Novel aspects of eicosanoid signaling through the use of gene-targeted mice

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Abstract

Eicosanoids are lipid mediators consisting of prostaglandins, leukotrienes, lipoxins and related compounds derived primarily from arachidonic acid and to a lesser extent from eicosapentaenoic and di-homo-gamma-linolenic acids. This large class of bioactive lipids is derived from the initial release of polyunsaturated fatty acid from the sn2 position of glycerophospholipids predominantly from cytosolic phospholipase A2 and subsequent conversion by either prostaglandin H synthases-1 and -2 (PGHS-1, PGHS-2; also known as COX-1, COX-2), lipoxygenases or various members of the cytochrome P450 family. Eicosanoids control a vast array of physiological functions, including female reproductive function and parturition, platelet aggregation and vascular homeostasis, as well as renal function and roles in inflammation initiation and resolution. The authors have been studying the functions and signaling of eicosanoids, in particular the prostaglandin class of molecules, using a series of induced mutant mouse strains created in the laboratory by manipulation of the PGHS-1 and PGHS-2 genes by gene targeting to unravel new insights into eicosanoid signaling.

Keywords: aspirin; cardiovascular; coxib; inflammation; prostaglandin

Abbreviations: AA: arachidonic acid; ASA: aspirin; BP: blood pressure; COX: cyclooxygenase; DA: ductus arteriosus; IEL: internal elastic lamina; KD: knockdown; KO: knockout; LOX: lipoxygenase; NSAID: non-steroidal anti-inflammatory drug; PG: prostaglandin; PGHS: prostaglandin H synthase; PGI2: prostacyclin; POX: peroxidase; TPA: tetradecanoyl phorbol acetate; TxA2: thromboxane A2; WT: wild-type.

Introduction

There are three major enzymic pathways of eicosanoid biosynthesis via dietary essential fatty acid-catalyzed conversion by prostaglandin H synthases (PGHS), lipoxygenases (LOX) and cytochromes P450. The PGHS pathways produce prostaglandins (PGs), prostacyclin (PGI2) and thromboxane A2 (TxA2), lipoxygenase pathways give rise to leukotrienes, lipoxins, and 5-, 12- and 15-hydroxy-eicosatetraenoic acids, while the cytochrome P450 pathway metabolites are hydroxylated or epoxy-metabolites of arachidonic acid (AA). These eicosanoids possess a vast array of biological functions in pain, inflammation and many disease settings. The focus of this short review is to illuminate new understanding of the oldest class of eicosanoids, e.g. prostaglandins, by using gene-manipulated mice to alter the expression of PGHS.

Non-steroidal anti-inflammatory drugs (NSAIDs) target PGHS enzymes, which exist in two isoforms, PGHS-1 and PGHS-2. Aspirin and other traditional NSAIDs in the class are non-selective inhibitors of both PGHS-1 and PGHS-2, while newer agents termed “coxibs”, including rofecoxib, valdecoxib and celecoxib, are selective inhibitors of PGHS-2. Both PGHS isoforms are bifunctional with a cyclooxygenase (COX) activity and a functional linked peroxidase (POX) activity. The NSAIDs, including aspirin and coxibs, act via inhibition of the COX activity, while leaving POX activity intact. Aspirin is the most widely used drug in the world, and low-dose aspirin use is effective in the secondary prevention of both heart attack and stroke (1–3) and in the primary prevention of coronary heart disease in at-risk individuals (4–6). The development of coxibs was based on the notion that PGHS-2 was the major source of PGE2 and...
PGI₂ (prostacyclin), which mediates inflammation, and that PGHS-1 was the dominant PG source for gastric cytoprotection (7). The authors recently created two novel mouse strains, referred to as “low-dose aspirin genetic mimic” or PGHS-1 knockdown (KD) (8) and “selective COX-2 inhibitor genetic mimic” or PGHS-2 Y385F (9, 10) for the study of prostaglandin functions.

Prostaglandin H synthase-1 knockdown mouse model mimics low-dose aspirin treatment

Insertion of a neomycin resistance cassette within intronic sequences has been reported previously to generate a hypomorphic allele or “knockdown” of gene expression to very low levels (11–13). The PGHS-1 KD mice were generated by insertion of this cassette into intron 10 of the PGHS-1 gene through homologous recombination in murine embryonic stem cells. Follow-up characterization of the resultant mice proved that PGE₂ production was diminished by 75% compared with wild-type (WT) in unstimulated peritoneal macrophages and PGHS-1 expression was suppressed similarly (70–73%) in the macrophages and other PGHS-1 KD mouse tissues tested as well, which indicates that this model is globally hypomorphic for PGHS-1 expression.

The PGHS-1 isoform, but not PGHS-2, is expressed in mature human platelets, which are the dominant source of TxA₂ biosynthesis in humans (14). Pharmacological studies suggest that inhibition of the capacity of human platelets to generate TxA₂ by more than 95% is necessary to accomplish inhibition of TxA₂-dependent platelet aggregation *ex vivo* (15). The plasma thromboxane metabolite, TxB₂, was depressed by 97% in PGHS-1 KD mice compared with WT controls, which is remarkably similar to the inhibition achieved by low-dose aspirin treatment in mice (96%) and agrees with the level of inhibition achieved in humans (15). Moreover, the platelets from both PGHS-1 KD and low-dose aspirin-treated WT mice (ASA/WT) failed to aggregate in response to various concentrations of AA that were effective in WT platelets (Fig. 1A).

The PGHS-1 KD mouse model mimics low-dose aspirin clinical treatment regarding platelet function.

Protection against thrombosis and impaired inflammatory response in prostaglandin H synthase-1 knockdown mice

Since platelets from PGHS-1 KD mice have defective generation of thromboxane and impaired aggregation responses *ex vivo*, the finding that PGHS-1 KD mice were resistant to AA-induced thrombosis *in vivo* (Fig. 1B), while nearly all the normal WT controls died at the tested AA doses (8), could perhaps have been anticipated. However, since the time to arterial occlusion by clot formation was markedly prolonged in PGHS-1 KD mice compared with WT mice in a model of oxidative stress-induced arterial photochemical injury (8), the
implications for thromboxane involvement in thrombosis are far reaching.

The role of PGHS-1-derived prostaglandins and low-dose aspirin in the setting of inflammation remains controversial. Several inflammatory models, including chemically evoked ear swelling assays by AA, capsaicin, phorbol esters and croton oil, and carrageenan-induced paw edema, were tested in PGHS-1 KD mice (8). PGHS-1 KD mice displayed a notably reduced inflammatory response to AA, capsaicin and carrageenan, but failed to demonstrate significant inflammatory protection in response to tetradecanoyl phorbol acetate (TPA) and croton oil, suggesting that PGHS-1-derived eicosanoids may be involved in some aspects of the inflammatory response in mice in addition to the well-known actions of PGHS-2-derived eicosanoids.

Normal parturition in prostaglandin H synthase-1 knockdown and low-dose aspirin-treated mice, but not in prostaglandin H synthase-1 null mice

During late gestation, PGHS-1 expression is up-regulated in the uterus then decreases to undetectable levels after delivery (16), fitting with its requirement for normal initiation of parturition in mice. PGHS-1-derived PGF$_{2\alpha}$ mediates the initiation of parturition, and exogenous administration of this eicosanoid can rescue the parturition defect observed in PGHS-1 null mice (16) and induce a decline in serum progesterone to initiate the birthing process (17). Prostaglandin F receptor-deficient mice exhibit parturition failure owing to impaired withdrawal of serum progesterone at term (18). Uterus PGHS-1 expression in PGHS-1 KD mice was about 12% of normal at day 17.5 of gestation, and only 8% PGF$_{2\alpha}$ in these mice sufficed to prepare for normal labor onset at day 19 of gestation (Table 1). By contrast, the residual synthetic capacity in PGHS-1 null mice (1.1% PGF$_{2\alpha}$) was unable to initiate normal parturition at term. Moreover, just before delivery at day 19, the striking serum progesterone fall accompanied by oxytocin induction in myometrium was observed in PGHS-1 KD mice as in WT mice. Similarly to PGHS-1 KD mice, low-dose aspirin treatment to WT mice (ASA/WT) did not affect initiation of full-term labor (Table 1). Use of these novel PGHS-1 KD mice has allowed an efficient titration of the amounts of prostaglandin necessary to achieve an important regulatory physiological process.

Prostaglandin H synthase-2 Y385F mice represent a novel genetic mimic of coxib administration

Tyrosine 385 of PGHS is a critical residue for initiation of COX catalysis, and the phenylalanine-385 (Y385F) mutant of PGHS lacks COX activity but retains POX activity (7, 19). [Note that in this review, the tyrosine residue of PGHS-2 is numbered in parallel with ovine PGHS-1, the recognized standard in numbering nomenclature of PGHS isoforms to facilitate comparison of important residues in catalytic function. Accordingly, the actual position in mouse PGHS-2 is Tyr-371 based on numbering the amino-terminal methionine of the signal peptide as residue number 1.] To generate a mouse model that might mimic coxib administration, a Y385F point mutation was inserted into the PGHS-2 gene (known as $Ptgs2$) through a gene-targeting strategy (10). Biochemical studies in vitro confirmed that peritoneal macrophages of PGHS-2 Y385F mice lack COX activity from inducible PGHS-2 protein without disruption of POX activity, which apparently replicates the scenario with selective PGHS-2 inhibitor (coxib) treatment (7).

Table 1. Normal parturition in PGHS-1 knockdown (KD) and low-dose aspirin-treated mice, but not in PGHS-1 knockout (KO) mice

<table>
<thead>
<tr>
<th>Paternal genotype</th>
<th>Maternal genotype</th>
<th>No.</th>
<th>Labor onset (days) [range]</th>
<th>Average litter</th>
<th>Survival for 24 h (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGHS-1 KD</td>
<td>PGHS-1 KD</td>
<td>8</td>
<td>19.6 ± 0.2 [19.0–20.5]</td>
<td>7.3 ± 1.1</td>
<td>90.0</td>
</tr>
<tr>
<td>ASA/WT*</td>
<td>WT</td>
<td>6</td>
<td>19.3 ± 0.1 [18.0–19.5]</td>
<td>8.4 ± 1.6</td>
<td>92.3</td>
</tr>
<tr>
<td>PGHS-1 KO</td>
<td>PGHS-1 KD</td>
<td>10</td>
<td>21.1 ± 0.3*** [20.0–22.5]</td>
<td>8.7 ± 0.7</td>
<td>54.0*</td>
</tr>
<tr>
<td>WT</td>
<td>WT</td>
<td>7</td>
<td>19.3 ± 0.2 [19.0–20.0]</td>
<td>6.8 ± 0.5</td>
<td>89.5</td>
</tr>
</tbody>
</table>

Data from Yu et al. (8).

*Low-dose aspirin was supplied to wild-type mice in their drink (30 mg l$^{-1}$), starting on gestation day 7.

PGHS: prostaglandin H synthase; ASA: aspirin; WT: wild-type.

***p <0.001, *p <0.05 vs PGHS-1 KD, ASA/WT and WT mating.
TxA2 is the major PGHS-1 product from platelets, while PGI2 is synthesized dominantly from PGHS-2, which is expressed in shear-stimulated endothelium (20). Coxibs can selectively suppress circulating prostacyclin in humans (21). Urinary thromboxane metabolite excretion is not affected by Y385F mutation in PGHS-2 Y385F mice. In contrast, prostacyclin metabolite excretion is dramatically diminished, which is similar to celecoxib-treated mice (9). PGHS-2 Y385F mouse appear to represent a genuine genetic model of coxib administration in mice.

Elevated blood pressure and thrombosis susceptibility in prostaglandin H synthase-2 Y385F mice

NSAIDs, including PGHS-2 selective inhibitors, have been associated with hypertension reports or deterioration of blood pressure (BP) control in patients receiving antihypertensive drugs (22, 23), and this hypertension shows a relationship to both selectivity of PGHS-2 inhibition and duration of drug exposure (24). Short-term studies of PGHS-2 inhibitors reveal that they evoke transient sodium retention (25). As expected, elevated BP, including both diastolic and systolic measures, was observed in PGHS-2 Y385F mutant and PGHS-2 selective inhibitor celecoxib-treated mice on a regular chow diet compared with WT controls (Fig. 2A).

Prostacyclin is the predominant PG product in endothelium, causing vasodilatation and inhibition of platelet aggregation, and preventing the proliferation of vascular smooth-muscle cells in vitro. In contrast, thromboxane has opposing actions, evoking platelet aggregation, vasoconstriction and vascular proliferation (20). Since the balance of these two eicosanoids was disrupted as measured by urinary metabolites, as mentioned above, the cardiovascular effects of thromboxane would be expected to be exaggerated. Consistent with these observations, genetically induced PGHS-2 inhibition in PGHS-2 Y385F mice augments the thrombotic response to the thromboxane agonist U46619 (Fig. 2B), and a highly selective PGHS-2 inhibitor, DFU, increases thrombosis susceptibility in a photochemical vascular injury model (9).

Normal neonatal ductus arteriosus remodeling in prostaglandin H synthase-2 Y385F, but not in prostaglandin H synthase-2 null mice

The fetal ductus arteriosus (DA) is an essential conduit for deoxygenated blood to bypass the collapsed lungs to enter via the descending aorta to the placenta. PGs are essential mediators of DA patency during fetal life and can modulate its constriction in the neonatal period (26). Mice deficient in PGHS-2 (10, 27) or lacking the PG receptor EP4 (28) predispose to failure of DA remodeling at birth. Surprisingly, patent DA and resulting neonatal mortality were not observed in PGHS-2 Y385F mice and celecoxib-treated mice (Fig. 3). Electron microscopic analysis of the DA 4 h after delivery revealed multiple endothelial cell layers oriented toward the lumen, fragmentation of
the internal elastic lamina (IEL) and smooth-muscle cell infiltration to the subendothelial region across the fragmented IEL in PGHS-2 Y385F and WT mice, indicating successful remodeling. In contrast, the patent DA in PGHS-2 null mice display a single layer of endothelial cells with an intact IEL. This normal DA remodeling in PGHS-2 Y385F mutant mice at birth might be explained by a novel means of PGHS-1/PGHS-2 heterodimer signaling in the DA at birth (10). Thus, molecular modeling and a variety of biochemical tests provided evidence that PGs can be synthesized from an active PGHS-1/mutant PGHS2 complex and that the two PGHS isoforms can associate (10).


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