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Role of Prostacyclin in the Cardiovascular Response to Thromboxane A_2

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Thromboxane (Tx) A_2 is a vasoconstrictor and platelet agonist. Aspirin affords cardioprotection through inhibition of TxA_2 formation by platelet cyclooxygenase (COX-1). Prostacyclin (PGI_2) is a vasodilator that inhibits platelet function. Here we show that injury-induced vascular proliferation and platelet activation are enhanced in mice that are genetically deficient in the PGI_2 receptor (IP) but are depressed in mice genetically deficient in the TxA_2 receptor (TP) or treated with a TP antagonist. The augmented response to vascular injury was abolished in mice deficient in both receptors. Thus, PGI_2 modulates platelet-vascular interactions in vivo and specifically limits the response to TxA_2. This interplay may help explain the adverse cardiovascular effects associated with selective COX-2 inhibitors, which, unlike aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), inhibit PGI_2 but not TxA_2.

Prostacyclin (PGI_2) is the major product of cyclooxygenase (COX) catalyzed metabolism of arachidonic acid in macrovascular endothelium (*1*, *2*). It is a potent inhibitor of platelet activation by all recognized agonists and a vasodilator. Biosynthesis of PGI_2 is increased in human syndromes of platelet activation (*3*), suggesting that it functions as a homeostatic response to accelerated platelet–vessel wall interactions. However, its importance in vivo has remained speculative. Recent studies of the coxibs, which are selective inhibitors of COX-2 (*4*) have suggested that this isozyme is a major source of PGI_2 under physiological conditions in humans (*5*, *6*), perhaps reflective of COX-2 induction in endothelial cells by hemodynamic shear (*7*). PGI_2 synthase preferentially couples with COX-2 rather than COX-1 in coexpression systems (*8*).

It is possible that PGI_2 modulates cardiovascular homeostasis in humans, as suggested by the outcome of a controlled comparison of a selective COX-2 inhibitor, rofecoxib, and an isoform nonselective inhibitor, naproxen (the VIGOR study). In this trial, rofecoxib was associated with a higher risk of myocardial infarction by a factor of 5 (*9*). Two mechanistic hypotheses have been advanced to explain these results: (i) A cardioprotective effect of naproxen, mediated by its inhibition of COX-1–dependent production of platelet TxA_2, and (ii) enhancement of the deleterious cardiovascular effects of TxA_2 in the patients on rofecoxib, which, in contrast to naproxen, inhibits only PGI_2 production, leaving TxA_2 unopposed (*6*).

To test the hypothesis that PGI_2 modulates the cardiovascular effects of TxA_2 in vivo, we generated mice with disordered expression of receptors for these eicosanoids and monitored the response to vascular injury. Both PGI_2 and TxA_2 activate G protein–coupled receptors, the prostacyclin receptor (the IP) and the thromboxane receptor (the TP), respectively (*10*, *11*). Others have shown that IP knockout mice (IPKOs) do not develop spontaneous thrombosis but are more susceptible to thrombotic stimuli than their wild-type (WT) littermates (*12*). TPKOs, by contrast, exhibit a mild bleeding tendency (*13*). Both IPKOs and TPKOs are normotensive.

Initially, we studied the impact of IP deletion on the proliferative response to catheter-induced carotid vascular injury. This model involves passage of a polyamide catheter containing a fine wire down the common carotid artery, causing endothelial denudation, neointimal formation, and vascular smooth muscle cell proliferation (*14*, *15*). Neointimal proliferation was not observed 2 weeks after surgery in sham-treated mice, but it was induced by injury in both IPKOs and their WT littermates (*15*). Deletion of the IP resulted in an enhanced proliferative response to injury: the intima-to-media ratio increased significantly in the IPKOs (Fig. 1A). The percentage of luminal stenosis was also increased after injury in the IPKOs [Web table 1 (*15*)]. We also assessed intimal cellular proliferation by bromodeoxyuridine (BrdU) labeling. There was no significant labeling in the intima or media of uninjured WTs. Injury caused a marked increase in labeling of both intimal and medial cells in WTs. However, intimal and medial proliferation was enhanced in IPKOs (Fig. 1B). In humans, vascular angioplasty is associated with an increase in platelet activation, reflected by urinary excretion of the Tx metabolite (Tx-M), 2,3-dinor-TxB_2 (*16*). Patients undergoing coronary angioplasty are given aspirin, which suppresses TxA_2 formation and reduces the periprocedural rate of myocardial infarction by ~50% (*17*). We found that catheter-induced carotid injury in the mouse also results in a significant increase in Tx biosynthesis (Fig. 1C), which was augmented in the IPKOs compared with IPWTs (Fig. 1D). These data support the hypothesis that PGI_2 modulates platelet activation by TxA_2 in vivo.

To address the functional importance of TxA_2 in this mouse model, we investigated the effect of S18886, a highly selective, long acting TP antagonist activity (*18*). We measured the impact of TP antagonism on the response to

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vascular injury in both WT mice and transgenic mice with vascular overexpression of the human TP (TPOEs) (*15*, *19*). Oral administration of S18886 (10 mg/kg body weight, per os) completely inhibited platelet aggregation induced by the TP agonist, U46619, in both WT and TPOEs (*20*). Both 10 mg/kg and 100 mg/kg of S18886 significantly decreased the intima-to-media ratio, percentage stenosis, and the number of intimal cells labeled with BrdU in WT mice (Table 1). The response to injury was markedly exaggerated in TPOEs (Fig. 2). Thus, the intima-to-media ratio (1.2 ± 0.1 versus 0.6 ± 0.1; $P < 0.01$), the percentage stenosis (77.6 ± 4.3 versus 31.7 ± 5.2; $P < 0.05$), and the number of intimal BrdU-labeled cells (198.8 ± 26.4 versus 94.9 ± 19.5; $P < 0.05$) were all exaggerated in the transgenic animals. The increase in platelet activation evoked by vascular injury, as reflected by urinary Tx-M, was also more pronounced in TPOEs (6.2 ± 1.1 times basal) than in WT mice (3.2 ± 0.6 times basal; $P < 0.05$). TP antagonism also reduced the response to vascular injury in the TPOEs (Fig. 2), although the dose-response relationship was modified compared with that in WT mice. Thus, oral administration of S18886 (100 mg/kg per day) reduced the intima-to-media ratio (0.5 ± 0.1; $P < 0.01$), the percentage stenosis (39.2 ± 9.7; $P < 0.001$), and intimal BrdU-labeled cells (52.9 ± 17.9; $P < 0.01$) evoked by injury in TPOEs. Although S18886 does not inhibit enzymes in the Tx biosynthetic cascade in vitro (*18*), treatment with the antagonist reduced the procedure-related increment in Tx-M (1.6 ± 0.4; $P < 0.01$ versus untreated TPOEs). This is likely to reflect interruption of the continued stimulation of platelets by TxA_2 released after TP-mediated platelet activation (*21*).

To exclude the possibility that properties unrelated to TP antagonism might account for the effects of S18886, we determined that TP deletion reduced the changes evoked in the intima-to-media ratio (Fig. 1A), percentage stenosis (Web table 1), and the number of BrdU-labeled cells (Fig. 1B) compared with WT littermates (*15*). Consistent with our observa-

Table 1. The effects of TP antagonism with S18886 on the proliferative response to vascular injury in WTs. Results are means ± SEM of values from eight animals in each group. *$P < 0.05$, **$P < 0.01$ compared with vehicle-treated group (one-way ANOVA analysis and Newman-Keuls multiple-comparison test).

	Intimal-to-medial area	Percent lumen stenosis	Proliferative cells (cells/cross section)
Vehicle	0.63 ± 0.09	40.8 ± 10.0	102 ± 17
S18886			
1 mg/kg	0.58 ± 0.11	45.1 ± 10.5	100 ± 25
10 mg/kg	0.30 ± 0.08*	22.5 ± 5.6*	50 ± 12*
100 mg/kg	0.26 ± 0.04**	19.0 ± 3.5*	43 ± 9*

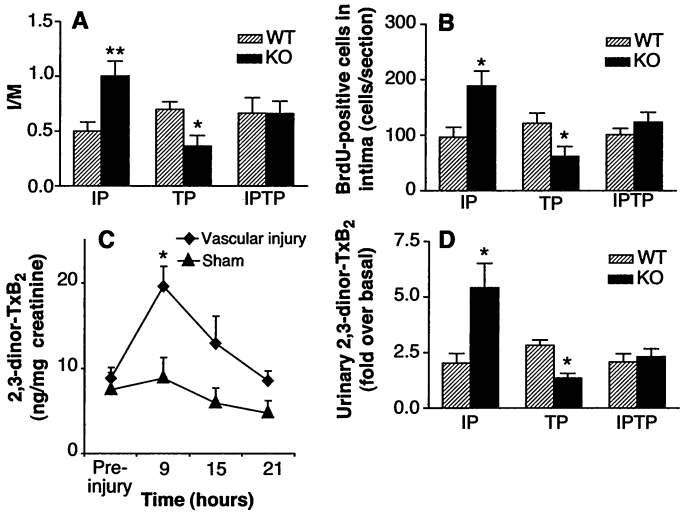

Fig. 1. Morphometric analysis of carotid arteries and Tx-M biosynthesis after vascular injury in WT and KO mice. (**A**) Intima-to-media ratio; (**B**) the intimal BrdU-labeled cell number. Results are means ± SEM of values from eight or nine animals in each group. *$P < 0.05$, **$P < 0.01$ compared with corresponding WTs group, respectively by Student's *t* test. (**C**) Urinary excretion of 2,3-dinor-TxB_2 (Tx-M) increased in response to vascular injury in WTs. The data are depicted at the midpoint of the timed collections. Tx-M increased significantly in the periprocedural aliquot of urine (*$P < 0.05$ versus preinjury by Kruskal-Wallis test). Tx-M did not change in the sham group. (**D**) The periprocedural increment in urinary Tx-M times baseline values in WTs versus KOs after vascular injury (*$P < 0.05$).

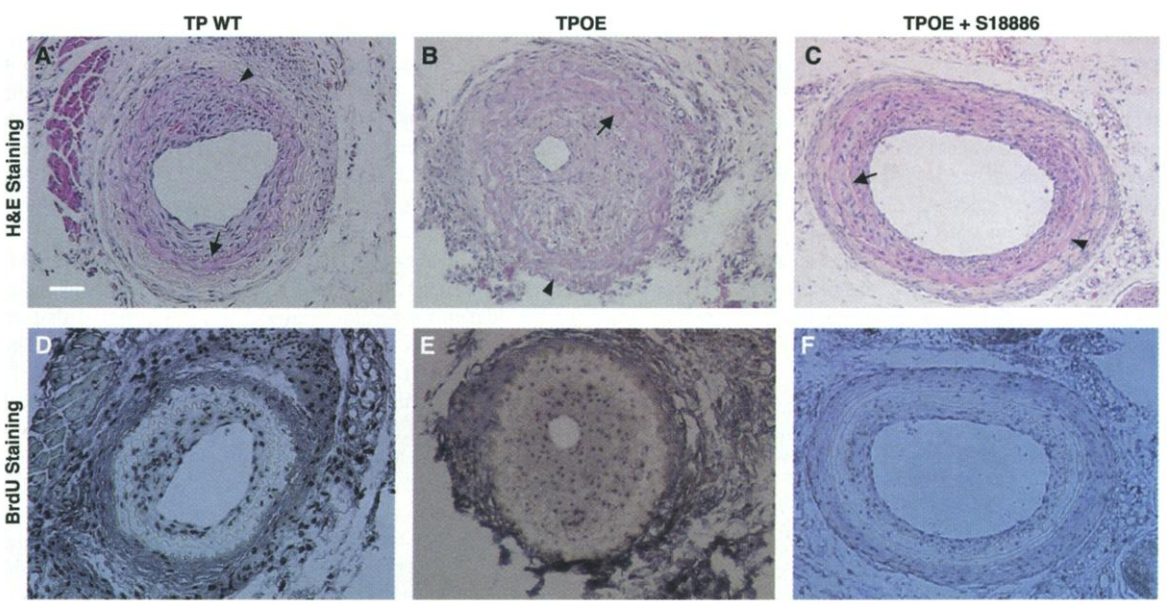

Fig. 2. The response to vascular injury in WT and TPOE carotid arteries and the effect of the TP antagonist, S18886. Injured artery in WTs (**A**), TPOEs (**B**), and TPOEs treated with S18886 (**C**) are representative segments from hematoxylin and eosin (H&E)–stained sections. The sections of injured artery in WTs (**D**), TPOEs (**E**), and TPOEs treated with S18886 (**F**) stained with BrdU. The arrows and the arrowheads indicate the internal elastic laminae and the external elastic laminae, respectively. Scale bar, 50 μm.

tions with TP antagonism, TP deletion also reduced the procedure-related increment in urinary Tx-M (Fig. 1D). To address the contribution of exaggerated Tx biosynthesis to the vascular proliferative response in IPKO mice, we studied coincidental deletion of the TP and the IP [TPIP double knockouts (DKOs)], which abolished the increased response to injury observed in the IPKOs (*15*) (Fig. 1, A, B, and D, and Web table 1). All three indices of proliferation were similar in TPIP DKOs and their WT littermates. Furthermore, the augmented, procedure-related increment in platelet activation, observed in IPKOs, was also abolished by concomitant TP and IP deletion (Fig. 1D).

Biosynthesis of PGI_2 is elevated along with TxA_2 in human syndromes of platelet activation (*22*, *23*), and the formation and activity of the two prostaglandins may interact via several mechanisms. TP agonists evoke PGI_2 release from endothelial cells in vitro (*24*), and platelet-derived endoperoxide precursors of TxA_2 may also be utilized by endothelial COX to generate PGI_2 in vitro (*25*). Pharmacological inhibition of Tx synthase enhances rediversion of endoperoxides to platelet inhibitory prostanoids, such as PGI_2, in vivo (*26*). Both the arachidonic acid in microparticles shed by activated platelets (*27*) and platelet-derived TxA_2 evoke COX-2–dependent PGI_2 formation by endothelial cells (*28*). Considerable evidence also exists for cross talk between IP- and TP-dependent signaling pathways. Thus, TP desensitization evokes sensitization to IP agonists (*29*), and IP activation evokes TP desensitization (*30*).

Our data establish that endogenous PGI_2 modulates the cardiovascular actions of TxA_2 in vivo. This interplay may be relevant to the cardiovascular effects of selective COX-2 inhibitors, which, unlike aspirin and NSAIDs, depress PGI_2 without coincidental inhibition of TxA_2 formed by COX-1 in platelets. Although our results suggest that the enhancement of the deleterious effects of TxA_2 is a plausible explanation for the cardiovascular outcome in the VIGOR trial, they do not exclude a contributory role for naproxen's cardioprotective effect. Indeed, both mechanisms may be important. If the estimate of the difference in incidence rates of myocardial infarction between the groups is accurate, it is roughly twice that expected if naproxen was affording cardioprotection via sustained inhibition of platelet COX-1 (*31*).

The response to carotid vascular injury was modified by both TP antagonism and TP deletion. Although our mouse models do not perfectly simulate percutaneous angioplasty on an atherosclerotic background in humans, it prompts reconsideration of the potential utility of TP antagonists in this setting. Two clinical trials have compared TP antagonists with aspirin for the prevention of restenosis after angioplasty (*32*, *33*). TP antagonists, in contrast to aspirin, do not depress PGI_2 formation. Patients received periprocedural aspirin before randomization to continuing aspirin or a TP antagonist for the following 6 months. However, the aspirin regimen used depresses the procedure-related increment in PGI_2 biosynthesis (*16*). Our results suggest that this may have conditioned the proliferative response to injury and may have undermined the comparison of the two treatments.

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Optical Projection Tomography as a Tool for 3D Microscopy and Gene Expression Studies

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Current techniques for three-dimensional (3D) optical microscopy (deconvolution, confocal microscopy, and optical coherence tomography) generate 3D data by "optically sectioning" the specimen. This places severe constraints on the maximum thickness of a specimen that can be imaged. We have developed a microscopy technique that uses optical projection tomography (OPT) to produce high-resolution 3D images of both fluorescent and nonfluorescent biological specimens with a thickness of up to 15 millimeters. OPT microscopy allows the rapid mapping of the tissue distribution of RNA and protein expression in intact embryos or organ systems and can therefore be instrumental in studies of developmental biology or gene function.

The ability to analyze the organization of biological tissue in three dimensions has proven to be invaluable in understanding embryo development, a complex process in which tissues undergo an intricate sequence of movements relative to each other. A related goal is the mapping of gene expression patterns onto these 3D tissue descriptions (*1*). This information provides clues about the biological functions of genes and also indicates which genes may interact with each other. Gathering this data has become one of the clear challenges of the genomics era.

A number of techniques for obtaining 3D information about biological tissue have recently been developed or improved (*2*–*7*). Methods for the digital reconstruction of thin serial sections have become increasingly automated (*2*,

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