Prostanoid and TP-receptors in atherothrombosis: Is there a role for their antagonism?

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Summary
Atherosclerosis and its clinical manifestations (i.e. myocardial infarction, stroke) are major causes of mortality and morbidity in Western countries. Endothelial dysfunction is considered the first step in the cascade leading up to coronary events. Increasing evidence suggests that direct inhibition of thromboxane A2/prostaglandin (TP)-receptors may not only have anti-platelet effects but also impact endothelial dysfunction as well as inflammatory component of atherosclerosis. While TP-receptor involvement in platelet function has received the greatest attention, more recent findings support the critical role of TP-receptor in other pathophysiological aspects of atherothrombosis. Prostanoids (i.e. TxA2, F2-isoprostanes, prostaglandins endoperoxides PGG2/PGH2) are known to promote the initiation and progression of atherosclerosis, not only via platelet activation, but through leukocyte-endothelial interactions and vasoconstriction. Dysfunctional endothelium, characterised by increased COX-activity, releases prostanoids that promote endothelial exposure to adhesion molecules and induce smooth muscle cell contraction. Plaque macrophages synthesise PGH2/PGG2 via COX-2; these potent prostanoids can trigger platelet activation and aggregation despite COX-1 inhibition by aspirin. TP-receptor inhibition has been reported to exert anti-atherosclerotic effects in pre-clinical model of disease. Reduction of plaque burden was associated with plaque stabilisation documented by the reduction in the content of macrophages, apoptotic cells, MMPs and endothelin-1, and the increase in smooth muscle cells content. TP-receptor blockade might have an anti-atherosclerotic and plaque stabilisation effect. The possibility of combining anti-platelet activity with an anti-atherosclerotic effect via selective TP-receptor inhibitors could have important implications especially in clinical conditions associated with increased production of prostanoids, such as diabetes.

Keywords
Atherothrombosis, endothelial cells, platelet physiology, prostanoids, TP-receptors

Introduction
Atherosclerosis and its clinical manifestations (i.e. myocardial infarction, stroke) are major causes of mortality and morbidity in Western countries (1). Atherogenesis is a pathological process characterised by the deposition of lipids and other blood-borne materials in the arterial wall (2). Endothelial dysfunction is considered the first step in the cascade leading up to coronary events (3).

A healthy endothelium maintains vascular tone and an anti-adhesive and anti-thrombotic surface, through the synthesis and release of nitric oxide (NO). Despite its very short life, NO is biochemically very active. NO prevents the adhesion and migration of leucocytes into the arterial wall, inhibits vascular smooth muscle cells (VSMC) proliferation, and together with prostacynin (PGI2), is a major inhibitor of platelet adhesion and aggregation (3–5). NO also mediates endothelial release of tissue plasminogen activator (t-PA), which maintains an anti-thrombotic surface (6). A dysfunctional endothelium, characterised by reduced NO availability, transforms the physiologic “anti-atherogenic” environment into a “pro-atherogenic” one. Endothelial dysfunction facilitates the penetration of plasma lipids into the sub-endothelial space where they accumulate and undergo oxidation. These events trigger a series of defensive endothelial responses, including the exposure of adhesion and chemotactic molecules (e.g. selectins, intracellular adhesion molecule – ICAM, vascular cell adhesion molecule – VCAM) on the endothelial surface. These molecules facilitate the homing and internalisation of monocytes into the intima where they transform into macrophages and engulf lipid material, becoming foam cells. Activated monocytes and macrophages generate and release inflammatory mediators that induce multiple effects, including change of VSMC from the quiescent “contractile” state to the active “synthetic” state that can migrate and proliferate from media to the intima (4). Lipid-rich macrophages may undergo apoptotic death, releasing cholesterol crystals, matrix metalloproteinases (MMPs), tissue factor (TF) and other products within the plaque, and generating the typical necrotic lipid core of advanced atherosclerotic lesions (7). The lytic activity of MMPs destabilises the vascular structure thereby increasing the instability of atherosclerotic lesions and the possibility of their rupture (4). Following plaque rupture, intraplaque TF interacts with blood initiat-
ing the acute thrombus formation associated with coronary syn-
dromes (8) (Fig 1). Another critical source of inflammation is
VSMC apoptosis/necrosis typically occurring in advanced plaques
(9, 10). Indeed, reduced phagocytosis within atherosclerotic
plaques may promotes chronic inflammation and plaque progres-
sion (10).

Increasing evidence suggests that direct inhibition of thromb-
oxane A₂ or T Prostanoid (TP)-receptors may not only have anti-
platelet effects but also impact endothelial dysfunction and plaque progression (10).

Figure 1: Endothelial dysfunction and TP receptor activation. Impaired
endothelial function allows subendothelial penetration of plasma lipids
where they undergo oxidation. Macrophages internalise these lipids, trans-
form into foam cells in the lipid rich necrotic core. A major feature of athero-
sclerosis is macrophage and VSMC apoptosis which promotes the release
of factors such as MMPs and TF Which facilitates plaque rupture leading to
thrombus formation. Dysfunctional endothelium, characterised by increased
COX activity, releases prostanoids which by activating TP-receptors induce
endothelial cell activation (i.e. adhesion molecules expression), vascular
smooth muscle cell contraction and platelet aggregation. Plaque macro-
phages synthetise PG₁ and PG₂ via COX-2. These potent prostanoids can
trigger platelet activation and aggregation, despite COX-1 inhibition by as-
pirin. EC: endothelial cells; MCP-1: monocytes chemoattractant protein-1;
MMPs: matrix metalloproteinases; NO: nitric oxide; PG₁: prostacyclin; PLT:
Platelets; TF: tissue factor; SMC: vascular smooth muscle cells.

TP receptors and their distribution

TP-receptors are membrane bound, G-protein-coupled, seven-
transmembrane receptors distributed widely in the cardiovascular
systems (13). Human TP-receptors exist in two isoforms, termed
TPα and TPβ, which differ in their C-terminal intra-cytoplasmic
region (14–16). The TPα receptor, originally cloned from placen-
ta, is commonly referred to as the TPα isoform, while the endothe-
rial receptor is referred to as the TPβ isoform. Endothelial cells express
only the TPβ isoform, whereas human platelets express both
isoforms.

Besides their different tissue distribution, TPα and TPβ exert
different effects since the C-terminal intra-cytoplasmic region acts
as a determinant of receptor-G-coupling efficiency. TPα and TPβ
isoforms show similar ligand binding and phospholipase C acti-
vation, but their effect on adenylyl cyclase is opposite: TPα stimu-
lates adenylyl cyclase activity, whereas TPβ inhibits it.

In addition to platelets and endothelial cells, TP-receptor are also
expressed in other cell types involved in atherothrombosis, such as
smooth muscle cells (17), macrophages and monocytes (18).

Known commonly also as thromboxane-receptors, TP-recep-
tors are in fact activated not only by thromboxane A₂ (TXA₂), but
also by prostaglandin (PG) D₂, E₂, F₂α, H₂, and isoprostanes. By
binding to TP-receptor, these molecules activate several signalling
cascades which regulate endothelial cell activation (i.e. adhesion
molecules expression), VSMC contraction and platelet aggre-
gation, thereby accelerating progression of atherosclerotic lesions
(12) (Fig 1).
TP-receptor signalling pathways

TP-receptor signal transduction involves calcium signaling (19, 20) which is responsible for platelet activation and VSMC contraction. Stimulation of Gq family protein causes activation of phospholipase C-β, resulting in accumulation of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG). This in turn activates calcium release from the endoplasmic reticulum along with protein kinase C (PKC). Calcium release is responsible for platelet activation and vascular smooth muscle cell contraction while DAG is associated with the process of platelet secretion. Stimulation of G12 family proteins modulates platelet shape change. The activated Gβγ also have a role as signalling molecules, causing activation of phosphatidylinositol 3-kinase (PI3K), phospholipase C-β2 and p44/42 mitogen-activated protein kinase (p44/42 MAPK)/extracellular signal-regulated kinase 1/2 (ERK1/2).

TP-signalling in endothelial cells

A main feature of endothelium dysfunction is an increased production of prostanoids (i.e. TXA2) (33), which facilitate the penetration of macrophages in the vessel wall (34). On endothelial cells, TXA2 activates the expression of adhesion proteins, such as ICAM-1, VCAM-1 and endothelial leucocyte adhesion molecule-1 (ELAM-1) (35). TP-receptor dependent expression of ICAM-1, VCAM-1 and ELAM-1 is mediated by PKC (36). TPβ-receptor activation also stimulates the expression of leucocytes adhesion molecules (LAM) on endothelial cells (37).

TP-signalling in vascular smooth muscle cells

Increased vascular tone due to generation of prostanoids is a main feature of endothelial dysfunction (33). Each component depends on...
on the activity of endothelial cyclooxygenase (COX)-1 and the activation of TP-receptors on smooth muscle cells (3). TP-receptor activation stimulate VSMC proliferation and hypertrophy (38), by potentiating the mitogenic effects of platelet derived growth factor (PDGF) and by increasing the synthesis and release of endogenous basic fibroblast growth factor (bFGF) (39, 40).

Relevance of TP-receptors in atherosclerotic disease

Endothelial dysfunction, platelet hyperactivity and inflammation play a critical role in atherogenesis (41) (Fig 1). Dysfunctional endothelium, characterised by increased COX activity, releases vasoconstrictor prostanoids that promote endothelial exposure of adhesion molecules and induce smooth muscle cell contraction (3). TP-receptor antagonists can inhibit prostanoid-mediated vasoconstriction associated with aging, diabetes and hypertension related to increased oxidative stress and consequent up-regulation of COX-1 and/or induction of COX-2 (33).

Reduced NO availability results in platelet hyperactivity which is exacerbated by endothelial-derived prostanoids. TxA2, derived from endothelial cells and activated platelets, is one of the most powerful agonists for platelet activation (42) and its inhibition by acetylsalicylic acid (aspirin) is effective in prevention of acute coronary syndromes (43). Plaque macrophages synthesise PGH2 and PGG2 via COX-2 which can trigger platelet activation and aggregation despite COX-1 inhibition by aspirin. Therefore, a direct inhibition of TP-receptors could exert a superior antiplatelet effect than aspirin, especially in high-risk conditions characterised by increased synthesis of prostanoids (44).

Terutroban’s dose-dependent antithrombotic effect has been demonstrated both in vitro and in vivo (45). Using the Badimon perfusion chamber in a porcine model, the inhibitory effect of terutroban on platelet and fibrin(ogen) deposition was observed at both high and low-sheet rates. In particular, the 100 μg/kg/day dose showed antithrombotic effect similar to clopidogrel, a more potent antplatelet agent than aspirin. These effects of terutroban have been more recently confirmed in a porcine model of intra-stent thrombosis (46). In this study, TP-receptor blockade resulted in a faster and greater platelet inhibitory effect than clopidogrel or aspirin alone and comparable to the combination of aspirin and clopidogrel.

The antithrombotic effects of increasing doses (1–30 mg/day) of terutroban have also been demonstrated in peripheral artery disease using a design based on the ex vivo evaluation of platelet aggregation. This effect was predictable, dose-dependent with maximal inhibition at 1 hour (h), and lasted for approximately 48 h at the oral dose of 30 mg (47).

TP-receptor inhibition has shown antiatherosclerotic effects in mice and rabbits (48–52). Our group previously reported regression of atherosclerotic lesions following six months of treatment with terutroban in a rabbit model of advanced atherosclerosis (44). Reduction of plaque burden was associated with plaque stabilization documented by the reduction in the content of macrophages, apoptotic cells, MMP-1 and endothelin-1, and the increase in smooth muscle cell content.

The potential mechanism underlying plaque regression and stabilisation could be ascribed to the beneficial effect of TP-receptor blockade on endothelial function (53). The resulting reduced expression of adhesion molecules on endothelial surface could account for the observed anti-inflammatory effect as shown by the reduced macrophage infiltration, which in turn abrogates the apoptotic phenomenon characterising plaque progression and instability (44). Inflammation plays a critical role in atherosclerosis and macrophages significantly contribute in maintaining this inflammatory status (54, 55). TP-receptor blockade, inhibiting the effect of macrophage-derived TxA2, could offer a significant therapeutic advantage over currently available treatments, such as aspirin. In fact, while aspirin inhibits TxA2 synthesis by platelets, it is ineffective in blocking COX-2-derived macrophage production of TxA2. Moreover, chronic COX-2 inhibition is associated with increased risk of adverse cardiovascular events (56, 57) and its role in atherosclerosis is still controversial. In fact, while TP receptor inhibition showed an anti-atherosclerotic effect, selective COX-2 inhibition, either alone or in combination with terutroban, failed to reduce plaque size in Apobec-1/LDLR DKOs mice (58). Additionally, the combination of COX-2 and TP receptor inhibition resulted in thinning of the fibrotic cap, suggesting increased plaque destabilisation in these experimental conditions.

Aside from the importance of endothelial dysfunction and inflammation in atherosclerosis, platelet activation significantly contributes to the genesis and progression of plaques (59). Another critical effect of prostanoids is the mitogenic and hypertrophic effect on VSMC (59). VSMC proliferation and hypertrophy is a well known feature of atherogenesis which, at least partly, is mediated by TP-receptors. Activated platelets, by releasing the content of their granules, increasing the expression of adhesive ligands (e.g. P-selectin), or binding molecules from the circulating blood (e.g. fibrinogen), provide the reactive surface for monocytes and lymphocytes recruitment (59). Activated platelets serve as a source for growth factors (i.e. platelet-derived growth factor), proinflammatory cytokines (such as CD40 ligand and IL-1) and chemokines (such as RANTES and platelet factor-4) (60). Platelets can also influence lipoprotein metabolism which affect the early changes characteristic of the atherogenic lesion. All these processes favour monocyte recruitment to the vessel wall, where they eventually undergo apoptosis and perpetuate the inflammatory milieu within the plaque. In addition, the interaction of activated platelets with endothelial cells can trigger endothelial dysfunction and inflammation (4, 44, 61).

Preclinical findings support a greater beneficial effect of TP receptor inhibition over aspirin in a rat model of ischaemic stroke (62). Therefore, TP-receptor antagonism could play a role in the clinical prevention of ischaemic stroke. In a double-blind, parallel group study involving patients with a history of ischemic stroke and/or carotid stenosis, terutroban demonstrated antithrombotic activity superior to aspirin and similar to clopidogrel plus aspirin (63). These encouraging data were the basis for undertaking the Prevention of cerebrovascular and cardiovascular Events of ischemic
origin with terutroban in patients with a history of ischemic stroke or transient ischemic attack (PERFORM) study. This trial was designed to demonstrate the superiority of terutroban over aspirin in secondary prevention of cerebrovascular and cardiovascular events among patients with ischaemic cerebrovascular disease. The trial, which is registered on www.controlled-trials.com (ISRCTN66615730), has been stopped presumably because terutroban was not shown to be superior to aspirin.

Interestingly, despite the well established higher antiplatelet activity of terutroban, it failed to prevent stroke in the study. These observations, combined with the results of the CAPRIE study (64), which failed to show clopidogrel’s superiority over aspirin in stroke patients, seem to suggest that the pathophysiology of ischemic stroke and coronary events may differ somehow.

This possibility is supported by the findings of the PLATO study (65), where newer and more potent P2Y12 inhibitor ticagrelor, failed to show superiority vs. clopidogrel in the secondary prevention of stroke.

In conclusion, the possibility of combining antiplatelet activity with an antiatherosclerotic effect via selective TP-receptor inhibition could have important clinical implications in conditions associated with increased production of prostanoids, such as diabetes. Aspirin treatment is less effective in reducing ischaemic events in diabetic patients than in non-diabetics because of enhanced COX-2 expression (66, 67). Therefore, TP receptor antagonism, alone or in combination with aspirin, might be more effective in diabetic patients in reducing the risk of cardiovascular events.

References
Giannarelli et al. TP-receptor antagonism in atherothrombosis