2 has a direct effect on postsynaptic signaling of excitatory neurons (Kaufmann et al, 1996). COX-2 expression is noted in the specific corticallaminae and subcortical nuclei. Within

the amygdala, COX-2 is observed in the caudal and posterior of the deep and cortical nuclei, and in the diencephalon COX-2 appears in the paraventricular nucleus of the hypothalamus and in the anteroventral region surrounding the third ventricle. In the brainstem of the rat, COX-2 appears in the dorsal raphe nucleus, the nucleus of the brachium of the inferior colliculus, and in the region of the subcoeruleus (Breder et al, 1995). The highest basal levels of COX-2 in the rat appear in the hippocampus, the pyramidal cells of the piriform cortex, neocortex, and amygdala complex (Breder et al, 1995; Yamagata et al, 1993). Lower levels have been found in the caudate-putamen, thalamus, hypothalamus, superficial layers of the neocortex, striatum, and preoptic area (Cao et al, 1995; Yamagata et al, 1993). Although the physiologic significance of constitutively expressed COX-2 is not known, varying levels of COX-2 expression in the brain suggest that it may have a variety of different functions under normal physiologic conditions.

Unlike COX-1, COX-2 is not diffusely located within the rat brain. Rather, it is discretely organized and may be localized in specific membrane compartments (Breder et al, 1995; Kaufmann et al, 1997). The location and distribution of COX-2 in rodent brains indicate that COX-2 may be involved in processing visceral and sensory input. In addition, COX-2 may play a role in the generation of autonomic, endocrine, and behavioral responses (Breder et al, 1995).

COX-2 also is expressed in the spinal cord of rats and may be involved in spinal nociception (Beiche et al, 1996; Hay et al, 1997; Ichitani et al, 1997; Willingale et al, 1997; Yamamoto and Nozaki-Taguchi, 1996). Studies using the rat have shown that COX-1 and COX-2 mRNA are expressed constitutively in the spinal cord and that COX-2 is the predominant isoform (Beiche et al, 1996; Willingale et al, 1997). These studies indicate that both cyclooxygenase isoforms may play a role in spinal nociception.

USE OF SELECTIVE COX-2 INHIBITORS IN ANIMAL MODELS

In newborn pigs, prostaglandin receptor densities were increased and prostaglandin E_2 and $F_{2\alpha}$ levels were significantly reduced by ibuprofen and selective COX-2 inhibitors, but were not affected by a COX-1-specific inhibitor. Treatment with ibuprofen and selective COX-2 inhibitors also increased blood pressure in the cerebral cortex of newborns (Li et al, 1997). It was postulated that these prostaglandins are important in maintaining cerebral blood flow in the newborn brain (Peri et al, 1995). In newborn pig cerebral microvasculature, selective COX-2 inhibition significantly decreased prostanoid synthesis and may have a substantial effect on newborn cerebral circulation (Parfenova et al, 1997).

Using the formalin test in rats as a model for pain, one study noted that a nonselective cyclooxygenase inhibitor, ibuprofen, effectively blocked prostaglandin E_2 release and decreased hyperalgesic response. Notably, administration of two different selective COX-2 inhibitors was ineffective, suggesting that in acute situations, spinal COX-1, but not COX-2, is responsible for synthesis of prostaglandin E_2 and nociception (Dirig et al, 1997).

In vitro studies have found that some NSAIDs may exhibit proinflammatory effects, such as generation of superoxide in neutrophil cells (Twomey and Dale, 1992). One analysis of rat mesangial cells found that treatment with a selective COX-2 inhibitor induced COX-2 mRNA (although the production of COX-2 metabolites was blocked) and significantly increased the level of inducible nitric oxide synthase produced by the cells. The authors of this study postulated that proinflammatory actions may account for unexplained adverse effects associated with selective COX-2 inhibition, although the effects may be restricted to the rat model. The clinical implications of these findings remain to be determined (Klein et al, 1998).

HYPOTHETICAL APPLICATION TO HUMANS OF PRECLINICAL RESULTS IN THE CENTRAL NERVOUS SYSTEM

Based on localization of COX-2 in the brain of animal models, particularly in newborns, it is possible that inhibition of COX-2 may be associated with gross sensory or behavioral changes. However, the role of COX-2 and the functional distinction between the two cyclooxygenase isoforms in the brain still remains unclear. Therefore, the effects of selective COX-2 inhibition in the human brain are unknown. Evidence from rat mesangial cells showed that selective COX-2 inhibition induces nitric oxide synthase and suggests that proinflammatory effects may be associated with adverse effects. The ability of COX-2 inhibitors to cross the blood-brain barrier remains to be investigated.

APPEARS THIS WAY ON ORIGINAL