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Dual Modulation of Cyclooxygenase and CYP Epoxygenase Metabolism and Acute Vascular Inflammation in Mice

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Abstract

Cyclooxygenase (COX)-derived prostaglandins and cytochrome P450 (CYP) epoxygenase-derived epoxyeicosatrienoic acids are important regulators of inflammation; however, functional interactions between these pathways in the regulation of vascular inflammation *in vivo* have not been studied. We investigated the relative and additive effects of endothelial CYP2J2 overexpression (*Tie2-CYP2J2*-Tr), global sEH disruption (*Ephx2*$^{-/-}$), and pharmacologic COX inhibition with indomethacin on the acute vascular inflammatory response to endotoxin in mice. Compared to vehicle-treated wild-type C57BL/6 controls, induction of myeloperoxidase (MPO) activity in lung and liver was similarly attenuated in *Tie2-CYP2J2*-Tr mice, *Ephx2*$^{-/-}$ mice and wild-type mice treated with moderate dose indomethacin. Dual modulation of both pathways, however, did not produce an additive anti-inflammatory effect. These findings demonstrate that both COX and CYP epoxygenase-mediated eicosanoid metabolism are important regulators of the acute vascular inflammatory response *in vivo*, and suggest that the anti-inflammatory effects of modulating each pathway may be mediated, at least in part, by overlapping mechanisms.

Keywords

CYP2J2; soluble epoxide hydrolase; cyclooxygenase; vascular inflammation; epoxyeicosatrienoic acid; prostaglandin

INTRODUCTION

Vascular inflammation is characterized by endothelial activation and subsequent infiltration of leukocytes into the surrounding tissue [1]. Nuclear factor-kappa B (NF-κB) initiates and propagates this coordinated process through transcriptional activation of cellular adhesion

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molecules (CAMs), chemokines, and cytokines [2,3]. This fundamental pathological process plays a pivotal role in the pathogenesis of numerous acute and chronic inflammation-related diseases such as sepsis, asthma, and cardiovascular disease (CVD). Thus, identification of the key pathways that regulate NF-κB-dependent vascular inflammatory responses offers enormous potential to facilitate the development of novel anti-inflammatory therapeutic strategies.

Eicosanoids are biologically active fatty acids derived through oxidative metabolism of arachidonic acid by the cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzyme systems [4]. It is well established that COX-derived prostaglandins are integral regulators of inflammation, such that inhibition of COX-mediated prostaglandin biosynthesis produces potent anti-inflammatory effects in preclinical models and humans [5]. Most notably, prostaglandin E2 (PGE2) and thromboxane A2 (TxA2) are key pro-inflammatory products of this pathway that activate NF-κB, promote leukocyte infiltration into tissue, and thus drive the vascular inflammatory response [6,7].

In parallel, CYP epoxygenases from the CYP2J and CYP2C subfamilies catalyze the metabolism of arachidonic acid into epoxyeicosatrienoic acids (EETs), which have become increasingly recognized to possess potent anti-inflammatory properties [8]. The life of circulating EETs, however, is ephemeral as they are quickly hydrolyzed into the less potent dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH, *EPHX2*). We have previously shown that potentiation of CYP epoxygenase-derived EETs by endothelial CYP2J2 overexpression (*Tie2-CYP2J2*-Tr), endothelial CYP2C8 overexpression (*Tie2-CYP2C8*-Tr) or global sEH disruption (*Ephx2*$^{-/-}$) attenuates NF-κB-dependent acute vascular inflammatory responses *in vivo* [9].

Past studies have shown that inhibition of one arachidonic acid pathway may shunt metabolism down another parallel path [10,11]. Furthermore, additive attenuation of lipopolysaccharide (LPS)-induced pain and hypotension from simultaneous augmentation of CYP-derived EETs in tandem with cyclooxygenase (COX) inhibition has been recently reported [12,13]. However, functional interactions between the COX and CYP epoxygenase pathways in the regulation of acute vascular inflammation *in vivo* have not been studied. Consequently, we investigated the relative and additive effects of endothelial CYP2J2 overexpression, global sEH disruption, and pharmacologic COX inhibition on the acute vascular inflammatory response to the endotoxin LPS, a well-established activator of NF-κB-dependent endothelial activation and leukocyte infiltration [14].

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise noted.

Animal

All experiments were completed in adult male and female mice on a C57BL/6 background. Transgenic (Tr) mice that express human CYP2J2 in endothelial cells under control of the murine *Tie2* promoter and full enhancer (*Tie2-CYP2J2*-Tr) were developed on a pure C57BL/6 background, as previously described [15]. A colony of mice with targeted disruption of *Ephx2* (*Ephx2*$^{-/-}$) was rederived and backcrossed onto a C57BL/6 genetic background for more than 10 generations [16,17]. We have previously reported significantly higher endothelial EET biosynthesis in *Tie2-CYP2J2*-Tr mice and circulating epoxide:diol ratios in *Ephx2*$^{-/-}$ mice, compared to wild-type (WT) littermates, consistent with CYP epoxygenase overexpression and sEH disruption, respectively [9,15,17,18]. All experiments

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with *Tie2-CYP2J2*-Tr mice used Tr mice from multiple founder lines and WT littermates as controls. Experiment 2, which did not involve the genetically-modified mice, utilized commercially available C57BL/6 mice (Taconic, Hudson, NY, USA). All mice had free access to food and water, and were housed in controlled conditions for temperature and humidity using a 12-hour light/dark cycle. All experiments were completed in accordance with the *US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill and the National Institute of Environmental Health Sciences.

Experimental Protocol

Wild-type C57BL/6, *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ mice weighing 18–31 grams were pretreated with the nonselective COX inhibitor indomethacin (1, 2.5, 5 or 10 mg/kg) or vehicle (0.5% carboxymethylcellulose) once daily for 3 days via oral gavage. On day 3, one hour following indomethacin or vehicle administration, mice received a single dose of *Escherichia coli* LPS (serotype O111:B4, 1,000,000 EU/mg; 10 mg/kg) or endotoxin-free phosphate-buffered saline (PBS) by intraperitoneal injection. Mice were euthanized by CO_2 inhalation 3 hours after LPS (or PBS) administration, as previously described [9]. Blood was collected by cardiac puncture, and plasma was separated by centrifugation. Lung and liver, highly vascularized tissues that exhibit prominent activation of NF-κB signaling and leukocyte infiltration *in vivo* upon LPS administration [14,19] were snap-frozen in liquid nitrogen and stored at −80°C for protein isolation.

Myeloperoxidase Activity

Myeloperoxidase (MPO) is a leukocyte-derived heme oxidase primarily carried by polymorphonuclear neutrophils [20]. Since MPO enzymatic activity in parenchyma predominantly reflects the presence of neutrophils, and correlates with immunohistochemical detection of neutrophil margination and tissue infiltration in this model [9], we utilized MPO functional activity as an *in vivo* biomarker of LPS-induced neutrophil infiltration and the primary index of vascular inflammation in these experiments. Briefly, frozen tissue (30 mg) was homogenized in 20 mM PBS with 10 mM N-ethylmaleimide (pH 7.4), and centrifuged at 10,000 x g for 15 minutes at 4°C. The pellet was resuspended in 50 mM PBS (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide, sonicated for 30 seconds, and centrifuged at 8,000 x g for 20 minutes at 4°C. MPO activity in the supernatant was quantified using the Fluorescent Myeloperoxidase Detection Kit (Cell Technology, Mountain View, CA, USA), according to the manufacturer's instructions. Fluorescence was quantified on a FLUOstar Omega Microplate Reader (IMGEN Technologies, Alexandria, VA, USA) at excitation/emission wavelengths of 530/590 nm.

Quantification of Eicosanoids

Plasma epoxy (8,9-, 11,12-, 14,15-EET), dihydroxy (5,6-, 8,9-, 11,12-, 14,15-DHET), and prostaglandin (PGE2, TxB2, 6-keto-PGF1α) metabolites of arachidonic acid were quantified using an established HPLC-MS/MS method, following solid-phase extraction, as described [17,21]. The sum total of EETs plus DHETs and the 14,15-EET:DHET ratio were used as biomarkers of CYP epoxygenase and sEH metabolic function, respectively [9,17]. In a subset of experiments, plasma PGEM (a stable metabolite of PGE2) and 11-dehydro-TxB2 (a stable metabolite of TxA2) concentrations were quantified using the Prostaglandin E Metabolite and 11-dehydro Thromboxane B2 EIA kits (Cayman Chemical, Ann Arbor, MI, USA), respectively, according to the manufacturer's instructions.

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Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) and were normalized to the PBS-treated WT control group unless otherwise indicated. Rank-transformed mean values were compared using a one-way ANOVA followed by Fisher's LSD post hoc test. Statistical analysis was performed using SAS 9.2 (SAS Institute, Cary, NC). P<0.05 was considered statistically significant.

RESULTS

Experiment 1: Relative effect of endothelial CYP2J2 overexpression and high-dose indomethacin

In pilot studies, high-dose (10 mg/kg) indomethacin significantly inhibited the LPS-mediated increase in plasma PGEM and 11-dehydro-TxB2 concentrations by 99±0.2% and 77±2%, respectively (p<0.05 versus vehicle, n=6–8 per group). The induction of lung MPO activity following LPS administration was significantly attenuated in both *Tie2-CYP2J2*-Tr and indomethacin (10 mg/kg)-treated WT mice compared to vehicle-treated WT mice (Figure 1, p<0.05 for each comparison). Furthermore, high-dose indomethacin attenuated the induction of MPO activity to a significantly greater degree than observed in *Tie2-CYP2J2*-Tr mice (67±9% versus 37±11%, respectively, p<0.05).

Experiment 2: Relative effect of incremental indomethacin doses

A dose-response experiment was conducted in order to identify an indomethacin dose that attenuated LPS-induced vascular inflammation to a similar degree as genetic potentiation of the CYP epoxygenase pathway. LPS-mediated induction of plasma PGEM (Figure 2A) and lung MPO activity (Figure 2B) was attenuated by indomethacin in a dose-dependent manner. Induction of liver MPO activity was also significantly attenuated by indomethacin at each dose compared to vehicle (Figure 2C); however, a dose-dependent attenuation was not observed. Since indomethacin 2.5 mg/kg produced a 38±5% attenuation of LPS-induced lung MPO activity, similar to the 37±11% attenuation observed in *Tie2-CYP2J2*-Tr mice from Experiment 1 and the attenuation observed in both *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ mice from prior studies [9], this "moderate" dose of indomethacin was utilized in subsequent experiments to evaluate the potential additive effect of simultaneously modulating both pathways.

Experiment 3: Relative and additive effect of endothelial CYP2J2 overexpression, global sEH disruption, and moderate-dose indomethacin

LPS-induction of lung MPO activity was significantly attenuated in indomethacin (2.5 mg/kg)-treated WT (47±3%), *Tie2-CYP2J2*-Tr (28±10%) and *Ephx2*$^{-/-}$ (31±8%) mice, compared to vehicle-treated WT controls (Figure 3A, p<0.05 for each comparison). MPO activity was decreased in indomethacin-treated *Tie2-CYP2J2*-Tr (46±5%) and *Ephx2*$^{-/-}$ (48±4%) mice to a level similar to that observed in indomethacin-treated WT mice; however, a significant additive anti-inflammatory effect was not observed (Figure 3A, p=0.848 and p=0.851, respectively, versus indomethacin-treated WT mice). Similar results across genotype and treatment groups were observed following LPS-induction of liver MPO activity (Figure 3B), although the observed attenuation in vehicle and indomethacin-treated *Tie2-CYP2J2*-Tr mice was not statistically significant (p=0.397 and p=0.054, respectively, versus vehicle-treated WT mice).

Indomethacin markedly inhibited the LPS-mediated induction of plasma PGEM, TxB2, and 6-keto-PGF1α levels in WT, *Tie2-CYP2J2*-Tr, and *Ephx2*$^{-/-}$ mice (Figure 4A–C, p<0.05 versus vehicle in each genotype group). In contrast, no differences were observed across genotype within the vehicle or indomethacin-treated groups (Figure 4A–C). Similar results

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were observed with plasma PGD2 and PGF2α levels, and no treatment or genotype differences in hepatic or pulmonary *Ptgs2* mRNA levels were observed (data not shown). The total sum of EET+DHET levels was approximately 1.3-fold higher in *Tie2-CYP2J2*-Tr mice compared to WT (p=0.064) and *Ephx2*$^{-/-}$ (p=0.047) mice (Figure 4D), consistent with prior studies [9,18]. The 14:15-EET:DHET ratio was drastically increased in *Ephx2*$^{-/-}$ mice compared to WT and *Tie2-CYP2J2*-Tr mice, irrespective of treatment (Figure 4E, p<0.05 for all comparisons). Indomethacin did not significantly alter the total sum of EET+DHET levels in *Tie2-CYP2J2*-Tr mice (p=0.092 versus vehicle) or the 14:15-EET:DHET ratio in *Ephx2*$^{-/-}$ mice (p=0.596 versus vehicle). Furthermore, no treatment or genotype differences in 5-LOX-derived 5-hydroxyeicosatetraenoic acid (5-HETE) or CYP ω-hydroxylase-derived 20-HETE levels were observed (data not shown).

DISCUSSION

Functional characterization of the key eicosanoid metabolism pathways that regulate vascular inflammatory responses *in vivo* offers enormous potential to facilitate the development of new anti-inflammatory therapeutic strategies. Consequently, we investigated the relative and additive effects of endothelial CYP2J2 overexpression, global sEH disruption, and pharmacologic COX inhibition on LPS-induced acute vascular inflammation in mice. Our studies demonstrate that: 1) high-dose indomethacin attenuates acute vascular inflammation to a significantly greater extent than genetic potentiation of the CYP epoxygenase pathway; 2) moderate-dose indomethacin attenuates acute vascular inflammation to a similar degree as either endothelial CYP2J2 overexpression or global sEH disruption; and, 3) dual modulation of the COX and CYP epoxygenase pathways does not additively attenuate the acute vascular inflammatory response to LPS. Collectively, these findings demonstrate that both COX and CYP epoxygenase-mediated eicosanoid metabolism are important regulators of the acute vascular inflammatory response *in vivo*, and suggest that the anti-inflammatory effects of modulating each pathway may be mediated, at least in part, by overlapping mechanisms.

Consistent with our prior studies and hypothesis, the acute vascular inflammatory response to LPS was significantly and similarly attenuated in *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ mice [9], further demonstrating that increased endothelial EET biosynthesis and decreased sEH-mediated EET hydrolysis elicit potent anti-inflammatory effects in the vasculature *in vivo*. Similarly, inhibition of COX-mediated prostaglandin biosynthesis with the nonselective COX inhibitor indomethacin significantly attenuated vascular inflammation, consistent with prior studies in various models of inflammation [5]. More specifically, administration of a moderate (2.5 mg/kg/day) dose of indomethacin elicited an approximate 80% reduction in LPS-induced plasma PGEM levels and a reduction in pulmonary and hepatic MPO activity equivalent to that observed in *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ mice. Administration of high (10 mg/kg/day) dose indomethacin, however, produced a complete abrogation of LPS-induced PGE2 biosynthesis and a significantly greater anti-inflammatory effect than genetic potentiation of the CYP epoxygenase pathway. These results suggest that maximizing inhibition of COX-mediated prostaglandin biosynthesis yields a greater anti-inflammatory effect than maximizing inhibition of EET hydrolysis via genetic disruption of *Ephx2*. Importantly, administration of high doses of nonselective COX inhibitors such as indomethacin produces substantial, and often intolerable, dose-dependent adverse effects. Consequently, identifying therapeutic strategies that enhance the anti-inflammatory effects of lower doses could expand the therapeutic window of COX inhibitors.

Arachidonic acid serves as a common substrate for the parallel biosynthesis of biologically active COX-, LOX- and CYP-derived eicosanoids. Two recent reports have demonstrated that COX and sEH inhibition can shunt arachidonic acid metabolism down parallel

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metabolic paths [10,11]. Thus, shunting metabolism away from pro-inflammatory COX-derived prostaglandins and toward anti-inflammatory CYP-derived EETs would be hypothesized to yield a greater anti-inflammatory effect than modulation of either pathway alone. Indeed, an additive attenuation of LPS-induced pain [13] and hypotension [12] following dual inhibition of COX and sEH has been reported, suggesting that functional interactions exist between COX- and CYP epoxygenase-mediated arachidonic acid metabolism (i.e., where modulation of one pathway enhances the effects of the other). The effects of COX inhibition in tandem with potentiation of CYP-derived EETs on vascular inflammation, however, had not been evaluated to date. The current investigation demonstrates that despite anti-inflammatory effects following modulation of each pathway alone, simultaneous inhibition of COX-derived prostaglandin biosynthesis and potentiation of CYP epoxygenase-derived EET levels does not additively attenuate the acute vascular inflammatory response to LPS *in vivo*. Although indomethacin has been previously reported to inhibit CYP epoxygenase activity *in vitro* [22], the observed IC50 in these experiments (70 μM) was more than 20-fold above the peak plasma concentrations following high dose (10 mg/kg) treatment in rodents [23]. In the current investigation, *in vivo* EET levels were not significantly suppressed by indomethacin in WT, *Tie2-CYP2J2*-Tr or *Ephx2*$^{-/-}$ mice. Moreover, an increase in metabolism down the parallel 5-LOX and CYP ω-hydroxylase pathways was not observed. Thus, the observed lack of an additive anti-inflammatory effect was not secondary to indomethacin-mediated inhibition of EET biosynthesis or shunting arachidonic acid metabolism down a parallel pro-inflammatory pathway. Although the specific mechanism remains unclear, these data collectively suggest that the vascular anti-inflammatory effects of COX inhibition and increasing EET levels may be mediated by an overlapping mechanism.

Prior evidence has demonstrated the presence of metabolic and functional crosstalk between the CYP epoxygenase and COX pathways, independent of arachidonic acid substrate shunting. Most notably, EETs have been reported to inhibit COX enzymatic activity in ram seminal vesicles, rat monocytes and murine vascular smooth muscle cells *in vitro* [24–26], suppress LPS-induction of hepatic and spinal COX-2 expression *in vivo* [13,27,28], and consequently attenuate the induction of pro-inflammatory prostaglandin biosynthesis [13,25–28]. These observations suggest that inhibition of prostaglandin biosynthesis may contribute to the anti-inflammatory effects of EETs. In the presence of genetic potentiation of EET levels, however, we did not observe any significant differences in prostaglandin levels. Similar results were recently reported following induction of tumor growth in these genetically-modified mice [18]. Collectively, this suggests that EET-mediated attenuation of prostaglandin biosynthesis does not contribute to the acute vascular anti-inflammatory phenotype observed in *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ mice.

It has also been reported that EETs induce COX-2 expression and prostacyclin production in cultured endothelial cells in the absence of an inflammatory stimulus [29], suggesting that EET-mediated modulation of prostaglandin biosynthesis may be dependent on the specific model or cell type under investigation. Endotoxin-induced hypotension and nociception are phenotypes primarily driven by inducible nitric oxide synthase-mediated vasodilation [30] and cAMP-mediated signaling [27,31], respectively, as compared to induction of CAM and chemokine-mediated endothelial activation and leukocyte infiltration into tissue [14]. Furthermore, the hypotension and pain phenotypes that were additively attenuated by dual modulation of the COX and CYP epoxygenase pathways were assessed 6 hours following LPS administration [12,13]. In contrast, we measured MPO activity, an established biomarker of neutrophil infiltration, 3 hours following LPS administration [9]. Since induction of inflammatory CAM, chemokine and cytokine expression peaks 2–4 hours following administration of LPS [32,33], functional interactions between EET and prostaglandin biosynthesis may be unmasked at later time points during resolution of the

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acute inflammatory response. These findings collectively highlight the complexity of initiation and resolution of the acute inflammatory response, and the potential contribution of temporal and phenotypic differences to the role of eicosanoid metabolism in the regulation of inflammation.

More recently, EETs have been reported to selectively antagonize recombinant Tx (TP) receptors, with little or no binding affinity for non-TP prostanoid or leukotriene receptors, and specifically inhibit TP receptor agonist-mediated constriction of isolated rodent resistance arteries, conduit arteries and bronchi [34]. In accordance, EETs also blocked TP receptor agonist-mediated constriction of isolated human bronchi, while administration of a CYP epoxygenase inhibitor increased TP-dependent bronchoconstriction [35]. Additionally, EETs inhibit arachidonic acid-stimulated platelet aggregation in isolated human platelets without changing Tx levels [24], further corroborating the potential presence of redundancy in the biological effects of increasing CYP-derived EET and decreasing COX-derived TxA2 levels. Importantly, activation of TP-receptor signaling also drives acute and chronic vascular inflammatory responses, including cytokine-mediated activation of leukocyte adhesion [7] and diabetes-induced atherosclerosis [36]. Considering the lack of an additive anti-inflammatory effect following dual modulation of the COX and CYP epoxygenase pathways in the current investigation, it is conceivable that attenuation of TP receptor signaling contributes, at least in part, to the vascular anti-inflammatory effects of both COX inhibition (via inhibiting TxA2 biosynthesis) and increasing CYP-derived EETs (via competitive inhibition of TP receptors). These observations collectively highlight the importance for future studies to elucidate functional interactions between EETs and TP receptor signaling in the regulation of vascular inflammation. Furthermore, thoroughly understanding the relative roles of, functional interactions between and mechanisms underlying the parallel CYP epoxygenase, CYP ω-hydroxylase, COX (including the specific prostaglandin synthases and receptors), and LOX pathways of arachidonic acid metabolism in the regulation of acute and chronic vascular inflammatory responses will be essential in order to maximize the anti-inflammatory therapeutic potential of targeting eicosanoid metabolism.

CONCLUSIONS

Although potentiation of the CYP epoxygenase pathway, through increased EET biosynthesis or decreased EET hydrolysis, and non-selective inhibition of COX-mediated prostaglandin biosynthesis each attenuated the acute vascular inflammatory response to endotoxin *in vivo*, dual modulation of both pathways did not result in an additive anti-inflammatory effect. These findings further demonstrate that both the COX and CYP epoxygenase pathways are important regulators of vascular inflammation, and suggest that the anti-inflammatory effect of modulating each pathway may be mediated, at least in part, by overlapping mechanisms. Future studies delineating the complex functional interactions between these parallel metabolic pathways in the regulation of inflammation are necessary.

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Highlights

- CYP2J2 overexpression and sEH disruption each attenuate acute vascular inflammation.
- Moderate dose indomethacin attenuates vascular inflammation to a similar degree.
- Modulation of both pathways did not produce an additive anti-inflammatory effect.
- These anti-inflammatory effects may be mediated by overlapping mechanisms.

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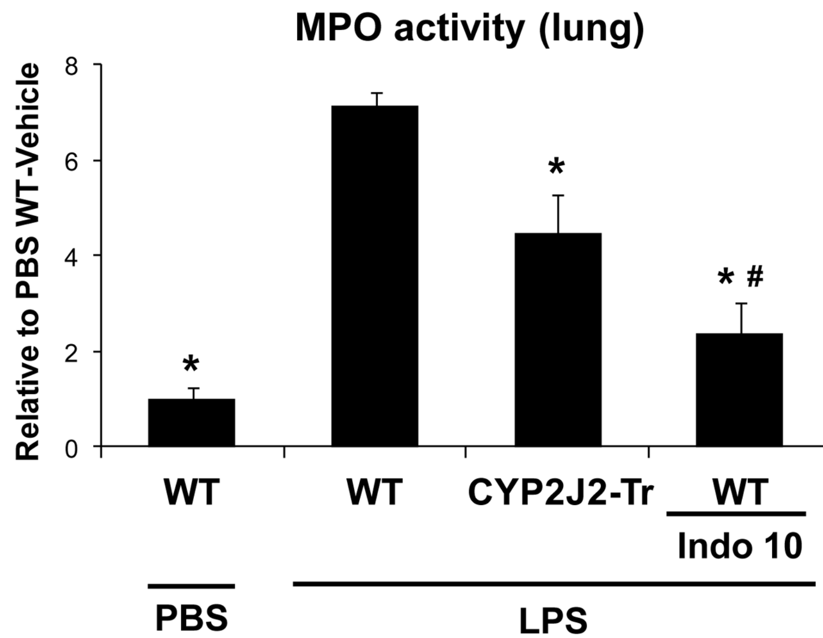

Figure 1. Relative effect of endothelial CYP2J2 overexpression and high-dose indomethacin on acute vascular inflammation

LPS-induced lung MPO activity in indomethacin- (10 mg/kg) and vehicle-treated wild-type (WT) mice and *Tie2-CYP2J2*-Tr mice. Data are expressed relative to saline (PBS)-treated WT controls. N=6–8 per group. *P<0.05 versus LPS WT-vehicle. #P<0.05, indomethacin versus CYP2J2-Tr.

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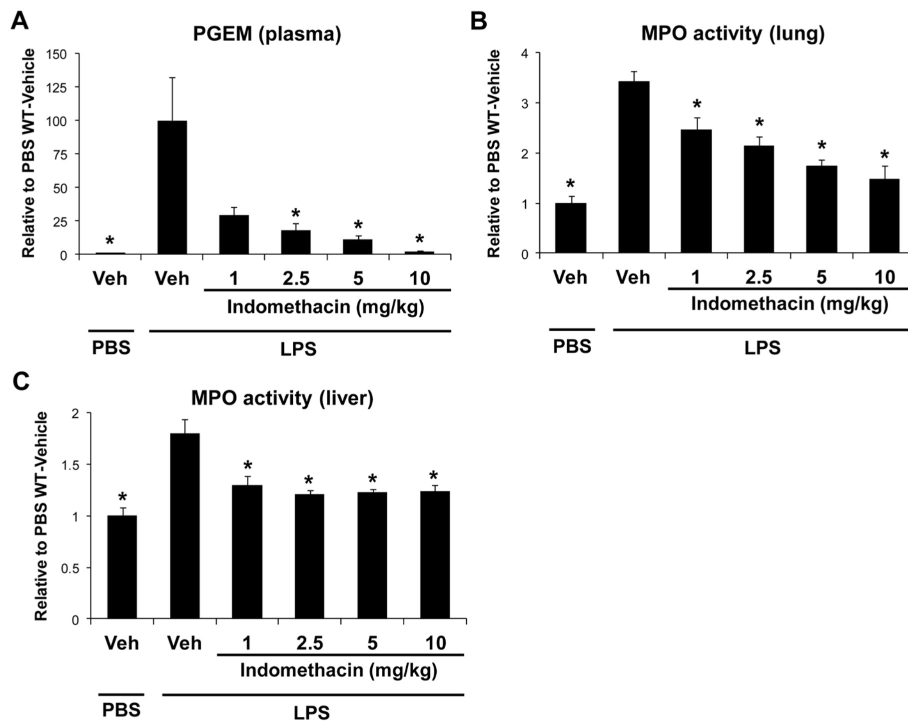

Figure 2. Dose-dependent inhibition of acute vascular inflammation by indomethacin

LPS-induced (A) plasma PGEM levels, (B) lung MPO activity, (C) and liver MPO activity following administration of vehicle (Veh) or incremental doses of indomethacin (1, 2.5, 5 or 10 mg/kg) to wild-type C57BL/6 mice. Data are expressed relative to saline (PBS) and Veh-treated controls. The mean PGEM levels in the PBS control group and LPS-treated vehicle group were 6.6 and 658 pg/mL, respectively. N=4–9 per group. *P<0.05 versus LPS-vehicle.

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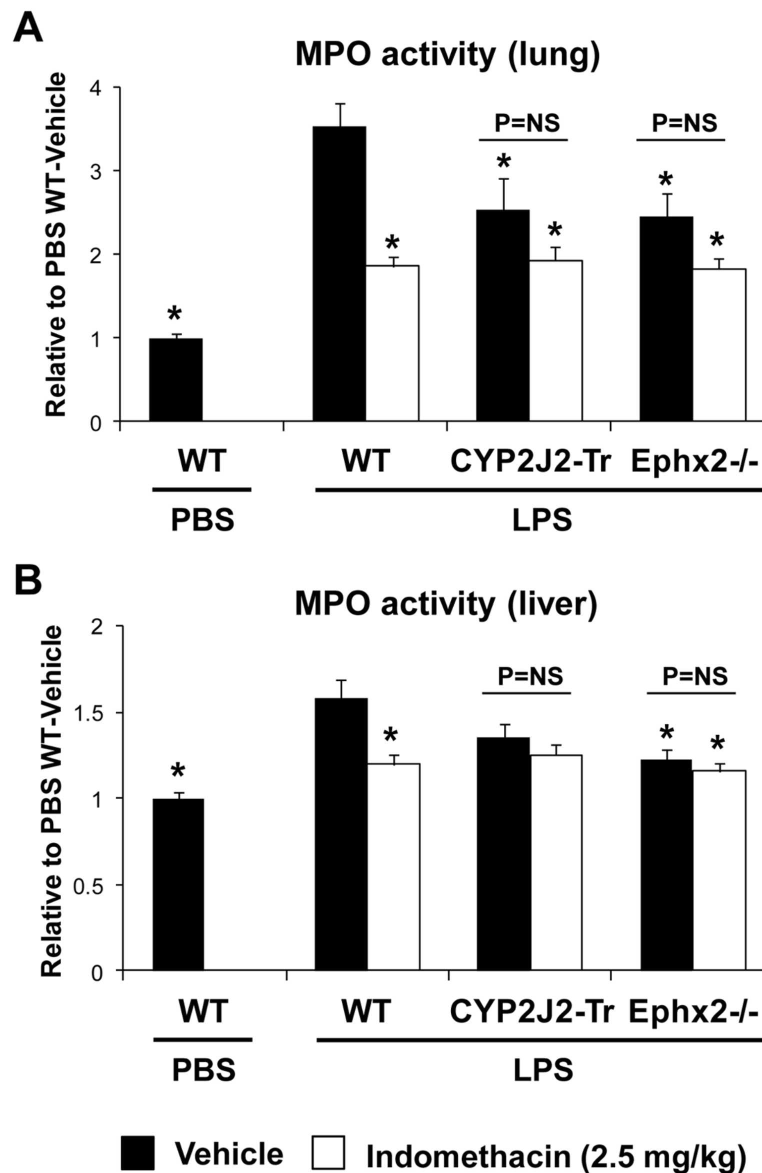

Figure 3. Relative effect of endothelial CYP2J2 overexpression, global sEH disruption, and moderate-dose indomethacin on acute vascular inflammation

LPS-induced (A) lung MPO activity and (B) liver MPO activity following administration of vehicle or indomethacin (2.5 mg/kg) to wild-type (WT), *Tie2-CYP2J2*-Tr, and *Ephx2*$^{-/-}$ mice. Data are expressed relative to saline (PBS)-treated WT controls. PBS: N=6 per group, LPS: N=9–18 per group. *P<0.05 versus LPS WT-vehicle. NS=not significant.

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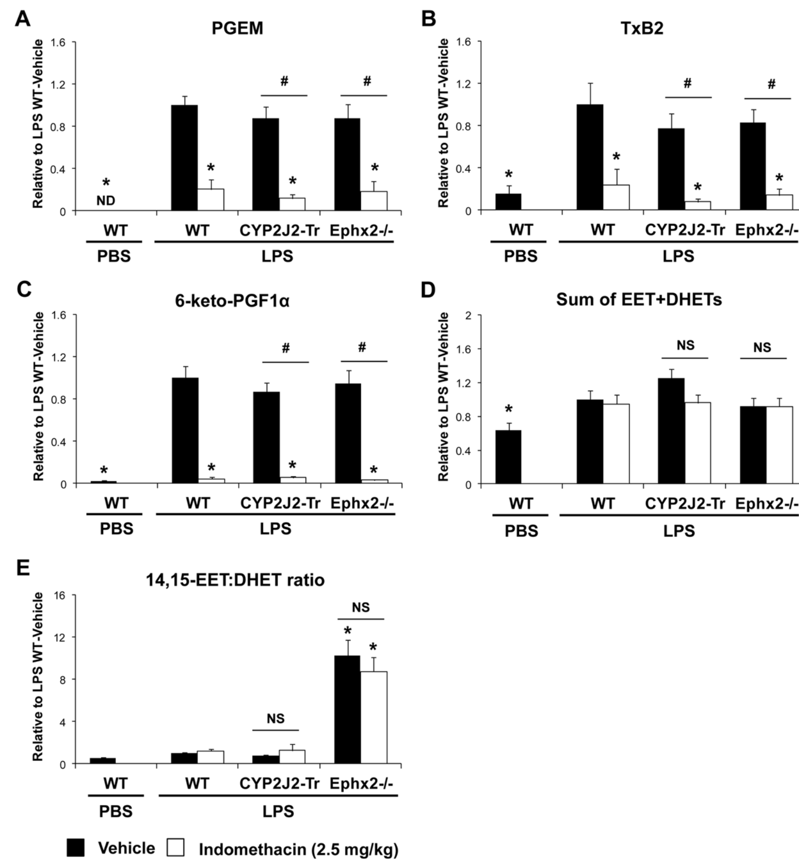

Figure 4. Relative effect of endothelial CYP2J2 overexpression, global sEH disruption, and moderate-dose indomethacin on eicosanoid metabolite levels

LPS-induced plasma (A) PGEM, (B) TxB2, and (C) 6-keto-PGF1α levels, (D) the total sum of EET+DHETs, and (E) the 14,15-EET:DHET ratio following administration of vehicle or indomethacin (2.5 mg/kg) to wild-type (WT), *Tie2-CYP2J2*-Tr, and *Ephx2*$^{-/-}$ mice. The mean eicosanoid levels for the PBS control group are as follows: (A) PGEM, below the limit of detection (the mean level in the LPS-treated WT group is 379 pg/mL); (B) TxB2, 214 pg/mL; (C) 6-keto-PGF1α, 49.5 pg/mL; (D) sum of EET+DHETs, 13.7 ng/mL; (E) 14,15-EET:DHET, 0.035 (ratio). PBS: N=5 per group, LPS: N=9–16 per group. *P<0.05 versus LPS WT-vehicle. $^{\#}$P<0.05, indomethacin versus vehicle within the *Tie2-CYP2J2*-Tr and *Ephx2*$^{-/-}$ genotype groups. ND=below the limit of detection. NS=not significant.