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 a fundamental process that characterises the disease and sets up the cascade of events leading to atherothrombotic events. The mechanism of endothelial dysfunction is multifactorial, involving a complex interplay between the risk factors of atherosclerosis and the vascular response to these factors.

Introduction
Atherosclerosis and its clinical manifestations (i.e. myocardial infarction, stroke) are major causes of mortality and morbidity in Western countries (1). Atherosclerosis 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 to coronary events (3).

A healthy endothelium maintains vascular tone and an anti-adhesive and anti-thrombotic potential, through the synthesis and release of nitric oxide (NO). Despite its short half-life, NO is biologically very active. NO prevents the adhesion and migration of leucocytes into the arterial wall, inhibits vascular smooth muscle cells (VSMC) proliferation, and together with prostacyclin (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 physiological “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 syndromes (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 progression (10).

Increasing evidence suggests that direct inhibition of thromboxane A2 or T Prostanoid (TP)-receptors may not only have antiplatelet effects but also impact endothelial dysfunction and the inflammatory component of atherosclerosis (11, 12). While historically TP-receptors’ involvement in platelet function has received the greatest attention, it is now clear that TP-receptors exhibit a wide distribution within the cardiovascular system (13).

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-cyttoplasmic region (14–16). The TPα receptor, originally cloned from placenta, is commonly referred to as the TPα isoform, while the endothelial 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 activation, but their effect on adenylyl cyclase is opposite: TPα stimulates 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-receptors 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 aggregation, thereby accelerating progression of atherosclerotic lesions (12) (Fig 1).

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, transform into foam cells in the lipid rich necrotic core. A major feature of atherosclerosis 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 prostanooids which by activating TP-receptors induce endothelial cell activation (i.e. adhesion molecules expression), vascular smooth muscle cell contraction and platelet aggregation. Plaque macrophages synthesise PG1, and PG2 via COX-2. These potent prostanooids can trigger platelet activation and aggregation, despite COX-1 inhibition by aspirin. 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-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 proteins 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 Gi family proteins modulates platelets 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 prostanooids (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 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 prostanooids is a main feature of endothelial dysfunction (33). Each component depends
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. TXA$_2$, 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 PGG$_2$ and PGG$_3$, 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-shear rates. In particular, the 100 µg/kg/day dose showed antithrombotic effect similar to clopidogrel, a more potent antiplatelet 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 TxA$_2$, could offer a significant therapeutic advantage over currently available treatments, such as aspirin. In fact, while aspirin inhibits TxA$_2$ synthesis by platelets, it is ineffective in blocking COX-2-derived macrophage production of TxA$_2$. 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 DKO 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 (ISRCTN66157730), 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.

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