Cyclooxygenase-2-Linked Attenuation of Hypoxia-Induced Pulmonary Hypertension and Intravascular Thrombosis

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ABSTRACT

Exogenous prostacyclin is effective in reducing pulmonary vascular resistance in some forms of human pulmonary hypertension (PH). To explore whether endogenous prostaglandins played a similar role in pulmonary hypertension, we examined the effect of deleting cyclooxygenase (COX)-gene isoforms in a chronic hypoxia model of PH. Pulmonary hypertension, examined by direct measurement of right ventricular end systolic pressure (RVESP), right ventricular hypertrophy (n = 8), and hematocrit (n = 3), was induced by 3 weeks of hypobaric hypoxia in wild-type and COX-knockout (KO) mice. RVESP was increased in wild-type hypoxic mice compared with normoxic controls (24.4 ± 1.4 versus 13.8 ± 1.9 mm Hg; n = 8; p < 0.05). COX KO mice showed a greater increase in RVESP following hypoxia (36.8 ± 2.7 mm Hg; p < 0.05). Urinary thromboxane (TX)B2 excretion increased following hypoxia (44.6 ± 11.1 versus 14.7 ± 1.8 ng/ml; n = 6; p < 0.05), an effect that was exacerbated by COX-2 gene disruption (54.5 ± 10.8 ng/ml; n = 6). In contrast, the increase in 6-keto-prostaglandin F1α excretion following hypoxia was reduced by COX-2 gene disruption (29 ± 3 versus 52 ± 4.6 ng/ml; p < 0.01). Tail cut bleed times were lower following hypoxia, and there was evidence of intravascular thrombosis in lung vessels that was exacerbated by disruption of COX-2 and reduced by deletion of COX-1. The TXA2/endothelium receptor antagonist ifetroban (50 mg/kg/day) offset the effect of deleting the COX-2 gene, attenuating the hypoxia-induced rise in RVESP and intravascular thrombosis. COX-2 gene deletion exacerbates pulmonary hypertension, enhances sensitivity to TXA2, and induces intravascular thrombosis in response to hypoxia. The data provide evidence that endogenous prostaglandins modulate the pulmonary response to hypoxia.

Pulmonary hypertension (PH) is characterized by an elevation in pulmonary vascular tone and a striking degree of pulmonary vascular remodeling. As a consequence, there is increased pulmonary vascular resistance and right ventricular hypertrophy that ultimately leads to right ventricular failure and death (Blumberg et al., 2002). Prostaglandins have been implicated in the pathogenesis of at least some forms of PH (Christman et al., 1992). These bioactive products are generated by the enzyme cyclooxygenase (COX), of which there are two isoforms, COX-1 and COX-2 (Hinz and Brune, 2002). COX-1 is expressed in many tissues, including platelets, and it is a major source of thromboxane (TX). COX-2 is largely absent in normal tissues, but it is induced by a range of factors, including hypoxia (Schmedtje et al., 1997), and it catalyzes the formation of, among other prostaglandins, prostacyclin (PGI2). These two products exhibit quite different effects in the vasculature. PGI2 is a vasodilator and inhibits platelet aggregation and thrombosis, whereas TXA2 is a vasoconstrictor and induces activation and aggregation of platelets (Cheng et al., 2006).

An imbalance between the production of TXA2 and PGI2 has been reported in pulmonary hypertension that potentially promotes platelet activation and increased pulmonary vascular resistance (Christman et al., 1992). Moreover, over-expression of prostacyclin synthase protects against the development of experimental pulmonary hypertension (Geraci et al., 1999), whereas intravenous iloprost (a PGI2 analog)
reduces pulmonary pressures in patients with primary pulmonary hypertension (Friedman et al., 1997). In contrast, thromboxane receptor activation potentiates pulmonary hypertension in the rat (Jankov et al., 2002), whereas inhibition of thromboxane reduces pulmonary hypertension induced by hypoxia in the pig (Fike et al., 2005).

Although the effects of eicosanoids in human and experimental models of PH may reflect alterations in pulmonary vascular tone, platelet activation and its regulation by PGH₂ and TXA₂ may be involved. Intra-arterial thrombosis has been reported in more than 60% of patients with hypoxia-related PH (Fedullo et al., 2001). Plasma and urinary levels of thromboxane metabolites are increased in patients presenting with various forms of PH (Christman et al., 1992), which would be consistent with increased activation of platelets, the major source of TXA₂ in vivo. Long-term infusion of PGH₂ normalizes plasma markers of platelet activation in patients with primary pulmonary hypertension (Friedman et al., 1997), and aerosolized iloprost induces a mild, but sustained inhibition of platelet aggregation (Beghetti et al., 2002). Nevertheless, it is unclear whether alterations in the generation of endogenous eicosanoids regulate platelet activity in PH. However, there is evidence that the formation of PGH₁ and TXA₂ modulates platelet activation in vivo in other settings (Cheng et al., 2002).

To further understand the role of endogenous eicosanoids in hypoxia-induced pulmonary hypertension, we explored the role of COX isofoms in a murine model, where pulmonary hypertension was induced by chronic hypoxia.

Materials and Methods

Drugs. Ifetroban (BMS 180,291), a thromboxane receptor antagonist, was kindly provided by Dr. Martin Ogletree (Bristol-Myers Squibb Co., Princeton, NJ). It was administered orally to the mice at a dose of 50 mg/kg/day in 0.1% methylcellulose (Tesfamariam and Ogletree, 1995).

Mice. Mice with targeted disruptions of the genes encoding either COX-1 or COX-2 were kindly provided by Dr. L. Ballou (The University of Tennessee Health Science Center, Memphis, TN). They were maintained on an outbred genetic background of C57/BL6 and DBAⅠ. Wild-type mice were generated from the COX-deficient lines by Robert Langenbach (Loftin et al., 2001), and the predicted alveolar PO2 of 55 mm Hg for 3 weeks. The animals were therefore exposed to poikilocapnic hypoxia, i.e., they were not given supplementary CO₂ to maintain normocapnia, so they were hypoventilated as a result of hypoxic stimulation of the arterial chemoreceptors. It has been shown that CO₂ is protective against pulmonary hypertension in so far as chronic hypercapnic hypoxia abolishes the pulmonary hypertension caused by poikilocapnic hypoxia (Oi et al., 2000). Age- and weight-matched controls were maintained in 21% oxygen and subjected to the same light/dark cycle. The chamber was opened every 4 days for 15 to 20 min for cleaning and replenishing of food, water, and drugs.

Right Heart Pressure Recordings. After 3 weeks of hypoxia, the mice were anesthetized with 0.1% fentanyl/fluanisone (Hypnorm, Janssen Pharmaceuticals, Oxford, UK), and right ventricular end systolic pressure (RVESP) was recorded by retrograde catheterization of the superior vena cava. Upon adequate exposure of the superior vena cava, a small transverse incision was made in the vein. A micro-tip pressure catheter (SPR-671, 1.4 French; Millar Instruments Inc., Houston, TX) was inserted and advanced into the right ventricle. Signals were continuously recorded with an ARIA pressure conductance system (Millar Instruments Inc.), coupled with a Powe-lab/4SP AD converter (ADInstruments, Colorado Springs, CO).

Right Ventricular Hypertrophy Measurements. Right ventricular hypertrophy measurements were carried out on the anesthetized mice after recordings of the right heart pressures were completed. The thoracic cavity was opened, and the hearts were removed. The right ventricle of each heart sample was isolated from the left ventricle and septum. Both heart sections were weighed, and right ventricular hypertrophy (RVH) was determined as a ratio of the weight of the left ventricle and septum to that of the right ventricle alone.

Measurement of Apoptosis. Right ventricular cellular apoptosis levels were measured in all mouse groups using an ApopTag in situ apoptosis detection kit (Chemicon International, Temecula, CA) (Mi-zumatsu et al., 2003). In brief, after treatment with 20 μg/ml proteinase K and 0% hydrogen peroxide, paraffin-embedded sections were treated with a digoxigenin-dNTP complex for 1 h at 37°C. This complex binds to free 3-OH termini of nucleosome-sized DNA fragments that are formed during apoptosis, and it is detected by an anti-digoxigenin conjugate antibody. The bound complex is then stained with a 3,3-diaminobenzidine-based substrate. Sections were counterstained in methyl green [0.5% (w/v)] in 0.1 M sodium acetate, mounted in DPX (Sigma-Aldrich, St. Louis, MO), and visualized on a Leica DNLB light microscope (Leica, Wetzlar, Germany) with color video attachment for recording. Staining was quantified using Image Pro-Plus software, version 4.0 (Media Cybernetics, Gelichen, Germany), and it was performed by an observer blinded to genotype. The total number of apoptotic and normal cells was counted over three high-powered fields per section. Each high-powered field was selected at random, and the number of apoptotic cells was expressed as a percentage of the total cell count.

Tail-Cut Bleeding Times. Tail-cut bleeding time was measured in all mouse groups as an in vivo assay of hemostasis, as described previously (Weiss et al., 2002; Hamilton et al., 2004). In brief, tails were transected 5 mm from the tip using a sterile blade. The bleeding tail was then immersed in phosphate-buffered saline. Time to cessation of flow (stoppage for more than 30 s) was measured. Assays were terminated at 3 min, and they were carried out by an observer blinded to genotype.

Immunohistochemistry. The right lobes of the lungs were fixed in formalin, and then they were embedded in paraffin for immunohistochemical staining. Paraffin sections (5 μm) were cut, and Vectastain Elite kits (Vector Laboratories, Burlingame, CA) were used for all staining protocols. Deparaffinized and rehydrated sections were incubated for 30 min in 3% H₂O₂ in methanol to quench endogenous peroxidase activity. Thereafter, nonspecific binding was blocked by incubating the sections in diluted normal serum for 30 min. The sections were then incubated in affinity-purified polyclonal antibodies against specific antigens: 1:800 anti-COX-1 from Santa Cruz Biotech (Santa Cruz, CA), 1:400 dilution anti-COX-2 from Cayman Chemical (Ann Arbor, MI), 1:5000 dilution anti-thromboxy- ceyes from WAK Chemie (Steinbach, Germany), and 1:1250 anti-fibrin from Abcam plc (Cambridge, UK). The sections were then incubated for 30 min with the biotinylated secondary antibody and then for 30 min further with streptavidin-biotin complex before incubation with 0.025% 3,3-diaminobenzidine for 2 to 15 min. Sec-
Cross-reactivity to other related eicosanoid compounds is negligible. Matocrit levels were elevated in wild-type (49.7 ± 2.4%) mice after a 24-h time period. After this time period, the excreted urine was collected and stored at –80°C for analysis of 6-keto-prostaglandin (PG) F1α, (the stable hydrolysis product of PGI2) and TXB2 metabolites by standard enzyme immunoassay (R&D Systems, Minneapolis, MN). Cross-reactivity to other related eicosanoid compounds is <0.01% for PGE2, PGD2, and 6-keto-PGF1α for the TXB2 antibody, and 0.2% for PGD2 and <0.01% for PGE2 and TXB2, respectively, for the 6-keto-PGF1α antibody. Intra-assay and inter-assay coefficients of variation were 2.9 and 6.0%, respectively, for the 6-keto-PGF1α assay, and 1.6 and 6.2%, respectively, for the TXB2 assay. Absorbance values were measured on a plate reader at 405 nm, with correction between 570 and 590 nm. Levels of sensitivity were calculated as 1.4 and 10.54 pg/ml for 6-keto-PGF1α and TXB2 assays, respectively. Controls used for each assay included a blank control (no sample, conjugate, or antibody) and a nonspecific binding control (conjugate only). Urinary metabolite levels were expressed as milligram of urinary creatinine.

**Statistical Analysis.** All data are expressed as mean ± S.E.M. Data were analyzed by one-way analysis of variance (InStat version 3.0; GraphPad Software Inc., San Diego, CA), with Bonferroni post-tests where appropriate, and also by paired Student’s t-test where appropriate.

**Results**

**Hypoxic Induction of Pulmonary Hypertension.** Hematocrit levels were elevated in wild-type (49.7 ± 1.3 versus 36.6 ± 2.5%; p < 0.01), COX-1 KO (48.8 ± 0.63%) and COX-2 KO mice (50.7 ± 2.2%) following hypoxia, as expected (n = 8/group). RVESP (n = 8/group; Fig. 1A) increased in wild-type mice following chronic hypoxia, compared with normoxic controls (24.3 ± 1.4 versus 13.8 ± 1.9 mm Hg). A more marked increase in RVESP was seen in mice with the COX-2 gene deletion following hypoxia (36.8 ± 2.7 mm Hg versus wild-type mice). Mice with targeted disruption of the COX-1 gene showed a less marked increase in RVESP than wild-type mice (18.6 ± 1.6 mm Hg); although the difference was not statistically significant.

The ratio of the weight of the right ventricle (RV) to the left ventricle and septum (LV + S) was recorded as an index of right ventricular hypertrophy (n = 8/group; Fig. 1B). Chronic hypoxia resulted in an increase in the RV/LV + S ratio in wild-type mice, relative to normoxic controls (0.34 ± 0.02 versus 0.26 ± 0.013). The RV/LV + S ratio was unaffected by COX-1 (0.35 ± 0.02) or COX-2 (0.36 ± 0.02) gene deletion under normoxic conditions or following chronic hypoxia exposure.

**Measurement of Right Ventricular Apoptosis.** Because COX-2 has been shown to protect against cell death in the heart (Dowd et al., 2001), we examined the effect of COX-2 gene disruption on apoptosis in right ventricular myocytes (Fig. 2; n = 8/group). The rate of apoptosis was unchanged by chronic hypoxia (Fig. 2, B versus A) in wild-type mice or following COX-1 gene disruption (Fig. 2C), but it was increased by COX-2 gene disruption (0.66 ± 0.04 versus 0.33 ± 0.04%) (Fig. 2, D and E).

**Immunohistochemistry for COX Isoform Expression.** COX-1 protein was ubiquitously expressed in the lung tissue of wild-type normoxic (Fig. 3A), wild-type hypoxia (Fig. 3B), and COX-2 KO (Fig. 3D) mice, and it was absent in the COX-1 knockout mice (Fig. 3C).

In contrast, the COX-2 protein was absent in the lung tissue of wild-type normoxic mice (Fig. 3E). COX-2 expression was induced by chronic hypoxia (Fig. 3, F and G), where it was mainly localized to the smooth muscle layer of pulmonary blood vessels, but it was absent in the COX-2 KO mice (Fig. 3H).

**Urinary Excretion of Prostanoid Metabolites.** Urinary excretion of 6-keto-PGF1α, was increased with chronic hypoxia (52.4 ± 4.6 versus 29.9 ± 3 ng/ml; n = 6). This was further increased following COX-1 gene disruption (not significant) and attenuated by COX-2 gene disruption (25.4 ± 4.2 ng/ml; n = 6) (Fig. 4A). Urinary 6-keto-PGF1α excretion was unaffected by ifetroban treatment in the COX-2 gene-disrupted mice following hypoxia (37.1 ± 8.2 versus 31.2 ± 3.7 ng/ml untreated).

Urinary TXB2 excretion was also increased by chronic hypoxia (44.6 ± 11.1 versus 14.7 ± 1.8 ng/ml in normoxic controls; n = 6). This was further increased by COX-2 gene deletion (54.5 ± 10.8 ng/ml; not significant), whereas COX-1 gene deletion caused a reduction in TXB2 excretion (4.9 ± 1.6 ng/ml; n = 6) (Fig. 4B). Urinary TXB2 excretion was unaf-
fected by ifetroban treatment in COX-2 gene-disrupted mice following hypoxia (42.7/3.3 versus 44.4/11006 3 ng/ml untreated).

Platelet Activity and Thrombosis. Tail-cut bleed times were recorded in all groups as an in vivo measure of platelet activity (Fig. 5; n = 6/group). Bleed times were reduced in wild-type mice after 3 weeks of chronic hypoxia, relative to normoxic controls (104 ± 3.6 versus 140 ± 11.5 s). COX-2 gene disruption further shortened the bleed time following hypoxia (89 ± 4.8 s; not significant); whereas in mice lacking COX-1, there was prolongation in tail bleed times (177 ± 1.7 versus 140 ± 11.5 s).

Immunohistochemical staining was performed on mouse lung tissue sections for markers of thrombosis (n = 5/group). Two primary antibodies were used: one antibody directed against thrombocytes, and the other antibody against fibrinogen. There was increased thrombocyte deposition within the vessels of wild-type mice following hypoxia, with a further increase following deletion of the COX-2 gene. Thrombocyte expression in the small pulmonary vessels of mice lacking COX-1 was similar to that of wild-type normoxic controls, and it was unchanged by chronic hypoxia. Staining intensity was quantified using Image-Pro Plus software (Fig. 6). Staining intensity was increased in wild-type hypoxic tissue sections in comparison with normoxic controls (437 ± 77 versus 41 ± 8 OD units; not significant), with a further increase in mice lacking COX-2 (1686 ± 372 OD units).

A similar trend was observed with fibrinogen, with in-
increased deposition seen following chronic hypoxia, relative to normoxic controls, and a further increase with COX-2 disruption. Fibrinogen deposition in COX-1 knockout mice was similar to the wild-type (data not shown). These findings suggest that TXA₂ overrides the inhibitory effect of PGI₂ on platelet activation, because increased thrombosis was observed in wild-type mice following chronic hypoxia, whereas urinary levels of both PGI₂ and TXA₂ were increased.

**Effects of Thromboxane/Prostaglandin Endoperoxide Receptor Antagonism on Right Heart Pressure Recordings and Platelet Deposition.** The further increase in RVESP seen following hypoxia in mice lacking COX-2 was attenuated by the thromboxane receptor antagonist ifetroban (50 mg/kg/day) (26.9 ± 11006 1.1 treated versus 35.3 ± 11006 3.3 mm Hg untreated; n = 6; Fig. 7A). No differences in RVESP were observed between untreated COX-2 KO hypoxic mice and those treated with the vehicle alone (35.3 ± 3.3 mm Hg untreated versus 36.8 ± 2.7 mm Hg vehicle). Ifetroban also reduced thrombocyte expression in the pulmonary vasculature following hypoxia (451 ± 157 versus 1579 ± 152 OD units untreated; n = 5; Fig. 7B).

**Discussion**

There is an imbalance in the generation of thromboxane A₂ and PGI₂ in pulmonary hypertension, with plasma and urine concentrations of TXB₂ (the stable metabolite of TXA₂) increased and those of PGI₂ reduced in patients with the disease (Christman et al., 1992; Fuse and Kamiya, 1994). Endogenous PGI₂ is a potential regulator of the pathogenesis of pulmonary hypertension because its analog, iloprost, reduces vascular resistance and improves hemodynamic parameters and survival in patients with the disease (McLaughlin and Rich, 1998). In contrast, the TXA₂ mimetic U-46619 has been shown to cause pulmonary hypertension in the conscious goat (Carrithers et al., 1994). In this study, we demonstrate that cyclooxygenases and their products, TXA₂ and PGI₂ modulate pulmonary hypertension in a hypoxic murine model. Hypoxia increased the expression of COX-2 protein in the smooth muscle layer of pulmonary vessels following 21 days of hypoxia, whereas expression of COX-1 was unaltered. Generation of TXB₂ and 6-keto-PGF₁α (metabolites of TXB₂ and PGI₂) was likewise increased in wild-type mice following hypoxia. TXB₂ and 6-keto-PGF₁α excretion was reduced in mice lacking the COX-1 and COX-2 gene, respectively. Thus, COX-1 was identified as the major source of TXA₂, whereas COX-2 was found to be responsible for the increased generation of PGI₂. Differential signaling by COX-1 and COX-2 is...
thought to be due to selective coupling to terminal isom-eras or reductases that convert PGH₂ to terminal prosta-noids. COX-2 seems to couple preferentially to PGE synthase or PGI₂ synthase (Smith et al., 2000). In the cardiovascular system, selective coupling to prostacyclin synthase has been reported previously (Catella-Lawson et al., 1999). Several conditions, such as protein-protein interactions, differences in kinetic properties, or colocalization of terminal prostaglan-din synthases (e.g., COX-1 and thromboxane synthase in platelets) may also result in selective coupling (Smith et al., 2000). Our findings are consistent with previous observations that selective pharmacological inhibitors of COX-2 decreased PGI₂ production in a rat model of PH, with a concomitant increase in TXA₂ generation (Pidgeon et al., 2004).

Hypoxia resulted in elevated hematocrit, right ventricular systolic pressure and right ventricular hypertrophy (RVH) following hypoxia exposure, reflecting the level of hypoxia and pulmonary hypertension. RVESPs was further increased in mice lacking the COX-2 gene, and it was unaffected in the group lacking COX-1. In contrast, RVH was unaffected by COX-2 gene deletion. The absence of a further increase in RVH in hypoxic mice lacking the COX-2 gene despite the further increase in RVESP may be explained in part by the increased apoptosis of the right ventricular cells. COX-2 has been shown to protect against apoptosis of myocardial cells in several settings, for example in response to oxidative stress (Dowd et al., 2001) and in myocarditis (Takahashi et al., 2005), and partly mediates the reduction in cardiac apoptosis seen with adiponectin in ischemia/reperfusion (Shibata et al., 2005). These findings suggest that COX-2-derived endoge-nous PGI₂ affects both the pulmonary and cardiac response to hypoxia and are consistent with the findings of a previous study demonstrating that transgenic overexpression of PGI₂ synthase protects against hypoxia-induced pulmonary hyper-tension (Geraci et al., 1999).

There are several mechanisms by which endogenous or exogenous PGI₂ may modify the pulmonary vascular re-sponse to hypoxia, including vasodilation, remodeling of the pulmonary vascular bed, and suppression of platelet activa-tion. Platelet activation is enhanced in patients with primary pulmonary hypertension (Farber and Loscalzo, 1999), and intravascular thrombosis has also been observed both in patients and in model systems. Moreover, experimentally induced thrombocytopenia reduces the development of pul-monary hypertension (Kanai et al., 1993). In this study, staining for thrombocytes (αIIBβ3) and fibrinogen showed platelet deposition and thrombosis in the pulmonary vasculature of wild-type hypoxic mice. There was also evidence of increased systemic platelet activation as indicated by a reduced tail-cut bleeding times (Weiss et al., 2002). As anticipated, increased tail-cut bleeding times were observed in COX-1 gene-disrupted mice, and there was reduced thrombo-cyte and fibrin deposition in pulmonary vessels. However, there was only a modest and nonsignificant reduction in RVESP. In contrast, there was a marked increase in the pattern and intensity of staining for thrombocytes in mice lacking the COX-2 gene. Under normal circumstances PGI₂ offsets the effect of TXA₂, thereby reducing its effect on platelet activation. In this model, COX-2 gene disruption and chronic hypoxia resulted in a significant inhibition of PG II production with a concomitant elevation (albeit nonsignifi-cant) in TXA₂, resulting in an altered balance of these pro-stanoids. This could favor elevated platelet activation and thrombosis, effects that were observed in our study. Following chronic hypoxia, it may be deduced that TXA₂ overrides the protective effect of PGI₂, because increased thrombosis was observed in wild-type hypoxic mice (where urinary levels of both TXA₂ and PGI₂ were increased).

The clinical use of selective COX-2 inhibitors has com-monly been associated with an increased risk of thrombosis (Nussmeier et al., 2005; Westgate and Fitzgerald, 2005), lending support to our observations. The mechanisms whereby these drugs contribute to cardiac complications may be through a disruption in the fine balance between PGI₂ and TXA₂, which acts to regulate blood clotting. In this study, the increase in RVESP and platelet deposition in the pulmonary vasculature of COX-2 KO mice following chronic hypoxia was abolished by the TXA₂/prostaglandin endoperoxide antago-nist ifetroban. This observation supports our theory that the increase in RVESPs following disruption of the COX-2 gene and consequent suppression of PGI₂ generation is at least partially TXA₂-mediated, possibly through platelet activa-tion. Although we cannot exclude the possibility of a nonspe-cific effect of the drug, ifetroban has previously been used to examine the effect of thromboxane receptor antagonism in a rat model of the disease, where it reduced effects attributed to overproduction of TXA₂ under hypoxia (Pidgeon et al., 2004). In a separate study, ifetroban restored endothelium-dependent relaxation in spontaneously hypertensive rats (Tesfamariam and Ogletree, 1995). In the absence of COX-2,
targeting the COX-1 pathway may be insufficient to affect thrombus formation because other platelet-activating mechanisms, such as ADP, serotonin, collagen, and thrombin, remain fully functional (Hennan et al., 2001). Our observations are consistent with recent findings in a murine model of carotid artery injury, where platelet deposition was enhanced following disruption of the prostacyclin receptor and suppressed by coincident disruption of the thromboxane receptor. The findings in both models suggest that endogenous PGI2 suppresses platelet activity in vivo, and it is particularly important in regulating the platelet response to TXA2 (Cheng et al., 2002). Our study also demonstrates that COX-1 is responsible for the enhanced TXA2 generation in this model. One potential tissue source is the platelet, in which TXA2 is the principle product. However, other tissues, including the lung, may generate TXA2. Indeed, we have observed expression of thromboxane synthase as well as COX-1 in the vasculature of this pulmonary hypertension model (data not shown).

Cyclooxygenases and their products influence other mechanisms that may contribute to our observations. Generation of COX-2-derived prostanooids has been shown to promote the expression of proangiogenic factors (Masferrer et al., 2000). Although a role for COX-1 in endothelial cell angiogenesis has been reported previously (Tsuiji et al., 1998), the majority of studies have indicated that the antiangiogenic effects of nonsteroidal anti-inflammatory drugs result mainly from COX-2 inhibition (Masferrer et al., 2000; Dornond et al., 2001). Downstream of the cyclooxygenases, TXA2 inhibits angiogenesis, an effect that is partly mediated by modifying the expression of vascular endothelial growth factor (Ashton and Ware, 2004). However, a proangiogenic role for TXA2 has also been described previously (Nie et al., 2000). TXA2 has also been shown to inhibit endothelial cell proliferation, and it may regulate vascular remodeling in this system (Ashton et al., 1999). Deletion of the prostacyclin receptor, or PGRII, suppression with a selective COX-2 inhibitor, resulted in increased vascular remodeling (Rudic et al., 2005). In contrast, thromboxane receptor deletion inhibited vascular remodeling in response to selective COX-2 inhibition. These mechanisms were not addressed in our study, although a decrease in vessel wall thickness following TXA2 receptor antagonism was observed (data not shown). Endothelial derived endoperoxides may also play a role in the response to chronic hypoxia. Hypoxia has been shown to cause inactivation of prostacyclin synthase, favoring PGH2 release and vasconstriction. The release of PGH2 into the plasma also triggers the formation of platelet-derived TXA2, resulting in vasospasm, platelet aggregation, and thrombosis (Zou and Bachschmid, 1999).

In conclusion, COX-2 gene deletion exacerbates pulmonary hypertension, enhances sensitivity to TXA2, and induces intravascular thrombosis in response to hypoxia. The data provide evidence that endogenous prostacyclin and thromboxane modulate the pulmonary response to hypoxia through their opposing effects on platelet activity.

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References


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