Deletion of Microsomal Prostaglandin E Synthase-1 Does Not Alter Ozone-Induced Airway Hyper-Responsiveness

Miao Wang, Philip R. Cooper, Meiqi Jiang, Hengjiang Zhao, Yiqun Hui, Yubing Yao, Joshua C. Tate, Gautam Damera, John A. Lawson, William F. Jester, Jr., Angela Haczku, Reynold A. Panettieri, Jr., and Garret A. FitzGerald


Received January 28, 2010; accepted April 2, 2010

ABSTRACT

Nonsteroidal anti-inflammatory drugs ameliorate pain and fever by inhibiting cyclooxygenase (COX) and suppressing prostanoid formation. Microsomal prostaglandin E synthase-1 (mPGES-1) catalyzes formation of PGE₂ from the COX product PGH₂ and has emerged as a therapeutic target. Inhibition of mPGES-1, however, renders the PGH₂ substrate available for diversion to other PG synthases. To address the possibility that substrate diversion augments formation of PGs that might modulate bronchial tone, we assessed the impact of mPGES-1 deletion in a mouse model of ozone-induced airway hyper-responsiveness. Ozone exposure increased total lung resistance to inhaled methacholine in wild-type mice. Deletion of mPGES-1 had little effect on total lung resistance in either naive or ozone-exposed animals. The carbachol-induced narrowing of luminal diameter in intrapulmonary airways of lung slices from acute ozone-exposed mice was also unaltered by mPGES-1 deletion. Likewise, although concentrations of PGE₂ were reduced in bronchoalveolar lavage fluid, whereas 6-keto-PGF₁α, PGD₂, and PGF₂α all were increased, deletion of mPGES-1 failed to influence cell trafficking into the airways of either naive or ozone-exposed animals. Despite biochemical evidence of PGH₂ substrate diversion to potential bronchomodulator PGs, deletion of mPGES-1 had little effect on ozone-induced airway inflammation or airway hyper-responsiveness. Pharmacologically targeting mPGES-1 may not predispose patients at risk to airway dysfunction.

Prostaglandins (PGs) are a group of bioactive lipids formed by the sequential enzymatic actions of cyclooxygenases (COXs) and terminal synthases that convert the COX product PGH₂ to specific PGs. Placebo-controlled trials have revealed that nonsteroidal anti-inflammatory drugs (NSAIDs) selective for inhibition of COX-2 confer a cardiovascular hazard (FitzGerald, 2007). Integration of diverse lines of evidence (Grosser et al., 2010) indicates that this is consequent to suppression of cardioprotective PGs, particularly prostacyclin (PGL₂). Microsomal PG synthase-1 (mPGS-1) (Jakobsson et al., 1999; Thoren et al., 2003), an enzyme downstream in the biosynthetic cascade, catalyzes the isomerization of PGH₂ into PGE₂ and is a member of the MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) superfamily. It has been suggested to be an anti-inflammatory drug target alternative to NSAIDs (Jakobsson et al., 1999; Samuelsson et al., 2007). Two other PGE synthases have been identified, mPGS-2 (Murakami and Kudo, 2006) and cysotolic PGES (Tanoioka et al., 2000; Pini et al., 2005). mPGES-1, however, is the dominant source of PGE₂ biosynthesis, at least in mice (Cheng et al., 2006). Although some have found that mPGES-1 deletion modulates experimentally evoked pain and arthritis to a degree indistinguishable from treatment with traditional NSAIDs (tNSAIDs)
mechanisms in response to acetyl-$\beta$-methylcholine chloride were made by using the FlexiVent system (SCIReq, Montréal, QC, Canada). FlexiVent uses a low-frequency forced-oscillation technique to measure respiratory system input impedance and evaluate the constant-phase model. Mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg body weight) and xylazine (10 mg/kg body weight) in saline. A tracheostomy was performed by inserting a 20-gauge polyethylene catheter into the distal trachea and ligating it around the catheter to avoid leaks or a disconnection from the ventilator. The animals were then mechanically ventilated with a computer-controlled small-animal ventilator (SCIReq) with a rate of 150 breaths/min, tidal volume of 10 ml/kg, and peak expiratory pressure of 2 to 3 cm of H₂O. Once ventilated, mice were paralyzed with 0.8 mg/kg pancuronium bromide to block spontaneous breathing. Using the custom-designed software FlexiVent 5.2 (SCIReq), e.g., SnapShot-150, resistance, elastance, and compliance were recorded, and input impedance was measured with Quick Prime-3, to distinguish between central airway and lung tissues. Before starting each dose measurement, total lung capacity was performed twice to open up the airspace and standardize lung volume for baseline determination. Then, aerosolized methacholine at doses of 0, 2.5, 5, 10, 20, 50, and 100 mg/ml was delivered via an in-line nebulizer in the ventilator circuit for 10 s. After methacholine exposure, 12 measurements were taken over 3 min for each dose.

**PCLS Preparation and Intrapulmonary Airway Function Assessment.** In a different group of mice PCLS were prepared immediately after ozone exposure (3 h, at 6 ppm), with slight modifications, as described previously (Ressmeyer et al., 2006; Cooper et al., 2010). In brief, the trachea was exposed and intubated with a cannula, and the lungs were inflated with 0.65 ml of 2% (w/v) low melting point agarose solution (37°C) followed by 0.1-litter bolus of air to force the agarose out of the airways and into the parenchymal tissue. After allowing the agarose to set at 4°C, the lobes were separated, and the largest lobe was embedded externally in agarose by using a tissue-embedding unit (TSE Systems, Chesterfield, MO). PCLS (thickness 250 μm) were prepared with a Krumdieck tissue slicer (model MD4000; Alabama Research and Development, Munford, AL) with the speed set to produce slices at approximately one every 30 s. Slices were transferred in sequence to wells containing supplemented Ham’s F-12 medium and then incubated at 37°C on a rotating platform in a humidified air/CO₂ (95:5%) incubator. Media were changed every hour for 4 h to minimize trauma, reduce airway tone, and remove any remaining agarose in the tissue. Media were also changed the next day. Up to four slices from each animal were placed in a 12-well plate in 1.0 ml of buffer and held in place by using a platinum weight with nylon attachments. Airway function was measured as described previously (Cooper and Panettieri, 2008; Cooper et al., 2009) by using a microscope (ECLIPSE, model TE2000-U; Nikon, Melville, NY) connected to a live video feed (RETIGA-2000R video recorder; QImaging, BC, Canada). Images were collected 4 min after each dose of carbachol or until no further contraction was evident. Log EC₅₀ and E_max values for each airway were derived from a concentration-response curve, and mean values for each animal were also derived.

**Measurement of Eicosanoids and Proteins.** BAL levels of 
PGD₂, 6-keto-PGF₁α, and thromboxane (Tx) B₂ (the hydrolysis product of 
TxA₂) were quantified by ultra high-pressure liquid chromatography/tandem mass spectrometry, using solid-phase extraction, negative ion electrospray introduction, and selected reaction monitoring techniques. Tetradeterated analogs of PGD₂, PGE₂, PGF₂α, and thromboxane (Tx) B₂ (the hydrolysis product of TxA₂) were quantified by ultra high-pressure liquid chromatography/tandem mass spectrometry, using solid-phase extraction, negative ion electrospray introduction, and selected reaction monitoring techniques.
acetonitrile in water for analysis. The instrument used was a Quantum Ultra interfaced to an Accela ultra high-pressure liquid chromatography system (Thermo Fisher Scientific, Waltham, MA). A 200-mm × 2.1-mm × 1.9-μm Hypersil Gold column (Thermo Fisher Scientific) was used. The mobile phase was generated from high-performance liquid chromatography-grade water (A) and 5% meth-

Fig. 1. Levels of prostanoids in BAL fluid. BAL samples were isolated from FA or ozone-exposed mPGES-1 WT [mPGES-1(+/+) ] and KO [mPGES-1(−/−)] mice (n = 10). *, p < 0.05; **, p < 0.01; ***, p < 0.001. These labels apply to all figures.

Fig. 2. Lung resistance (a) and dynamic compliance (b) measured after exposure of mice to methacholine. Response to each methacholine dose was plotted as the mean ± S.D. for each group (n = 5). FA: control for ozone exposure; BL: baseline. One outlier at a dose of 50 mg/ml that gave an enormously high value to the group of WT plus ozone was excluded. There is no statistical significance between WTs and KOs at any given dose of methacholine in control or ozone exposure condition.
anol/95% acetonitrile (B), both containing 0.005% acetic acid adjusted to pH 5.7 with ammonium hydroxide. The flow rate was 350 μl/min using a segmented linear gradient starting at 20% (T = 0), ramping to 35% B (T = 15 min), 40% B (T = 16 min) then 70% B (T = 23 min). The transitions monitored were m/z 384 → 272 (d₄-PGD₂ MO and d₄-PGE₂ MO), m/z 380 → 268 (PGD₂ MO and PGE₂ MO), m/z 357 → 197 (d₄-PGF₂α), m/z 353 → 193 (PGF₂α), m/z 402 → 173 (d₄-TxB₂ MO), m/z 398 → 169 (TxB₂ MO), m/z 402 → 372 (d₄-6-keto-PGF₁α MO), and 398 → 368 (6-keto-PGF₁α MO). The collision gas was argon, 1.5 mTorr. The collision energy was 18 eV for PGD₂, PGE₂, 6-keto-PGF₁α, and TxB₂, and 24 eV for PGF₂α. Source offset was 6 V. Quantitation was by peak area ratios. Levels of mouse CXC chemokine KC and total protein in BAL fluid were determined with an enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) and Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA), respectively.

**Statistical Analysis.** Data are expressed as means ± S.E.M. unless indicated otherwise. Statistical comparisons among treatment groups in reactivity to methacholine were performed by two-way ANOVA, followed by the Newman-Keuls post hoc test for more than two groups. Comparisons of multiple groups were performed by one-way ANOVA and Bonferroni post-ANOVA test when the ANOVA was deemed significant. When only two mean values were compared, the two-tailed Mann-Whitney t test was used. In all cases, statistical significance was defined as p < 0.05.

**Results**

Deletion of mPGES-1-Modulated Airway Production of Prostanoids. Evidence for substrate diversion consequent to mPGES-1 deletion was sought in BAL (Fig. 1). In both FA and ozone-exposed animals, deletion of mPGES-1 significantly reduced PGE₂, while augmenting biosynthesis of PGI₂, PGD₂, and PGF₂α.

Deletion of mPGES-1 Had Little Effect on Modulating Ozone-Induced Airway Responsiveness. To study whether targeting mPGES-1 may affect airway function in a predisposed high-risk condition, ozone-induced airway hyper-responsiveness was evaluated in mice deficient in mPGES-1 (Fig. 2). Enzyme deletion did not affect total lung resistance after challenge with increasing doses of methacholine in either naive or ozone-exposed animals, despite a hyper-responsiveness induced by ozone exposure (Fig. 2a). Likewise, mPGES-1 deletion failed to modulate significant compliance in naive or ozone-exposed animals (Fig. 2b).

Although ozone treatment increased carbachol-induced...
contraction of intrapulmonary airways in sliced lung sections, as reflected by a significant decrease in EC_{50} (log EC_{50} from 0.04 ± 0.11 to −0.36 ± 0.12 μM), mPGES-1 deletion had little effect on the sensitivity to carbachol-induced intrapulmonary airway contraction (Fig. 3).

**Deletion of mPGES-1 Did Not Affect Ozone-Induced Airway Inflammation.** Ozone exposure increased inflammatory cell trafficking into the BAL (by ~6 fold) as shown in Fig. 4a. The inflammatory cell infiltrate was composed primarily of neutrophils (~55%) and macrophages (~43%) (Fig. 4b). However, no differences in total BAL cell count or the differential macrophage, eosinophil, neutrophil, or lymphocyte counts were observed between wild-type (WT) and knockout (KO) animals (Fig. 4). Consistently, levels of KC (a neutrophil chemoattractant) and total protein in BAL were not changed in the KO animals (Supplemental Figure).

**Discussion**

Selective inhibitors of COX-2 were developed with the aim of conserving efficacy but reducing the incidence of serious gastrointestinal adverse events consequent to use of tNSAIDs, such as ibuprofen, which inhibited coincidentally COX-1 and COX-2. Although two of these newer drugs, rofecoxib and lumiracoxib, have been shown in randomized trials to cause serious gastrointestinal complications less frequently than tNSAID comparators, evidence has emerged that selective inhibition of COX-2 by the newer coxibs and some tNSAIDs may result in a cardiovascular hazard attributable to suppression of PGI_{2} (Grosser et al., 2010). mPGES-1 deletion, by contrast, augments PGI_{2} formation caused by diversion of the PGH_{2} substrate to PGI isomerase. Ironically, this tends to undermine the original intent of developing mPGES-1 inhibitors as PGI_{2}, such as PGE_{2}, that can mediate pain and inflammation (Murata et al., 1997; Honda et al., 2006). However, evidence in mice suggests that augmentation of PGI_{2} synthesis may not just attenuate the thrombogenic and hypertensive hazard seen with COX-2 inhibition, but actually re-strains atherogenesis and aneurolysis formation, suggesting that mPGES-1 inhibitors may have a role in altering cardiovascular inflammation.

Although the emphasis has been on substrate diversion to PGI_{2}, we also found (Wang et al., 2008) that biosynthesis of PGD_{2} was augmented by mPGES-1 deletion. Although activation of the adenylate cyclase-coupled platelet DP1 receptor for this PG may contribute to cardioprotection, this finding also raised the possibility of pulmonary adverse effects of mPGES-1 inhibitors. Others have shown that augmented PGD_{2} can provoke airway constriction, and PGE_{2}, another potential product of substrate rediversion, also causes bronchoconstriction in vitro (Mathé et al., 1973). Here, we confirmed that deletion of mPGES-1 did indeed cause substrate diversion in the lung, as reflected by measurement of eicosanoids in BAL. We also found that residual PGE_{2} (approximately 50% of the total PGE_{2}) was present in the mPGES-1 KO mice. PGE_{2} can be also formed by mPGES-2 and cytosolic PGE synthase. These would be unaffected by the gene deletion or the specific mPGES-1 inhibitors under development. Despite the presence of these enzymes, however, deletion of mPGES-1 and/or its inhibition resulted in analgesic and anti-inflammatory effects in a variety of models (Xu et al., 2008; Mbalaviele et al., 2010). These studies indicate that the reduction of PGE_{2} levels in the KO mice is biologically significant.

Despite increased formation of both PGD_{2} and PGF_{2α} in the mPGES-1 KO mice, airway function at baseline or in response to provocation with ozone was unaltered by mPGES-1 deletion. Our results may reflect a balanced increase in the bronchoconstrictor prostanoids and the bronchodilator PGI_{2}. Indeed, recently, it has also been reported that PGI_{2} inhibits allergen-induced airway inflammation (Jaffar et al., 2002; Takahashi et al., 2002; Nagao et al., 2003; Idzko et al., 2007), and despite the impact of DP1 deletion on allergen-induced airway hyper-responsiveness (Matsuoka et al., 2000), an anti-inflammatory function of PGD_{2}, acting on its DP1 receptor on bone marrow-derived cells, has been described previously (Hammad et al., 2007). Furthermore, although PGE_{2} might act as a bronchodilator via its EP2 and EP4 receptors, it may also have contributed to bronchoconstrictor tone via the EP1 and EP3 receptors (Narumiya et al., 1999; Tilley et al., 2003). It is likely that PGE_{2} does not act as a direct dilator but as an endogenous “brake” on bronchoconstrictive agonists (Sestini et al., 1996). Prostanoids differentially generated along the COX-1 and COX-2 pathways may have distinct roles in airway inflammation and hyper-responsiveness (Swedin et al., 2009). BAL fluid is an indirect reflection of prostanoid generation in the lung and may not reflect regional disparities in product formation relevant to airway responsiveness. However, the altered impact on both constrictor and dilator prostanoids reflected in BAL may also explain the failure of mPGES-1 deletion to alter airway function.

Ozone-induced airway hyper-responsiveness in mice is but one model of evoked bronchoconstriction. However, at least in the present studies, despite substrate diversion to other prostanoids, suppression of PGE_{2} by deleting mPGES-1 provided no evidence suggestive of enhanced airway dysfunction.

**Acknowledgments**

We thank Christopher Kirby, Homer Fogle, Helen Zou, and Wenxuan Li-Feng for technical support.

**References**


