Cytosolic phospholipase A2 (cPLA2) is the rate-limiting enzyme responsible for the generation of prostaglandins (PGs), which are bioactive lipids that play critical roles in maintaining gastrointestinal (GI) homeostasis. There has been a long-standing association between administration of cyclooxygenase (COX) inhibitors and GI toxicity. GI injury is thought to be induced by suppressed production of GI-protective PGs as well as direct injury to enterocytes. The present study sought to determine how pan-suppression of PG production via a genetic deletion of cPLA2 impacts the susceptibility to COX inhibitor–induced GI injury. A panel of COX inhibitors including celecoxib, rofecoxib, sulindac, and aspirin were administered via diet to cPLA2−/− and cPLA2+/+ littermates. Administration of celecoxib, rofecoxib, and sulindac, but not aspirin, resulted in acute lethality (within 2 weeks) in cPLA2−/− mice, but not in wild-type littermates. Histomorphological analysis revealed severe GI damage following celecoxib exposure associated with acute bacteremia and sepsis. Intestinal PG levels were reduced equivalently in both genotypes following celecoxib exposure, indicating that PG production was not likely responsible for the differential sensitivity. Gene expression profiling in the small intestines of mice identified drug-related changes among a panel of genes including those involved in mitochondrial function in cPLA2−/− mice. Further analysis of enterocyte mitochondria showed abnormal morphology as well as impaired ATP production in the intestines from celecoxib-exposed cPLA2−/− mice. Our data demonstrate that cPLA2 appears to be an important component in conferring protection against COX inhibitor–induced enteropathy, which may be mediated through affects on enterocytic mitochondria.

Key Words: cytosolic phospholipase A2; COX inhibitor; mitochondria; intestine.

Cytosolic phospholipase A2 (cPLA2) is the rate-limiting enzyme in the generation of arachidonic acid (AA), which is further metabolized by cyclooxygenase (COX) enzymes to generate prostaglandins (PGs). PGs are bioactive lipids that have been shown to be important in maintaining gastrointestinal (GI) homeostasis (Dey et al., 2006). They have been shown to play roles in regulating epithelial cell proliferation and apoptosis as well as in maintaining intestinal mucin production and vascular integrity (Dey et al., 2006; Wang and Dubois, 2006; Wang et al., 2005). The exposure of intestinal tissue to COX inhibitors reduces basal levels of PGs and thus can disrupt their ability to confer GI protection (Thiefin and Beaugerie, 2005; Whittle, 2004).

Adverse GI events in patients have been associated with the long-term use of nonselective COX inhibitors (Bjarnason et al., 1993; Davies et al., 2000; Thiefin and Beaugerie, 2005; Whittle, 2004). The damage profiles found in the small intestine of patients include ulceration, bleeding, stricture formation, increased intestinal permeability, and, in extreme cases, intestinal perforation that leads to peritonitis and sepsis (Davies et al., 2000). The development of selective COX-2 inhibitors such as celecoxib largely circumvented GI toxicity; however, there is a subpopulation that still experiences adverse events when exposed to this drug class (Bombardier et al., 2000; Juni et al., 2002; Reuben and Steinberg, 1999; Silverstein et al., 2000).

Non-steroidal anti-inflammatory drug (NSAID) toxicity to the GI mucosa is generally attributed to two underlying mechanisms: (1) compromised production of key PGs (e.g., PGE2) that are important in maintaining gut epithelial integrity and (2) a “topical” effect resulting from direct toxicity. The topical effect is associated with inhibition of enterocytic oxidative phosphorylation with accompanying mitochondrial vacuolization and decreased ATP production (Krause et al., 2003; Somasundaram et al., 1997, 2000). These effects can reduce cellular integrity and lead to increased intestinal permeability (Krause et al., 2003; Somasundaram et al., 1997, 2000). In the present study, we have evaluated the effects of NSAID exposure on mice with a compromised ability to generate prostanoids.

During the course of these studies, we observed a remarkable acute COX inhibitor–induced lethality that was dependent...
upon cPLA<sub>2</sub> genotype and limited to exposure of celecoxib, rofecoxib, and sulindac. Further examination using celecoxib revealed that lethality was associated with severe GI damage resulting in bacteremia and sepsis. Surprisingly, injury was unrelated to PG status as levels were equivalently reduced after drug exposure regardless of cPLA<sub>2</sub> status. Morphological and functional studies showed abnormalities in intestinal mitochondria after celecoxib exposure in cPLA<sub>2</sub><sup>−/−</sup> mice. Our results suggest that in the absence of cPLA<sub>2</sub>, COX inhibitors induce severe GI injury that may be related to impairment of enterocytic mitochondria.

**MATERIALS AND METHODS**

**Administration of COX inhibitors.** To determine the effects of COX inhibitors on mouse viability, cPLA<sub>2</sub><sup>−/−</sup> and cPLA<sub>2</sub><sup>+/−</sup> (n = 100) littermates were administered either control diet (5001 LabDiet, Purina Mills, St Louis, MO) or diet incorporated with one of the following drugs: 0.0075% rofecoxib (Merck & Co., Whitehouse Station, NJ), 0.15% celecoxib (Pfizer Inc., La Jolla, CA), 0.015% sulindac (Sigma-Aldrich, St Louis, MO), or 0.05% aspirin (Bayer, West Haven, CT) at clinically relevant doses previously reported in the literature (Gupta et al., 2004; Oshina et al., 2001; Perkins et al., 2003). For studies examining microsomal PG<sub>E</sub> synthase 1 (mPGES-1), a total of five mPGES-1<sup>−/−</sup> mice were administered 0.15% celecoxib-incorporated chow or control chow for a period of 2 weeks. Mice were housed in a ventilated, temperature-controlled (23°C ± 1°C), and immediately transferred to a Bactec Peds Plus/F bacterial culture bottle. Prior to peritoneal lavage, ethanol. Blood was collected by cardiac puncture in a sterile needle and syringe and aspirated immediately before transfer to a bacterial culture bottle. A sterile needle was inserted into the abdominal cavity, and sterile 1 ml containing bacterial growth broth (BD Biosciences). Prior to peritoneal lavage, blood was collected by cardiac puncture, and immediately transferred to a Bactec Peds Plus/F bacterial culture bottle. Samples were incubated for 20 min at 40°C and then dried under N<sub>2</sub> gas. Samples were analyzed using a temperature program of 10°C to 50°C followed by 5°C/min to 280°C with the final
Electron microscopy. In order to determine whether morphological abnormalities occur within intestinal mitochondria, 8 h after celecoxib-incorporated or control chow administration, a 5-cm piece of proximal small intestines from cPLA\(^2-/\) and cPLA\(^2+/+\) mice (n = 2) were excised and flushed with PBS and incubated in fixative comprised of 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4, for 3 h. Fixed tissues were then transferred to 0.1M cacodylate buffer. Small pieces of the intestinal mucosa were cut from proximal, middle, and distal portions, rinsed again with 0.1M cacodylate buffer, postfixed for 2 h in 1% osmium tetroxide/0.8% potassium ferri cyanide in 0.1M cacodylate, and stained in block with 1% aqueous uranyl acetate. The samples were then dehydrated with graded concentrations of ethanol, embedded in Polybed epoxy resin (Polysciences, Warrington, PA), and polymerized at 60°C. One-micron sections were cut with a glass knife using a Reichert Ultracut E ultramicrotome, collected on glass slides, stained with methylene blue-azure II, and observed under a light microscope. Thin sections (~70 nm) were cut from selected areas with a diamond knife, collected on Formvar-carbon–coated 200-mesh nickel grids, stained with uranyl acetate and lead citrate, and observed in a Philips CM10 transmission electron microscope (TEM). Criteria for abnormal mitochondria include swelling, paling of the matrix, disrupted cristae, or altered shapes.

**Gene expression profiling and data analysis.** Following 3 days of celecoxib or control treatment, intestines from cPLA\(^2-/\) and cPLA\(^2+/+\) mice (n = 4) were removed, and RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, CA) to examine gene expression changes induced by celecoxib exposure. All tissue samples were free of gross pathology to ensure no artificial alterations as a result of tissue injury. RNA samples were analyzed on an Agilent 2100 Bioanalyzer, and only those samples with an RNA integrity number greater or equal to 7 and a 28S:18S ratio greater than 1.7 were used for the arrays. The RNA was amplified and labeled with biotin using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin, TX). Biotinylated complementary RNA (750 ng) was mixed with 10 μl of the Illumina hybridization mix, and the samples were hybridized to MouseRef-8 v1.1 Sentrix BeadChip Array at 58°C for 16 h. The BeadChips were then washed, stained with streptavidin-cyanine3, and scanned on the Illumina BeadArray Reader. Images were then analyzed with the Illumina Bead Studio software, and values were reported as average signal for each gene. All data were then normalized using the rank invariant method. False signals were filtered out by eliminating all signals with a detection p value greater than 0.001. Significant differences in gene expression were determined by performing one-way ANOVA, and genes considered for analysis had a p value of ≤ 0.05. Significantly altered genes were entered into GeneSight 4.1.6 software (BioDiscovery, Inc., El Segundo, CA), and heat maps indicating gene signal intensity were generated by K-means clustering algorithm to determine differentially expressed gene patterns.

**Mitochondrial isolation and ATP measurement.** cPLA\(^2+/+\) and cPLA\(^2-/\) mice (n = 6) were given either control diet or diet containing 0.15% celecoxib or 0.05% aspirin 3 days prior to sacrifice in order to measure ATP production in intestinal mitochondria. All tissue samples were free of gross pathology to ensure no artificial alterations as a result of tissue injury. Upon sacrifice, small intestines were excised and enterocytic mitochondria were isolated as previously described (de Talamoni et al., 1985). Briefly, intestinal mucosa was scraped and homogenized in a dounce homogenizer containing Buffer A (0.23M mannitol, 0.07M sucrose, 0.1M EDTA, and 0.1M Tris-HCl). Homogenates were subjected to differential centrifugation to isolate mitochondria and resuspended in Buffer B (Buffer A without EDTA). ATP levels were measured using the ATP determination kit (Invitrogen) as per manufacturer’s instructions. Sample concentrations were calculated in relation to a standard curve using purified ATP. ATP concentrations were normalized to mitochondrial protein concentration determined by the Bradford method (Bradford, 1976).

**RESULTS**

**COX Inhibitors Induce Acute Mortality in cPLA\(^2-/\) Mice**

In order to test the impact of cPLA2 deletion on sensitivity to COX inhibitors, we administered a panel of COX inhibitors, including celecoxib, rofecoxib, sulindac, and aspirin to cPLA\(^2-/\) and cPLA\(^2+/+\) littermates at clinically relevant doses as previously reported in the literature (Gupta et al., 2004; Oshima et al., 2001; Perkins et al., 2003). Administration of celecoxib, rofecoxib, and sulindac, but not aspirin, produced the rapid onset of lethality (within 2 weeks) in cPLA\(^2-/\) mice but had no effect on cPLA\(^2+/+\) littermates (Figs. 1A–D). cPLA\(^2-/\) mice began to show signs of morbidity within 5 days (weight loss and lethargy) and remained moribund throughout the study period.

To establish the specificity of this effect, celecoxib-incorporated chow (0.15%) was administered to mice with a genetic deletion of mPGES-1 (n = 5), the rate-limiting enzyme in PGE\(_2\) production, for 2 weeks. Neither morbidity nor mortality was observed throughout the experimental period (data not shown).

**Celecoxib Induces Damage to the GI Tract of cPLA\(^2-/\) Mice**

In order to determine the cause of COX inhibitor–induced death in cPLA\(^2-/\) mice, cPLA\(^2+/+\) and cPLA\(^2+/+\) littermates were administered 0.15% celecoxib ( celecoxib was used as a representative drug for all subsequent studies) and mice were sacrificed immediately upon evidence of toxicity (weight loss exceeding 10%, lethargy, and dehydration determined by “tenting”), typically occurring between 5 and 9 days after the start of drug treatment. At necropsy, severe GI damage, including black intestinal content and marked distention of the small bowel, was observed only in cPLA\(^2-/\) mice (Figs. 2A and B). This gross pathology extended from the stomach to the ileocecal junction in a fairly even distribution, sparing the colon that was devoid of luminal contents. The observation that damage was limited to the small intestine may result from extensive drug absorption in the upper GI tract. The small intestine was fragile and upon closer inspection revealed multiple strictures and perforations (Figs. 2C and D). Histological analysis of the small intestine revealed areas of severe ulceration, peritonitis, and fecal material on the peritoneal side of the intestine, indicating that intestinal material had leaked out of the lumen and into the peritoneum.
In addition, we observed thymic atrophy and splenomegaly that was related to an expansion of the white pulp (data not shown). In contrast, there were neither adverse effects observed in the cPLA$^{+/+}$ mice treated with celecoxib nor was there evidence of intestinal damage in untreated mice of either cPLA$_2$ genotype.

(Figs. 2E and F). In contrast, there were neither adverse effects observed in the cPLA$^{+/+}$ mice treated with celecoxib nor was there evidence of intestinal damage in untreated mice of either cPLA$_2$ genotype.
The observed damage to the small intestine raised the possibility that lethality may occur as the direct result of translocation of bacterial species into the peritoneum. Thus, bacterial cultures of both the blood and the peritoneum were prepared from celecoxib-treated cPLA$_2^+/+$ and cPLA$_2^{-/-}$ mice. Bacteremia and peritonitis were identified only in the celecoxib-treated cPLA$_2^+/+$ group. As shown in Table 1, the spectrum of pathogens that were recovered from the peritoneum and blood suggest their intestinal origin, which included Escherichia coli, Enterococcus gallinarum, Streptococcus, and Clostridium perfringens. Bacterial cultures for control or celecoxib-administered cPLA$_2^+/+$ mice were negative (Table 1). The identification of these species outside of the intestines indicated a dramatic increase in intestinal permeability. The occurrence of sepsis was investigated in celecoxib-administered mice by the measurement of blood serum cytokine levels using ELISA. These analyses showed that whereas administration of celecoxib to wild-type mice had no effect on cytokine levels (Fig. 3A), significant elevation of the proinflammatory cytokines, MCP-1 and IL-6, and a trend for a reduction in the anti-inflammatory cytokine IL-10 were observed in cPLA$_2^{-/-}$ administered celecoxib for 5–9 days relative to the control diet group (Fig. 3B).

As cardiovascular toxicity is an important adverse effect of COX-2–selective inhibitors, we examined whether celecoxib-induced mortality was exacerbated by cardiovascular injury in cPLA$_2^{-/-}$ mice (Breyer, 2005; Grosser et al., 2006). Measurement of cardiac function using a working heart model as an indicator of myocardial infarction was tested in cPLA$_2^+/+$ and cPLA$_2^{-/-}$ mice before and after celecoxib administration. No differences were found among genotypes in the panel of heart function indices that were examined (Supplementary table 1). Thus, the acute lethality observed was likely to be independent of direct damage to cardiac tissue.

**cPLA$_2$ Status Affects AA Production after Celecoxib Exposure**

cPLA$_2$ is the rate-limiting enzyme in the release of free AA; therefore, we determined how genetic deletion of cPLA$_2$ would impact AA production in mice. AA levels were measured by GC/MS in the intestines of cPLA$_2^+/+$ and cPLA$_2^{-/-}$ mice with or without celecoxib exposure for 3 days. All tissue samples were free of gross pathology to ensure no artificial alterations as a result of tissue injury. As shown in Figure 4, under control conditions, cPLA$_2$ status did not impact intestinal AA production. However, after celecoxib exposure, there was a marked increase in AA levels by ~30% in cPLA$_2^+/+$ mice, an effect that was absent in cPLA$_2^{-/-}$ mice.

**Celecoxib Treatment Reduces PG Levels Independent of cPLA$_2$ Status**

The important roles of COX-derived PGs in the physiological function and maintenance of the intestinal epithelium have been well documented (Stenson, 2007; Wang et al., 2005). To provide further insight into underlying mechanisms of celecoxib-induced GI damage, we compared PG levels within

### TABLE 1

<table>
<thead>
<tr>
<th>Species</th>
<th>cPLA$_2^+/+$</th>
<th>cPLA$_2^{-/-}$</th>
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<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Enterococcus gallinarum</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>-</td>
<td>+</td>
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<tr>
<td><em>Clostridium perfringens</em></td>
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*Note.* Blood and peritoneal fluid were cultured from cPLA$_2^{-/-}$ and cPLA$_2^+/+$ mice following celecoxib administration (5–9 days) to determine whether bacterial species were present as described under Materials and Methods section.
the intestinal tissue of cPLA$^{+/+}$ and cPLA$^{-/-}$ mice before and after drug administration. All tissue samples were free of gross pathology to ensure no artificial alterations as a result of tissue injury. In the absence of drug, the levels of PGD$2$ and PGE$2$ were significantly higher in the cPLA$^{+/+}$ mice compared with cPLA$^{-/-}$ littermates (Fig. 5A). In addition, the levels in both prostaglandin F$_2\alpha$ and 6-keto-PGF$_1\alpha$ trended higher in the cPLA$^{+/+}$ intestines (Fig. 5A). Upon administration with celecoxib, however, intestinal PG levels were reduced to a comparable extent regardless of cPLA2 genotype (Fig. 5A).

To eliminate the possibility that sulfonamide-induced suppression of PG formation may underlie the drug-induced GI damage, PG levels were compared in mice administered aspirin as well. Whereas aspirin causes no apparent GI damage or lethality in these mice (Fig. 1D), previous studies have shown that aspirin reduces PG levels in the intestinal mucosa (Frieboes et al., 2001; Krishnan et al., 2001) and can thus serve as an appropriate comparison. Consistent with these earlier studies, liquid chromatography/mass spectrometry analysis revealed that aspirin reduces PG levels to a similar extent regardless of cPLA2 genotype (Fig. 5A).

Effects of Celecoxib Exposure on Intestinal Gene Expression Profiles

In the following experiment, global gene expression profiling was performed on intestinal tissue isolated from mice of both genotypes (cPLA$^{+/+}$ and cPLA$^{-/-}$) at 3 days after celecoxib exposure. A control group of mice fed regular diet was used for comparison. Analysis of heat maps generated by Genesight software showed 1579 genes that were significantly different when comparing the four groups as determined by one-way ANOVA (Fig. 6A). Further analysis of the 1579 genes identified a subset of 23 genes that were upregulated to a greater extent after drug exposure in cPLA$^{-/-}$ mice as compared with cPLA$^{+/+}$ mice (Fig. 6B). Many of these genes encode for proteins involved in mitochondrial function including oxidase (cytochrome c) assembly 1-like (Oxa1l) and aldolase C, fructose-bisphosphate (Aldoc) as well as other cellular processes including...
cellular transport and Golgi and endoplasmic reticulum (ER) function as shown in Figure 6B.

**Mitochondrial Abnormalities Are Induced after Celecoxib Exposure in cPLA$_2^{−/−}$ Mice**

As we observed differential expression of genes involved in mitochondrial function between genotypes and COX inhibitors have been shown to induce enterocytic mitochondrial abnormalities after short-term exposure, we determined whether morphological changes were occurring in the mitochondria. In order to examine early mitochondrial changes in the intestines of mice, we exposed cPLA$_2^{+/+}$ and cPLA$_2^{−/−}$ mice to celecoxib-incorporated or control chow for 8 h and analyzed morphology by TEM. As shown in Figures 7A and 7B, enterocytes from cPLA$_2^{+/+}$ mice exhibited normal appearing mitochondria both under control conditions and after celecoxib exposure. Mitochondria from enterocytes of untreated cPLA$_2^{−/−}$ mice appear to have slight abnormalities, which may represent an underlying susceptibility to drug-induced damage. However, mitochondria from celecoxib-treated mice frequently exhibited abnormal mitochondria, including swelling and paling of the matrix, as well as disrupted cristae or altered shapes (criteria are described in detail under Materials and Methods section) (Figs. 7C and D).

We next sought to determine whether early morphological changes in mitochondria were associated with impaired mitochondrial function at a later time point of drug exposure. To test this possibility, mitochondria were isolated from...
intestinal enterocytes after 3 days of celecoxib exposure and the rate of ATP production was determined in vitro. All tissue samples were free of gross pathology to ensure no artificial alterations as a result of tissue injury. As shown in Figure 8A, celecoxib exposure to cPLA2−/− mice resulted in a ~60% reduction in ATP production, whereas drug treatment had no effect on ATP generation in mitochondria isolated from of wild-type littermates (Fig. 8A). No effect on ATP generation was found in either genotype after aspirin administration (Fig. 8B).

**DISCUSSION**

The present study demonstrates that genetic deletion of cPLA2 results in a lethal susceptibility to certain COX inhibitors including celecoxib. This lethality was associated with severe GI damage associated with bacteremia and sepsis. Gene expression analysis in the intestines showed a number of differentially expressed genes including a set associated with mitochondrial function after celecoxib exposure. Further examination of intestinal mitochondria revealed abnormalities in morphology and function after celecoxib exposure in cPLA2−/− mice.

Previous studies have shown nonselective COX inhibitors to induce direct damage to enterocytic mitochondria when orally or directly administered to the intestines of rats, known as a topical effect (Somasundaram _et al._, 1997, 2000). Analysis of mitochondrial morphology by electron microscopy revealed vacuolization and ballooning of the organelle with a concomitant uncoupling of oxidative phosphorylation determined by measuring oxygen consumption as a marker for respiration in isolated mitochondria (Somasundaram _et al._, 1997, 2000). These studies also demonstrated that orally administered aspirin did not induce this type of
mitochondrial injury. Aspirin and other NSAIDs differ largely in their structure and metabolism. Many NSAIDs, including those examined in the present study, have a sulfur group as part of their structure, whereas aspirin does not. It is believed that this drug class can act as protonophores, which are classic uncouplers of oxidative phosphorylation and accumulate at high concentrations within the mitochondrial membrane thus disturbing the critical balance of the proton gradient (Krause et al., 2003). Additionally, unlike aspirin, a number of these inhibitors have been shown to undergo enterohepatic recirculation, thereby prolonging exposure of the small intestine to drug damage (Huntjens et al., 2008).

Our study is the first to demonstrate this type of injury to the rodent intestine using a selective COX-2 inhibitor. As we have observed a very similar pattern of damage with resulting deficiencies in mitochondrial function, including a reduction in ATP generation, we speculate that the selective COX-2 inhibitor, celecoxib, is acting as a classic uncoupler of oxidative phosphorylation in the mouse intestine in the absence of cPLA_2_. These previous studies indicate likely non–COX-related effects of these drugs on the intestinal mucosa. Given our findings of mitochondrial injury as well as the observation that there is an equivalent reduction of PG levels in both genotypes after drug exposure (Fig. 5), we suspect that the phenotype observed in cPLA_2^{−/−} mice could be independent of COX-related activity.

ATP production is a critical component to normal cellular function and integrity. Disturbances in mitochondrial membrane permeability are important steps leading to cell death (Lemasters, 2005). Mitochondrial function in intestinal epithelial cells is of particular importance given their role in maintaining gut barrier function (Nazli et al., 2004; Somasundaram et al., 2000). A number of studies have shown that perturbations in ATP generation can lead to impaired cellular respiration and loss of gut barrier function. For example, in vivo exposure of the intestinal mucosa to 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, resulted in increased junction permeability determined by ion conductance and transepithelial flux of horseradish peroxidase or 51CrEDTA (Nazli et al., 2004; Somasundaram et al., 2000). Given that we observe impairment in ATP generation after celecoxib exposure in those mice experiencing GI damage, it is possible that a reduction in ATP production leads to loss of gut integrity in our model.

The exact mechanism by which cPLA_2_ deletion confers susceptibility to COX inhibitors is not clear. Given that mitochondria have been shown to utilize FAs to generate ATP, especially under pharmacological stress, it is possible that the inability to generate FAs impair proper mitochondrial function in our model (Hagen et al., 2002; Virmani et al., 2003, 2005). It has been demonstrated that L-carnitine, a carrier molecule that is critical for the transport and oxidation of FAs into mitochondria, elicits a protective effect in neuronal cells following exposure to drugs which impair mitochondrial enzyme function (Hagen et al., 2002; Virmani et al., 2003, 2005). These studies suggest that a shift occurs in the source for energy generation from glucose to FAs under pharmacological stress.

Given the important role of cPLA_2_ in releasing AA, we examined AA production in mouse intestines. Under control conditions, cPLA_2_ status did not impact basal AA levels. This finding indicates that cPLA_2_ might not be critical to generate AA in the steady state. However, after drug challenge, we observed impaired AA production in cPLA_2^{−/−} mice. This observation suggests that the enzymatic activity of cPLA_2_ is most critical when the gut is exposed to exogenous insult. Additionally, it could be reasoned that other PLA_2_ such as the secretory PLA_2_ could have compensatory enzymatic actions to generate AA under basal conditions but are not sufficient to release AA upon drug challenge. Although we did not find a difference in the levels of AA under control conditions, we did find impaired intestinal PG production in cPLA_2^{−/−} mice. Studies have demonstrated that upon stimulation, cPLA_2_ and COX-2 colocalize at organelle membranes to generate PGs (Bidgood et al., 2000; Grewal et al., 2005). Loss of cPLA_2_ in our model would not allow for colocalization of these two enzymes and might account for the impaired PG production regardless of AA levels.

Global gene expression profiling of intestinal tissue identified a large number of expression changes induced by celecoxib exposure. Many of these genes are found to be involved in mitochondrial function including Oxa1l and Aldoc, which are known to be important mediators of energy generation via mitochondrial respiration (Jian et al., 2010; Stiburek et al., 2007). These genes were all found to be more markedly upregulated after drug exposure in cPLA_2^{−/−} mice, which might reflect a tissue response to attempt to increase rates of mitochondrial respiration. However, there was impaired ATP production in these same groups; therefore, it is possible that in the absence of cPLA_2_, other steps in the energy generation pathway are defective. Although in this study we have focused primarily on celecoxib-induced alterations in mitochondrial function, it is possible that celecoxib may also affect other key signaling pathways that may contribute to the exaggerated lethality observed in the cPLA_2^{−/−} mice. A number of genes encoding proteins that are involved in cellular transport processes, Golgi and ER function, as well as a number of transmembrane proteins (Tmems), were all found to be induced after celecoxib exposure to a greater extent in the cPLA_2^{−/−} mice. These differentially expressed genes include ATP-binding cassette transporter class f (Abcf) and solute carrier family 31 that differ in their expression profiles.
transport, assembly of Golgi apparatus, and protein accumulation in the ER, respectively (Kanekura et al., 2009; Saito et al., 2009; Xiang and Wang, 2010). Additionally, genes encoding several Tmems were also found to be more highly expressed in the cPLA$_2$$^{-/-}$ mice. These genes include Tmem 111 and Tmem66, a set of proteins that play a role in organelle stress response as well as in maintenance of the cellular redox environment (Nayak et al., 2009; Romanuik et al., 2009). Each of these processes may be critical in maintaining cellular homeostasis, and the toxicological outcome of their dysregulation as a result of COX inhibitor administration is likely exaggerated by the absence of cPLA$_2$. Although the use of coxibs has largely circumvented the GI toxicity associated with COX inhibition, a subset of patients have continued to experience adverse GI events, suggesting the possibility of an underlying genetic susceptibility (Bombardier et al., 2000; Juni et al., 2002; Reuben and Steinberg, 1999; Silverstein et al., 2000). The basis to this differential response to coxibs is unknown but could involve dysregulation of genes within the AA cascade, including the COXs and PLA$_2$ isoforms. cPLA$_2$ appears to be the most critical PLA$_2$ in the release of AA and, positioned upstream of the COX enzymes, directly controls the release of AA substrate. Several studies have identified the presence of genetic polymorphisms on the cPLA$_2$ gene within human populations, including CA and poly A repeats within the promoter region, although these genetic variants were not shown to lead to any adverse clinical events (Chowdari et al., 2001; Pae et al., 2004; Tay et al., 1995). More recently, a case was reported of a patient who had diminished basal cPLA$_2$ expression as a result of two heterozygous single basepair mutations on the cPLA$_2$ gene. This defect was associated with spontaneous small intestinal ulcerations (Adler et al., 2008). Based in part on these recent data, we postulate that a deficiency in cPLA$_2$ might partially explain individual susceptibility to drug-related GI toxicity.

Our findings have demonstrated that certain COX inhibitors induce severe damage to the GI tract of cPLA$_2$$^{-/-}$ mice, leading to death. We show that in the absence of cPLA$_2$, intestines are susceptible to a number of genetic alterations including those involved in mitochondrial function. These gene changes are associated with abnormalities of mitochondrial morphology and ATP generation. We propose that impaired mitochondrial function leads to reduced cellular integrity and as a result produces septicemia with concomitant acute lethality. Susceptibility to COX inhibitor–induced GI injury in mice with a genetic deletion in cPLA$_2$ might serve as a representative model for the human subpopulation described.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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