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Thromboxane and the thromboxane receptor in cardiovascular disease

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Abstract

Thromboxane A₂ (TXA₂), the primary product of COX-1-dependent metabolism of arachidonic acid, mediates its biological actions through the TXA₂ receptor, termed the TP. Irreversible inhibition of platelet COX-1-derived TXA₂ with low-dose aspirin affords protection against primary and secondary vascular thrombotic events, underscoring the central role of TXA₂ as a platelet agonist in cardiovascular disease. The limitations associated with aspirin use include significant gastrointestinal toxicity, bleeding complications, potential interindividual response variability and poor efficacy in some disease states. This, together with the broad role of TXA₂ in cardiovascular disease beyond the platelet, has refocused interest towards additional TXA₂-associated drug targets, in particular TXA₂ synthase and the TP. The superiority of these agents over low-dose aspirin, in terms of clinical efficacy, tolerability and commercial viability, remain open questions that are the focus of ongoing research.

Keywords

aspirin; cardiovascular disease; cyclooxygenase; platelets; thromboxane A₂

Thromboxane A₂ (TXA₂) is a member of the prostanoid family of arachidonic acid metabolites generated by the sequential action of three enzymes – phospholipase A₂, COX-1 or COX-2 and TXA₂ synthase (TXAS). TXA₂ directs multiple biological processes [1] via its cell surface receptor, termed the TP [2]. The biosynthesis of TXA₂ [3-9] as well as isoprostanes [5,10, 11], nonenzymatic free radical-derived products of arachidonic acid that can activate the TP *in vivo* [12], is elevated in numerous cardiovascular and inflammatory diseases, as is expression of the receptor itself [13]. These, and other studies [14-18], have established TXA₂ as a mediator of cardiovascular disease (CVD). Irreversible inhibition of platelet COX-1-derived TXA₂ with low-dose aspirin is currently used as an antiplatelet therapy for the prevention of primary and secondary vascular thrombotic events [19-22], reflecting the central role of TXA₂ as a platelet agonist in CVD. TXA₂ also affects vasoconstriction [23-25], endothelial adhesion molecule expression [15,26] and cell migration [27], proliferation [14,28-30] and hypertrophy [31,32], in accordance with its role in CVD beyond the platelet. This article describes the current understanding of TXA₂ and the TP in CVD, and examines potential new drugs targeted at this pathway.

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Thromboxane biosynthesis

Biosynthesis of the prostanoids, including TXA₂, has been described thoroughly elsewhere (Figure 1) [1,33-35]. Of the two evolutionarily conserved COX isoforms [36], COX-1 and COX-2, the former, constitutively expressed in platelets, is a dominant source of TXA₂ biosynthesis in humans [37,38]. TXA₂ acts in a paracrine manner activating adjacent platelets to generate more TXA₂ [39] and amplify the action of other, more potent, platelet agonists. The inducible COX-2 isoform is found in only a small fraction of circulating platelets under normal conditions. Its higher expression in newly formed platelets suggests a greater role for this isozyme in conditions of high platelet regeneration [40,41]. Monocytes/macrophages are the second largest contributors to TXA₂ biosynthesis [42]. TXA₂ generation in these, and other, extra-platelet sources can occur via COX-1 or COX-2, with the latter enzyme becoming more relevant during inflammation or in other evoked settings [5]. COX-2 is also the physiological source of prostacyclin (PGI₂) [43], a second major vasoactive prostanoid that mediates vasodilation [14,44] and platelet inhibition [45,46], and was demonstrated to restrain the cardiovascular effects of TXA₂ *in vivo* [14].

Thromboxane receptor

Thromboxane A₂ directs its biological actions at, or close to, the site of its formation. As for all the prostanoid receptors [47], the TP is a member of the G-protein-coupled receptor (GPCR) superfamily [2]. It is expressed widely in a variety of tissues and cells including platelets, vasculature (smooth muscle and endothelial cells), lungs, kidneys, heart, thymus and spleen [48-50]. Two highly related isoforms of the human TP (α and β) arise through differential mRNA splicing [51], a characteristic that is peculiar to the human receptor. Transcription of the isoforms is driven through distinct upstream promoters [52,53], suggesting at least some independent functions. In most tissues, both isoforms are expressed, although platelets express only the TP α [54]. Although numerous differences exist between the TP α and TP β , including isoform-specific postranslational modifications, cellular trafficking, interacting proteins, G protein-coupling and receptor regulation have been reported [55-64], the physiological and pathophysiological relevance of the two TP isoforms remains unclear. One exception to this is the potential differential role of the α and β isoforms in angiogenesis (see later).

The multiple cellular signaling and regulatory mechanisms activated through TP were recently reviewed comprehensively [65]. The TP can couple with multiple G proteins [57,66-68], however signaling through Gq and G12/13 appear most relevant to TP function [69]. In platelets, for example, G12/13-mediated stimulation of RhoA signaling induces myosin light-chain phosphorylation leading to platelet shape change, with subsequent activation of Gq-PLC β signaling leading to aggregation [70]. Similar to other GPCRs, the TP is regulated rapidly in response to activation through phosphorylation-dependent desensitization [54,71]. Subsequent sequestration of the receptor away from the plasma membrane, and the fate of the sequestered protein, diverges between the two receptor isoforms. Thus, the TP β is more readily internalized under basal [55] and activated [72] conditions while levels of TP β expression are, at least in part, controlled through proteosomal degradation [73].

Thromboxane & other vascular mediators

The TXA₂-TP system operates through a complex set of signaling, regulatory, intermolecular and intercellular mechanisms that fine-tune the physiological and pathophysiological functions. During increased oxidant stress, a feature of CVD, isoprostane generation is elevated [5,10,11], leading to TP activation [12]. The TP α and β isoforms do not differ in their sensitivity to isoprostanes [74,75]; however, their physical association to form heterodimers was shown to increase sensitivity to at least two isoprostanes in transfected cells [75]. In addition to being *in vivo* markers of oxidant stress [76], isoprostanes are propagators of CVD [17,77], potentially

through activation of TP α TP β heterodimers. Interestingly, reactive oxygen species (ROS), generated downstream of TP activation, or made available from other sources, can stabilize the TP protein leading to elevated expression [78,79]. Thus, during CVD, when TXA₂, isoprostanes and ROS are elevated, TP expression and activation are augmented, driving disease processes. TXA₂ has also been implicated in Ang II-induced hypertension [80] and neovascularization [81]. In addition, reports indicate an interplay between TXA₂ and prostaglandin (PG) D₂, a prostanoid that is derived primarily from inflammatory cells and is a critical component of allergy [82]. Metabolites of both mediators can activate each other's receptors – 9 α 11 β PGF₂, a product of PGD₂ metabolism, can activate the TP [83] while hydrolysis of TXA₂ generates 11-dehydro TXB₂, a weak agonist for the DP₂ receptor for PGD₂ [84]. The implications of these observations during conditions of elevated PGD₂ and/or TXA₂ generation, such as inflammation or platelet activation, are currently unknown.

A complex interaction exists between nitric oxide (NO) and the prostanoids [85]. In several studies, COX-1/2 activity was increased in response to NO, whether exogenously administered or endogenously generated [86-88]. Others report NO-mediated inhibition of COX, particularly COX-2, activity [89,90]. The outcome for prostanoid biosynthesis appears dependent on the amount of NO released, the COX isoform, the downstream prostanoid biosynthetic enzyme involved and the cellular redox state. Indeed, platelet COX-1 can be stimulated by endogenously formed peroxynitrite, the product of NO and superoxide interaction, leading to enhanced TXA₂ generation and platelet aggregation [91]. Interestingly, although typically considered a platelet inhibitor, dual actions of NO on platelet reactivity have been reported [92,93], with concentration-dependent pro- and antithrombotic effects evident in mouse studies [94]. Prostanoids can, in turn, modulate NO biosynthesis. TXA₂-dependent NO generation is evident in endothelial cells [95] and platelets [96].

Substantial attention has focused on the interplay between TXA₂ and PGI₂ [14,97]. Mice lacking the PGI₂ receptor (IP) display cardiovascular phenotypes converse to TP knockouts [14-16], concordant with their *in vivo* interplay. Antagonism or deletion of TP blunted the proliferative and platelet response to vascular injury in mice [14]. The opposite phenomenon was evident in IP-deficient mice, with normalization of the response in double knockouts, demonstrating *in vivo* restraint imposed through the IP on TP function. In addition to functional opposition, the antioxidant effects of PGI₂ offset isoprostane generation *in vivo*, limiting this pathway to TP activation [98,99]. Furthermore, the TP α , but not TP β , is desensitized by both NO and PGI₂, terminating TP α activity [100]. The TP α also associates with the IP, to form a heterodimer with distinct signaling and regulation characteristics [101,102]. When heterodimerized to the IP, the TP signaled in a PGI₂-like manner in response to either TXA₂ analogs or isoprostanes. In addition, the IP-TP heterodimer underwent agonist-induced internalization as a unit, directing the TP to accompany the activated IP along its endocytotic pathway. The reciprocal relationship between TXA₂ and PGI₂ has been validated through clinical experience with selective COX-2 inhibitors. Selective inhibition of COX-2-derived PGI₂, with unrestrained biosynthesis of mediators that promote CVD, including COX-1-derived TXA₂, is supported strongly across basic, animal and clinical studies as the mechanism responsible for the cardiovascular hazard associated with this class of drugs [103,104]. This does not reflect a 'balance' between PGI₂ and TXA₂ but rather the biological consequence of depressing biosynthesis of one mediator, PGI₂, which acts to restrain endogenous promoters of platelet activation, atherogenesis and hypertension.

Thromboxane & the thromboxane receptor in cardiovascular disease

Thromboxane receptor-deficient mice display a reduced response to thrombotic stimuli and prolonged bleeding times [14], concordant with TXA₂'s established role in platelets. TP-null mice are normotensive, however in models of hypertension, a role for TXA₂ in elevating blood

pressure is evident [80,105]. Expression of both TXAS and TP is elevated in atherosclerotic lesions [106], consistent with retarded atherogenesis in TP-deficient mice [15,77], or treated with a TP antagonist [17]. In addition, the proliferative and platelet responses to vascular injury [14] or remodeling [98] were reduced in TP-deficient mice, while COX-2-derived TXA₂ contributed to oxidant stress and isoprostane generation, leading to increase cardiomyocyte apoptosis and fibrosis and heart failure in a mouse model [107]. TP antagonism also attenuated renal oxidant stress and proteinuria in diabetic hyperlipidemic mice [108]. In humans, a functional single point mutation in the TP's first cytoplasmic loop (arginine⁶⁰ to leucine) caused a mild bleeding disorder [18].

There is conflicting data linking TXA₂ with angiogenesis. Both TXA₂ [109,110], and isoprostanes, acting at the TP [111], can inhibit angiogenesis in *in vitro* and *in vivo* models. However, a number of studies, particularly those focused on tumor angiogenesis, suggest that TXA₂ is a positive regulator of blood vessel growth [27,112]. These contrasting results may reflect differential contributions of the TP α [113] and TP β [109,110] isoforms to angiogenesis, an important consideration when extrapolating data from animal models that lack TP β to humans. Indeed, while no role for TP was apparent in a mouse model of cardiac ischemia–reperfusion [16], evidence for suppressed placental vascularization in mice transgenic for vascular overexpression of the human TP β [114] strengthens the notion of isoform-specific, and hence uniquely human, role for TXA₂ in blood vessel growth. Further studies will be necessary in order to delineate this complex biology in human physiology and disease.

Translation therapeutics of thromboxane & the thromboxane receptor

NSAIDs range in activity from COX-1/COX-2 nonselective to the selective COX-2 inhibitors [103]. Selective inhibition of COX-2 predisposes to myocardial infarction (MI), heart failure, hypertension and stroke and has been convincingly associated with a cardiovascular hazard in seven placebo-controlled trials [115–120]. Conversely, low-dose aspirin is associated with a 25% reduction of secondary MI and stroke and 30% reduction of primary MI [121]. Aspirin inhibits COX-1, and at higher concentrations COX-2 [122,123], through irreversible acetylation of a serine residue in the catalytic channel [124,125]. This translates into permanent suppression of TXA₂ generation in platelets, which lack a nucleus and therefore cannot regenerate COX-1 during their lifetime, fully explaining the cardioprotective effects of low-dose aspirin. Secondary effects of platelet inhibition, including reduced ROS, inflammatory cytokine and growth factor generation and improved endothelial function, likely contribute to the overall beneficial effect. However, the reduced risk of arterial thrombosis cannot be dissociated from an increased risk of bleeding complications. This is particularly problematic in the upper gastrointestinal (GI) tract and in the brain. For secondary prevention, the benefit from aspirin outweighs the risk, but in low-risk populations, the risk of intracranial bleeds and serious GI adverse reactions is numerically balanced with the benefit [121].

For decades, researchers have considered targeting directly TXAS and/or the TP in CVD. Previous attempts to develop TXAS inhibitors or TP antagonists failed owing to poor pharmacodynamic properties. Persistent and complete platelet inhibition, as is afforded by aspirin, was not achieved with these compounds. The competition by low-cost aspirin in the 1980s brought the clinical development of these compounds to a halt. Despite the lack of clinical success thus far, TXAS/TP remains an attractive target for several reasons. In contrast to aspirin, these targets avoid collateral inhibition of other COX-derived products. Inhibition of cytoprotective prostanoids (PGE₂ and PGI₂) generated in the GI mucosa has been posited as a contributor to the GI toxicity associated with low-dose aspirin [126]. However, given that other antiplatelet agents and anticoagulants are associated with comparable risks [127], it is likely that increased bleeding owing to platelet inhibition underlies this risk. In this case, superior GI tolerability of TXAS inhibitors or TP antagonists, compared with low-dose aspirin,

would not be expected. As they act downstream of COX, TXAS/TP-directed drugs would also be effective systemically against TXA₂ generated via either COX isozyme, while avoiding the interference with desirable COX-1/COX-2-derived products, such as PGI₂, that is associated with higher doses of aspirin and nonselective NSAIDs [43,128]. Therefore, TXAS/TP inhibition might provide systemic beneficial effects beyond the platelet, including inhibition of vasoconstriction and improved endothelial function. Moreover, in inflammatory situations where extra-platelet sources of TXA₂, in particular monocytes and macrophages, become more significant, the systemic action of TXAS/TP-targeted agents seems highly desirable.

Reversible nonselective NSAIDs such as ibuprofen [129] and naproxen [130] share a common target – the COX catalytic channel – allowing them to prevent the action of aspirin, potentially blunting its clinical effects [131]. TXAS inhibitors and TP antagonists do not carry this concern and would, in theory, be superior choices for combination therapy with NSAIDs. Finally, TP antagonists have a strong theoretical advantage over both COX and TXAS inhibitors because they block the action of all TP agonists, including the isoprostanes, which are nonenzymatically derived and thus not blocked by inhibition of either COX or TXAS, and the immediate COX product PGH₂, is itself a TP agonist. Indeed, continued generation of PGH₂ may limit the potential usefulness of TXAS inhibitors. However, diversion of PGH₂ to PGI₂ generation [132,133] may enhance the desirable cardiovascular effects of TXAS inhibition, a concept that remains to be experimentally verified. Substrate redirection is evident with PGE₂ synthase inhibition in animal models [134,135], demonstrating the potential complexity associated with synthase inhibitors. The ultimate product profile, and the biological consequences, are likely to be tissue- and context-dependent and should be considered carefully for beneficial and deleterious effects.

Animal models broadly support the concept that direct targeting of the TP may provide superior beneficial cardiovascular effects compared to inhibition of its biosynthesis. Across several mouse models of atherosclerosis, COX inhibitors produce mixed results accelerating, retarding or leaving unaltered atherogenesis [77,136]. By contrast, antagonism or deletion of the TP consistently abrogated atherogenesis [15,77] and was superior to COX inhibition [77,137]. In addition, a mixed TXAS inhibitor and TP antagonist reduced development and progression of atherosclerosis in mice [138]. Despite preclinical evidence, there is limited indication for superiority of TXAS inhibitors, TP antagonists or dual-inhibitors of both targets, compared with aspirin [139]. It seems that in peripheral artery disease and in CVD associated with diabetes, in which the efficacy of aspirin may be limited [140,141], dual TXAS/TP acting agents may provide a significant benefit [142]. A recently developed orally available TP antagonist, Terutroban (Servier Laboratories, UK), shows promise in preclinical studies. With a relatively long half-life of 6–10 h, Terutroban displays strong and persistent antithrombotic effects in animal models and humans [143,144]. In addition, Terutroban had antivasoconstrictive and antiatherosclerotic effects in animal models [145] and improved endothelial function in patients with coronary artery disease treated with aspirin [146]. Currently, the potential superiority of Terutroban over aspirin is under investigation in the large multicenter Prevention of Cerebrovascular and Cardiovascular Events of Ischemic Origin with Terutroban in Patients with a History of Ischemic Stroke or Transient Ischemic Attack (PERFORM) trial [147].

Beyond classical antagonism of the TP, potential novel targets continue to emerge from preclinical studies (Table 1). For example, the molecular pathways that link ROS to enhanced TP stability [78] remain ill-defined, as does the potential role of TP homo- and hetero-oligomerization [75,101] in this receptor's function and regulation. In addition, efforts to separate the divergent biological effects of TP α versus TP β expression and activation, particularly in regulation of new vessel growth [109,110,113], may identify specialized isoform-specific targets. Indeed, a recent study specifically implicated the TP β in bladder cancer progression [148]. In addition, a number of TP-interacting proteins, some α or β -isoform selective, have

been implicated in receptor expression, signaling and regulation [59,72,73,149-151], providing potential novel drug targets for interference with TP function (Table 1). Whether delineation of these and other poorly characterized aspects of TXA₂-TP biology can provide alternative, or superior therapies for CVD remains an open question.

Conclusion

Thromboxane A₂ has been unequivocally implicated in a range of cardiovascular diseases, owing to its acute and chronic effects in promoting platelet aggregation, vasoconstriction and proliferation. The documented success of low-dose aspirin in prevention of atherothrombosis can be explained fully by inhibition of TXA₂ biosynthesis via platelet COX-1 [104]. The limitations associated with aspirin use, which include significant GI toxicity, unwanted bleeding, potential interindividual response variability and poor efficacy in some disease states, together with the broad role of TXA₂ in CVD and mechanistic opportunities that exist beyond the platelet COX-1 pathway, has refocused interest in additional TXA₂-associated drug targets, in particular TXAS and the TP. Ongoing research will indicate whether these efforts will yield drugs that are clinically superior to low-dose aspirin in terms of clinical efficacy and commercial viability.

Future perspective

The therapeutic potential of the TXA₂ pathway is illustrated by the clinical efficacy of low-dose aspirin in prevention of atherothrombosis [121]. Many challenges and opportunities face novel drugs that inhibit TXAS and/or antagonize the TP. *Ab initio*, it will be important to determine, in large enough populations, whether TXAS/TP-directed therapies display superior GI tolerability, and less bleeding complications, compared with low-dose aspirin. Open questions remain regarding potential interindividual variability in the clinical outcome and adverse effects of low-dose aspirin [128], and whether these issues will extend to TXAS/TP inhibitors. Common variations in TXAS and PGIS have been associated with risk of MI [152], while the response to aspirin may be modified in individuals carrying a variant COX-2 gene [152,153]. Furthermore, gender-based differences in the human response to low-dose aspirin [121], and in animal models examining prostanoids in CVD [99,154], remain poorly understood. As our understanding of variability in human populations grows critical considerations will include how naturally occurring TXAS/TP mutations and gender may impact the clinical efficacy TXA₂-directed therapies. New therapeutic opportunities in this pathway, as well as a better understanding of disease-related modifications, continue to emerge from basic science studies. These include understanding the transcriptional and post-translational control of TXAS and TP expression, and examining the extent to which participation of the TP in multimeric receptor assemblies impacts its function in normal and disease states, as well as its response to agonists and antagonists. However, it may be that these efforts will serve simply to reinforce the position enjoyed by the unassuming aspirin, which continues to resist defeat by the newcomers.

Executive summary

Thromboxane biosynthesis

- Thromboxane A₂ (TXA₂) is a member of the prostanoid family of lipid mediators generated by the action of COX on arachidonic acid.
- Platelet COX-1 is the primary source of TXA₂ and is irreversibly inhibited by low-dose aspirin.

Thromboxane receptor

- A single gene encodes the TXA₂receptor (TP), a member of the G-protein-coupled receptor superfamily that exists as two human isoforms, TP α and TP β in humans.
- The TP is activated by TXA₂and some isoprostanes leading to platelet aggregation, vasoconstriction, cell proliferation and cell migration.

Thromboxane & other vascular mediators

- TXA₂, reactive oxygen species and isoprostanes are concomitantly elevated during cardiovascular disease.
- TXA₂has been implicated in the hypertensive action of Ang II.
- Vasoactive mediators including nitric oxide and prostacyclin (PGI₂) can act to counter regulate the deleterious effect of pro-platelet mediators including TXA₂.

Thromboxane & thromboxane receptor in cardiovascular disease

- *In vitro*, animal and human studies have unequivocally established the role of TXA₂and the TP propagating in cardiovascular disease.

Translation therapeutics of thromboxane & the thromboxane receptor

- Prevention of atherothrombosis by low-dose aspirin can be explained fully by inhibition of TXA₂biosynthesis via platelet COX-1.
- Drugs aimed at targets downstream of COX, such as TXA₂synthase and/or the TP, may prove useful in the prevention and/or treatment of cardiovascular disease, but their superiority over low-dose aspirin remains an open question.

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Figure 1. Simplified scheme of TXA₂ generation and TP signaling

Platelet COX-1-dependent metabolism of AA is the dominant source of TXA₂. Activation of the TP (agonists shown in pale blue boxes) induces biological effects that drive CVD. Multiple signaling pathways have been described for TP but the primary ones associated with TXA₂ biological function are activation of RhoA or PLCβ. ROS-dependent upregulation of TP expression may increase TXA₂/isoprostane responses during CVD/oxidant stress. TP signaling is limited by its desensitization and downregulation.

AA: Arachidonic acid; EC: Endothelial cell; PGH₂: Prostaglandin H₂; ROS: Reactive oxygen species; TP: TXA₂ receptor; TXA₂: Thromboxane A₂; TXAS: TXA₂ synthase.

Table 1

Potential novel targets in the TXA₂-TP pathway.

Class	Specific target	Role in TXA ₂ -TP function	Ref.
TP α interacting proteins	14-3-3 ζ	Erk activation	[149-151,155,156]
TP β interacting proteins	Nm23-H2	TP β endocytosis	
	Rab 11	TP β endocytosis	
	RACK-1	TP β cell surface expression	
	Peroxyredoxin-4	TP β degradation	
Dimeric partners	TP	Dimerization of α and β isoforms may increase isoprostane responses.	[75,101,102]
	IP	Modified TP signaling/regulation	
ROS-dependent targets	Unidentified	Stabilization of TP protein leading to enhanced expression	[78,79]

IP: PGI₂ receptor; ROS: Reactive oxygen species; TP: Thromboxane A₂ receptor; TXA₂: Thromboxane A₂.