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TP receptors and oxidative stress: hand in hand from endothelial dysfunction to atherosclerosis

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Abstract

Thromboxane A₂ and TP receptors are important mediators of platelet aggregation and therefore thrombosis, but it is now clear that TP receptors also mediate vascular wall pathology including impaired endothelium-dependent vasodilation, increased oxidant generation, and increased adhesion molecule expression. The beneficial vascular wall effects of TP antagonists which attenuate these features of vascular disease are not shared by aspirin, and in fact are active in patients treated with aspirin, indicating that the potential beneficial effects of TP antagonists are mediated by mechanisms different from the antithrombotic actions of aspirin. Our studies have demonstrated the vascular wall benefits of TP antagonists in experimental animals, particularly in models of diabetes mellitus, in which elevated levels of eicosanoids play a role in not only vascular, but also in renal, and other tissue pathologies. This suggests that TP blockade protects against fundamental and widespread tissue dysfunction associated with metabolic disease including hyperlipidemia and hyperglycemia. TP receptor antagonists represent a promising avenue for the prevention of vascular disease in part because of these pleotropic actions that extend beyond their antithrombotic properties.

Keywords

Thromboxane; prostacyclin; atherosclerosis; hypertension; inflammation; endothelium; oxidants

1) Introduction

The excess burden of atherosclerotic cardiovascular disease in the Western world can be attributed to the increased incidence of risk factors of hyperlipidemia, diabetes, and hypertension associated with obesity and metabolic syndrome. Smoking, sedentary life style and inappropriate diet are important, but avoidable accelerating risk factors, aging is a progressive factor with ever advancing life span, and family history of cardiovascular disease is a predictor that is unavoidable for the individual patient. The prevention of cardiovascular disease by risk factor control, particularly hyperlipidemia, diabetes, smoking, and hypertension cannot be over stressed. In addition, drug therapies have now been proven

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to add significant protection against cardiovascular disease, particularly in the case of HMG CoA reductase inhibitors (statins) and inhibitors of the renin-angiotensin system, especially angiotensin converting enzyme inhibitors (ACEI). The antiatherosclerotic effects of statins exceed their ability to lower cholesterol, and those of ACEI exceed their antihypertensive effects. In each of these two cases, a large body of experimental evidence supports pleiotropic effects exerted at the vascular cell level that inhibit inflammation and growth factor signaling that are key to the progression of vascular disease. However, since neither of these two classes of drugs has important antithrombotic effects, in large numbers of patients at risk for cardiovascular disease, aspirin is administered to prevent thrombotic and embolic complications of atherosclerosis.

TP antagonists were developed as anti-platelet agents principally to prevent recurrent embolic stroke. Animal studies showed that TP antagonists have potent anti-platelet actions that are attributed to their ability to specifically target the platelet TP receptor, which is stimulated by platelet-derived thromboxane A_2 . In addition, animal and cell studies have revealed effects that may add significantly to the advantages of treating patients with arteriosclerotic cardiovascular disease with TP antagonists. The benefits of blocking TP receptors may arise as a result of activation of eicosanoid production that accompanies the widespread vascular and organ inflammation and oxidant stress associated with vascular disease (Figure 1). As a result of inflammation, activation of phospholipases release arachidonic acid, which serves as substrate for eicosanoid products. As reviewed below, the inflammation also increases the production of oxidants that shift the production/effects of the eicosanoids generated from vasodilatation and anti-thrombosis to vasoconstriction, inflammation and pro-thrombosis.

2) Arachidonic acid derivatives and oxidative stress

Arachidonic acid is released from membrane phospholipids by phospholipases and metabolized by cyclooxygenases (COXs), lipoxygenases and cytochrome P450 monooxygenases. Two different cyclooxygenases (COX-1 and COX-2) have been cloned and characterized. In most tissues, COX-1 is expressed constitutively, while COX-2 is often induced at sites of inflammation. However, COX-2 is also expressed constitutively in several organs and cell types, including the endothelial cells. In the vascular wall, both endothelial and vascular smooth muscle cells express COXs, however, in healthy blood vessels, endothelial cells contain much more of the enzyme than the surrounding smooth muscle cells. Various biologically active eicosanoids are formed from short-lived but biologically active PGH_2 , through the action of various synthases PGD , PGE , PGF , PGI and thromboxane synthases. Prostaglandins interact with G-protein-coupled receptors, classified in five subtypes DP, EP, FP, IP and TP receptors in function of their preferential affinity towards the five primary prostaglandins PGD_2 , E_2 , $F_{2\alpha}$, I_2 (prostacyclin) and thromboxane A_2 , respectively [Tsuboi et al., 2002].

In most blood vessels, prostacyclin is the principal metabolite of arachidonic acid, the endothelium being the major site of its synthesis. By stimulating its preferential IP receptor, PGI_2 is a potent inhibitor of platelet adhesion to the endothelial cell surface and of platelet aggregation, and generally acts as an endothelium-derived vasodilator and an inhibitor of vascular smooth muscle migration and proliferation (Moncada and Vane, 1979; Fetalvero et al., 2007). The genetic deletion of IP receptors is associated with increased injury-induced restenosis (Cheng et al., 2002), thrombotic events (Murata et al., 1997), atherosclerosis (Egan et al., 2004; Kobayashi et al., 2004) and reperfusion injury (Xiao et al., 2001).

In the cardiovascular system, thromboxane A_2 is predominantly derived from platelet COX-1, but can also be produced by other cell types including the endothelial cells. The

stimulation of TP receptors elicits not only platelet aggregation and smooth muscle contraction, but also the expression of adhesion molecules and the adhesion and infiltration of monocytes/macrophages (Nakahata, 2008). Although thromboxane A₂ is the preferential physiological ligand of the TP receptor, PGH₂ and the other prostaglandins, with a various range of potency, can activate this receptor. Additionally, isoprostanes and hydroxyeicosatetraenoic acids are also potent endogenous agonists at TP receptors (Félétou et al., 2010). Mice deficient in TP receptors are normotensive but have abnormal vascular responses to thromboxane A₂ and show a tendency to bleeding (Thomas et al., 1998). The deletion of TP receptors decreases vascular proliferation and platelet activation in response to intimal lesions (Cheng et al., 2002), delays atherogenesis in apoE^{-/-} mice (Kobayashi et al., 2004), prevents angiotensin-II- and L-NAME-induced hypertension and the associated cardiac hypertrophy (Francois et al., 2004, 2008). TP knockout mice are also protected against various LPS-induced responses such as the increase in iNOS expression (Yamada et al., 2003), acute renal failure (Boffa et al., 2004) and inflammatory tachycardia (Takayama et al., 2005).

Reactive oxygen species, such as superoxide anion (O₂^{-•}) and hydrogen peroxide (H₂O₂), are derived from multiple sources within inflammatory leukocytes and vascular tissues including NADPH oxidase, uncoupled endothelial and inducible endothelial nitric oxide (•NO) synthase (eNOS, iNOS), xanthine oxidase, cyclooxygenases, lipoxygenases, cytochrome P450 monooxygenases and excess substrate utilization by mitochondria. Additionally, •NO react with O₂^{-•} to form the extremely potent oxidant, peroxynitrite (ONOO⁻). Reactive oxygen species can inhibit endothelium-dependent vasodilator pathways [i.e. the NO pathway and the endothelium-derived hyperpolarizing factor (EDHF) pathways] and shift the balance in eicosanoids action from vasodilatation and anti-thrombosis toward vasoconstriction and thrombosis. Superoxide anions reduce the bioavailability of NO, inhibit its main target, soluble guanylyl cyclase, and inactivate calcium-activated potassium channels. Peroxynitrites inhibit guanylyl cyclase, superoxide dismutases and decrease the EDHF component of flow-mediated vasodilatation (Félétou and Vanhoutte, 2006). The PGI₂ synthase is amongst the most sensitive targets of peroxynitrites and is inactivated by concentrations as low as 50 nM⁴⁻⁶. When PGI₂ synthase is inactivated, the excess PGH₂ is shunted into other products that can activate TP receptors and are, in general, deleterious to vascular function.

Reactive oxygen species enhance the stability and increase the density of functional TP receptors at the cell membrane (Valentin et al., 2004; Wilson et al., 2009) and, in endothelial cells, the activation of TP receptors inhibits NO production (Liu et al., 2009). The generation of deleterious eicosanoids, the post-transcriptional stabilization of TP receptors and the decreased production of NO, are reactive oxygen species-dependent feed-forward loops further altering the unbalance between relaxing/anti-thrombosis and contracting/pro-thrombosis pathways. Taken in conjunction, this experimental evidence indicates that TP receptors are very likely to play a pivotal role in cardiovascular diseases (Félétou et al., 2010).

3) Vascular function

The understanding of vascular regulation was revolutionized by the discovery of Robert Furchgott, who recognized that the normal arterial endothelium released the vasodilator, nitric oxide (•NO) when stimulated with agents that include acetylcholine, bradykinin, or the calcium ionophore, A23187^{8,9}. It was soon recognized that diseased arteries had diminished endothelium-dependent vasodilator responses while, at least in the early stages of the diseases, retaining the ability of their smooth muscle cells to relax normally to •NO donors such as nitroglycerin and nitroprusside. In many respects, Furchgott's discovery

brought the focus of pathophysiology of vascular disease to the endothelium, and generated the concept of endothelial dysfunction.

3.1) Hypertension

In the aorta of spontaneously hypertensive rats (SHR), when compared to that of normotensive Wistar Kyoto rats (WKY), it was first shown that the impaired endothelium-dependent relaxations were restored by the presence of COX inhibitors (Luscher and Vanhoutte, 1986). The endothelial dysfunction was associated with the generation of a diffusible endothelium-derived contracting factor(s) (EDCFs) that opposes the relaxing effect of nitric oxide with no or little alteration in its production. In healthy blood vessels, the release of EDCF is tempered by the presence of NO [Tang et al., 2005] and EDHF [Michel et al., 2008]. In SHR aorta, the sequence of events leading to endothelium-dependent contractions requires an exacerbated increase in endothelial intracellular calcium concentration, the phospholipase A2-dependent mobilization of arachidonic acid, the activation of COX-1 and the resulting production of reactive oxygen species along with that of eicosanoids. These EDCFs diffuse toward the vascular smooth muscle cells and directly activate the TP receptors. Reactive oxygen species stimulate COX-1 in the smooth muscle (with subsequent stimulation of TP receptors by the produced prostanoids) and/or are involved in a positive feedback loop on the endothelial cells by further activating COX.

Inhibition of thromboxane A₂ synthesis does not affect the endothelium-dependent contractions to acetylcholine but partially inhibits those in response to the calcium ionophore, A23187, to ADP and to endothelin-1, indicating that thromboxane A₂ is only one of the EDCFs that can be released from SHR aortic endothelial cells. The other EDCFs released by ACh have been identified as PGH₂ and prostacyclin. This is due importantly to the abundance of prostacyclin synthase in the endothelial cells, compared to the other specific synthases, and thus to the overwhelming production and release of prostacyclin [Tang & Vanhoutte, 2008]. The contribution of prostacyclin to EDCF-mediated responses may seem paradoxical, as one would expect the prostanoid to rather contribute to endothelium-dependent relaxations. However, a characteristic of the SHR [but also of aged normotensive rats] is that their vascular smooth muscle cells have lost the responsiveness to IP receptor activation, and that prostacyclin, being a weak activator of the TP-receptors, produces contraction [Gluais et al., 2005].

In the SHR aorta, the endothelial production of PGE₂ and PGF_{2α} is also observed. These prostaglandins can be produced from PGH₂ by specific synthases or even spontaneously. In this blood vessel, both prostaglandins can also directly activate TP receptors [Gluais et al., 2005]. However, although in a rat model of diabetes, PGE₂, which activates EP receptors, is an EDCF [Shi et al., 2007], in the SHR aorta the involvement of this prostaglandin either via EP or TP receptor activation has been ruled out [Tang et al., 2008]. Similarly, although in genetically modified mice PGF_{2α} and the FP receptor have been associated to hypertension and atherosclerosis [Yu et al., 2009], and in the hamster aorta, PGF_{2α}, via the activation of the TP receptors, is the predominant EDCF [Wong et al., 2009], in the SHR aorta, the contribution of PGF_{2α} to EDCF-mediated responses either via the FP or the TP receptors, is at best marginal.

The contribution of EDCF to endothelial dysfunction was first observed in the SHR but has been since reported in numerous other models of hypertension. In SHR endothelial cells, the mRNA and protein expression of COX-1 are enhanced when compared to that of WKY, and in both strains they are augmented by aging. However, COX-2-derived contractile prostanoids can also be produced in arteries of both WKY and SHR as well as in various other models of hypertension and/or aging. The identity of the eicosanoids associated with EDCF-mediated responses depends on the model and the stimulating agent, but the

activation of the TP receptors is ultimately involved in the endothelial dysfunction (Félétou et al., 2010). In most rat models of hypertension (SHR, Dahl-salt sensitive, DOCA-salt, and reno-vascular hypertensive rats) the production of prostacyclin from the aortic wall is enhanced when compared to that of normotensive controls (Ishimitsu et al., 1991), but this prostaglandin will only be involved in EDCF-mediated responses when the IP receptor is defective.

In hypertensive patients, but not in healthy subjects, the reduced vasodilatation in response to acetylcholine is improved by the administration of indomethacin, a non-specific COX inhibitor. Interestingly, indomethacin and vitamin C also restore the inhibitory effect of a NO-synthase inhibitor on acetylcholine-induced vasodilatation, indicating that, as in the SHR, the activation of COX generates not only EDCF but also reactive oxygen species that reduce the bio-availability of NO. In normotensive subjects, aging mainly affects the formation of NO and EDCF production only appears in old age. However, the presence of hypertension seems to cause an earlier onset of alteration in the L-arginine–NO pathway and also earlier formation of vasoconstrictor prostanoids, suggesting that the endothelial dysfunction observed in essential hypertension could be a mere acceleration of the changes seen in aging (Virdis et al., 2010). In these hypertensive patients, the selective inhibition of COX-1 partially restores the impaired acetylcholine-induced increase in forearm blood flow while the selective inhibition of COX-2, which does not produce any adverse effects in the forearm of healthy subjects (Verma et al., 2001), further reduces the acetylcholine-induced vasodilatation. These results indicate that COX-1 derived contractile prostaglandins contribute to the endothelial dysfunction and that the reduced production of vasodilator prostaglandins (prostacyclin?) secondary to COX-2 inhibition is of minor importance in subjects with normal endothelial function, but becomes relatively more important in hypertensive patients with endothelial dysfunction, where they could play a beneficial compensatory role (Bulut et al., 2003). Similarly, diminished prostacyclin receptor signaling, as observed in patients with a dysfunctional IP receptor mutation, results in accelerated thrombosis [Arehart et al., 2008].

In hypertensive patients, when compared to normotensive subjects, prostacyclin plasma levels are generally decreased (Frolich, 1990; Kuklinska et al., 2009). Since the endothelial generation of prostacyclin is only a fraction of the total synthesis of this prostaglandin, the plasma levels may not adequately represent the endothelial production. Alternatively, in hypertensive patients, the duration of the disease far exceeds what is generally observed in the rodent models of hypertension and a prolonged exposure to oxidative stress may lead to tyrosine nitration of the PGI₂ synthase and the redistribution of PGH₂ metabolism toward other prostaglandins, such as PGE₂ and PGF_{2α}, that also activate TP receptors (Zou et al., 1999; Bachschmid et al., 2003).

In line with the phenotype observed in TP receptor knockout mice, TP receptor antagonists given *in vivo* evoke no or only minor changes in arterial blood pressure, but they limit the endothelial dysfunction associated not only with hypertension but also, as described in the following paragraphs, in diabetes and atherosclerosis.

3.2) Diabetes

Arteries from diabetic rabbits^{10–15} and diabetic atherosclerotic mice also demonstrated abnormal acetylcholine-induced relaxations, and in mice were prevented by oral treatment with the TP antagonist, S18886 (Figure 2)¹⁹. The fact that the TP antagonist added *in vitro* could immediately prevent the abnormal relaxations in arteries from untreated diabetic mice, strongly suggested that the release of a vasoconstrictor eicosanoid is responsible¹⁹. As in arteries from hypertensive animals, it became clear early on that the prostanoid that countered the effects of •NO in arteries from diabetic animals was not thromboxane A₂,

because thromboxane synthase inhibitors did not prevent the abnormality. Instead, the vasoconstrictor activity could be ascribed to the product of cyclooxygenase, prostaglandin endoperoxide (PGH₂)^{10,11,20,21} or other eicosanoids, such as 12- and 15-HETE¹³, whose production increases as a result of shifting eicosanoid production away from PGI₂ synthase. As mentioned earlier, the cause of this shift in PGH₂ levels has been attributed to increased production of OONO⁻ and inactivation of PGI₂ synthase in diseased arteries. Depending on the type of pathology, mitochondria, NADPH oxidase^{22–24}, or eNOS^{4,5} can produce increased amounts of O₂^{-•} in diseased arteries. High levels of oxidants also increase the formation of non-enzymatic oxidation products of arachidonic acid, the isoprostanes, which are potent activators of TP receptors. In addition to the mechanisms mentioned above, we found that exposure of human endothelial cells to inflammatory cytokines or high glucose decreases the expression of eNOS, and that the decrease can be prevented by S18886. Therefore, it is likely that multiple mechanisms contribute to the improvement in vascular function associated with TP receptor blockade.

Although these studies were conducted in experimental animals, it is highly likely that vasoconstrictor eicosanoids contribute to vascular dysfunction in human patients. This is no better demonstrated by the fact that impaired acetylcholine-induced vasodilation in patients with coronary artery disease are immediately improved by TP blockade with S18886²⁵. The fact that the patients in this study were already treated with aspirin suggests that COX-2 activity, rather than COX-1, may be the main source of the vasoconstrictor prostanoids involved in diminishing vasodilation in the patients with coronary artery disease. Indeed, in patients with severe coronary artery disease, COX-2 inhibition improved flow-mediated dilatation (Chenevard et al., 2003). Nevertheless, for the reasons mentioned above, it is also possible that HETE's or other eicosanoids, such as isoprostanes, are involved.

4) Vascular inflammation

Activation of TP receptors may be directly implicated in the chronic inflammatory response^{19,26} which contributes to advancing atherosclerotic vascular disease. TP agonists such as U46619 are potent stimulators of the expression of vascular cell adhesion molecule-1 (VCAM1), a principal mediator of leukocyte adhesion to the endothelium^{19,26}. •NO, oxidants, and eicosanoids also modulate the inflammatory response of the endothelium to cytokines and metabolic factors such as elevated glucose and fatty acids. As an integral part of the inflammatory response, iNOS is induced which is responsible for the production of both •NO and O₂^{-•}, NADPH oxidase is activated which produces more O₂^{-•} and H₂O₂, and phospholipases are activated which liberate more arachidonic acid. This being the case, perhaps it is not surprising that TP antagonists decrease the inflammatory response in endothelial cells^{19,27}. As an example, human endothelial cells cultured in high glucose media that simulates the pro-inflammatory diabetic milieu show increased surface expression of VCAM1, and TP blockade inhibits the increased expression (Figure 3)¹⁹. Our studies thus stress the importance of the endothelial TP receptor for the regulation of adhesion molecules that are essential for mediating inflammation.

Stimulating TP receptors also increases VCAM1 expression in smooth muscle cells²⁸. Although leukocyte adhesion to endothelium is the primary event in inflammation, smooth muscle inflammation is thought to be important in enhancing the deleterious influx and retention of leukocytes in the vascular wall. TP receptor stimulation enhanced VCAM1 expression in smooth muscle cells, not by stimulating NFB, the prototypical inflammatory transcription factor, but rather by stimulating jun kinase and the transcription factor, AP1. The importance of this mechanism is seen in the aorta of diabetic hyperlipidemic apolipoprotein E deficient mice in which atherosclerosis is dramatically accelerated compared with non-diabetic mice, and which express VCAM1 throughout the aortic wall

(Figure 3). Treating the mice with S18886 prevented the VCAM1 expression indicating that TP receptors are stimulated by endogenous eicosanoids throughout the vascular wall which can contribute to the pathology and explain the anti-atherogenic actions of TP blockade ²⁸.

5) Atherosclerosis

Because of the role of TP receptors in regulating the vasomotor and inflammatory events in blood vessels and endothelium, it might be suspected that changes in •NO and adhesion molecules observed in *in vitro* studies of cultured cells and arteries might also be found in longer term *in vivo* studies. Indeed, treating hyperlipidemic apolipoprotein E deficient mice with S18886 led to a 25% decrease in early atherosclerotic lesion development in the root of the aorta without affecting the hypercholesterolemia in these animals (Figure 4)²⁶. Because of the anti-platelet actions of the TP antagonist, mice were also treated with aspirin. The high dose of aspirin used inhibited thromboxane A₂ production during platelet aggregation to an even greater extent than S18886, and yet atherosclerotic lesions were unaffected²⁶. Although other studies in another mouse model did identify anti-atherogenic actions of aspirin that are compatible with a role of platelets in atherogenesis²⁹, our results strongly point to a mechanism of action of S18886 to decrease atherosclerotic lesion development that is distinct from platelet aggregation. Supporting a direct vascular effect of the TP antagonist, we found that serum soluble intercellular adhesion molecule-1, which is shed by endothelial cells into the blood, was decreased by S 18886, but not by aspirin. These results strongly suggest that at least some of the anti-atherosclerotic action of S 18886 is distinct from its anti-platelet activity.

In a subset of New Zealand white rabbits that lack TP receptors only in the vasculature, when compared to control rabbits, a cholesterol-enriched diet produces a less severe impairment of endothelium-dependent relaxations and the incidence of aortic lesions caused by the diet is diminished (Pfister, 2006). Pharmacological studies with TP receptor antagonists have confirmed the deleterious effect of TP receptor activation. In hypercholesterolemic rabbits, the inhibition of plaque formation by S 18886 is accompanied by a decreased infiltration of macrophages ³⁰. In long term atherosclerotic rabbits, S 18886 treatment was able to cause regression of atherosclerotic lesions transforming them into a more stable phenotype ³¹. The beneficial effect of blocking TP receptors on the development of atherosclerosis has been confirmed by the demonstration that apolipoprotein E mice that are genetically deficient in TP receptors also develop less atherosclerosis³². Although the platelet function of TP receptor deficient mice were inhibited as expected, studies in which TP receptor intact or deficient bone marrow was transplanted into TP receptor intact or deficient mice showed that the anti-atherosclerotic protection was conferred by the lack of vascular TP receptors, but not the lack of TP receptors on bone marrow derived platelets or leukocytes ³³. This result confirms that a TP antagonist can inhibit atherosclerosis development independently of its anti-platelet effects.

The role of TP receptors in the marked deterioration of endothelial vasodilator function and inflammation associated with hyperglycemia and diabetes also predicts that TP receptor blockade might be particularly effective in combating the accelerated atherosclerosis associated with diabetes. Indeed, in apolipoprotein E deficient mice induced to have type-1 diabetes with streptozotocin, atherosclerotic lesions were increased at least 3-fold (Figure 5) ^{19,34}. Treatment with S18886 completely prevented this dramatic enhancement of atherosclerosis caused by diabetes, indicating that TP receptors played an essential role¹⁹. The finding is all the more impressive, because there was no effect of the TP antagonist on the hyperlipidemia or hyperglycemia in the mice. The TP antagonist not only decreased lesions throughout the aorta, but also prevented the decrease in eNOS expression and the increase in vascular VCAM1 expression. In addition, the accumulation of nitrotyrosine and

advanced glycation end products in the aorta were prevented. Nitrotyrosine accumulates in tissues and cells exposed chronically to oxidants and reactive nitrogen species including OONO^- , and advanced glycation end products are generated as a result of inflammation and oxidants, particularly in the setting of hyperglycemia. These results indicate that not only do *in vivo* studies recapitulate the results of studies in cultured endothelial cells exposed to hyperglycemic conditions in which beneficial effects of S18886 on eNOS expression and inflammation were observed, but blocking TP receptors also attenuates deleterious tissue consequences of inflammation mediated by oxidants.

Of course, the tissue consequences of the metabolic dysfunction and inflammatory activation in diabetes may be widespread. As a case in point, the pathophysiology of diabetic nephropathy includes oxidant activation and eicosanoid generation which is stimulated by angiotensin II and prevented by ACEI³⁵. In apolipoprotein E deficient mice, we found that the induction of diabetes led to a more than 10-fold increase microalbuminuria³⁶. Treatment with S18886 significantly prevented the albuminuria as well as the accompanying increases in $\text{TGF}\beta$ expression, collagen matrix deposition, and defects in glomerular morphology that were associated with the diabetic state³⁶. There were also dramatic increases in inflammatory enzymes in the kidney of diabetic apolipoprotein E deficient mice including the p47^{phox} subunit of NADPH oxidase, iNOS, and 12-lipoxygenase. The latter enzyme is the mouse homologue of human 15-lipoxygenase, both of which can elaborate HETE's that stimulate TP receptors. There was a 5-fold increase in urinary 12-HETE, potentially explaining the beneficial effects of S18886 on albuminuria. In addition, there was an approximate 10-fold increase in 8-iso-PGF_{1 α} , one of the many isoprostanes produced by the actions of oxidants on arachidonic acid which also stimulate TP receptors. These studies point out that in diabetes not only is there increased production of multiple eicosanoids that stimulate TP receptors, but also that treatment with the TP antagonist is associated with broad effects to decrease inflammation and activation of oxidant pathways in tissues. Thus, treatment with S18886 may positively reinforce the effect of blocking TP receptors by decreasing the production of TP stimulating eicosanoids. These effects go far beyond those of inhibiting platelet aggregation. This was demonstrated directly by the fact that diabetic animals treated with aspirin did not show any benefit observed on either atherosclerotic lesions (unpublished studies) or nephropathy³⁶ that was seen in the diabetic animals treated with S18886. The protective renal actions of S 18886 have also been observed in the uni-nephrectomized obese Zucker rat³⁷, and the TP antagonist attenuated renal damage in the double transgenic hypertensive rat harboring the human renin and angiotensinogen genes³⁸. Thus, protective renal actions of S 18886 extend to both type 1 and type 2 diabetes and renovascular hypertension, point out the role of TP receptors in these pathologies, and are particularly advantageous in treating complex cardiovascular disease that is usually of a systemic nature.

6) Implications for clinical usefulness of TP antagonists

Anti-platelet treatment is a proven therapeutic modality in patients with arteriosclerotic cardiovascular disease. The benefits include the prevention of recurrent thromboembolic stroke and myocardial infarction³⁹. The mechanism of the therapeutic effect is largely attributed to preventing the formation of platelet thrombo-emboli. Our animal and cellular studies have revealed that treatment of vascular disease with a TP antagonist has actions far beyond its antithrombotic effect exerted on platelets, and can be attributed to direct effects on endothelial and smooth muscle cells within the blood vessel wall. These include effects on vascular adhesion molecules, eNOS expression and function, oxidant production, and accumulation of extracellular matrix and advanced glycation endproducts. In addition, the effects on tissue appear to extend beyond the vasculature, particularly in diabetes in which hyperlipidemia and hyperglycemia induce tissue damage such as that observed in the kidney

of diabetic hyperlipidemic mice. The importance of the TP receptor in the pathogenesis of vascular and tissue pathology, particularly in diabetes, may be due to the fact that not only thromboxane A₂, but other eicosanoids including HETE's and isoprostanes are produced in diseased tissues to such an extent as to activate the TP receptor. Aspirin has no effect on isoprostanes which are formed non-enzymatically from arachidonic acid, and aspirin can actually increase HETE production by cyclooxygenase⁴⁰. Thus, the broad ability to block the actions of many eicosanoids that activate the TP receptor may account for the added benefit of TP blockade. Of course, in addition, blocking platelet TP receptors inhibits platelet aggregation. Therefore it is reasonable to anticipate that treatment of patients with a TP receptor antagonist will result in therapeutic benefits additional to those of aspirin. Furthermore, the addition of TP antagonism to COX-2 inhibitory activity may improve the cardiovascular risk profile of COX-2 inhibitors [Rovati et al., 2010].

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Figure 1.

Role of TP receptors in Cardiovascular Disease. Cardiovascular risk factors, including hyperglycemia, hypertension, and hyperlipidemia increase (plus sign) the expression and activity of endothelial and inducible nitric oxide synthase (eNOS, iNOS) and NADPH oxidase. As a result the levels of nitric oxide ($\cdot\text{NO}$), superoxide ($\text{O}_2^{\cdot-}$), and hydrogenperoxide (H_2O_2) increase. Peroxynitrite (OONO^-) the reaction product of $\cdot\text{NO}$ and $\text{O}_2^{\cdot-}$ also increases. OONO^- tyrosine nitrates (nY) PGI₂ synthase (PGIS) decreasing the production of PGI₂. As a result arachidonic acid derived endoperoxide (PGH₂) is shunted to other prostaglandins (PG's), thromboxane A₂ (TxA₂), hydroxyeicosatetraenoic acids (HETE's), and 8-isoprostanes (isoP), all of which stimulate TP receptors. TP activation can increase inflammation and oxidants, accentuating the pathology of atherosclerosis, hypertension, and diabetes. These pathologies contribute to the production of oxidants as well as the activation of phospholipases that is responsible for increased generation of arachidonic acid products.

Figure 2.

High glucose and diabetes impairs endothelium-dependent vasodilation. A. A normal rabbit aortic ring (top) is contracted with phenylephrine and then fully relaxed by increasing concentrations of acetylcholine which releases •NO from the intact endothelium. After the ring below was exposed to 800 mg/dL glucose (44 mmol/L) for 6 hours, phenylephrine caused a similar contraction, but acetylcholine caused less relaxation and each concentration caused a contraction due to the release of vasoconstrictor eicosanoids. From ref¹⁵. B. Rings of aorta of apolipoprotein E deficient (Apo E^{-/-}) mice made diabetic for 6 weeks with streptozotocin were similarly contracted and relaxed by acetylcholine. Aorta of diabetic apolipoprotein E deficient mice relaxed significantly less than that of non-diabetic mice. Treatment of the diabetic mice with S18886 during the 6 weeks of diabetes or incubation of the ring of aorta from an untreated diabetic mouse with S18886 *in vitro* improved the vasodilator response to acetylcholine so that there was no longer a significant difference with that of non-diabetic mice. From ref.¹⁹.

Figure 3.

S18886 decreases VCAM-1 expression. A. Human aortic endothelial cells in culture were incubated for 3 days in normal (5 mmol/L) or elevated (30 mmol/L or 600 mg/dL) glucose for 6 days. High glucose significantly increased VCAM-1 expression assessed by immunoblot, whereas a similar osmotic concentration of mannitol had no effect. S18886 (1 – 10 μmol/L) decreased the expression of VCAM-1 and normalized the expression in cells exposed to high glucose. From ref.¹⁹. B. VCAM-1 expression demonstrated by immunohistochemistry is increased throughout the vascular wall of diabetic apolipoprotein E deficient mice, but treatment of diabetic mice with S18886 prevents the increase in VCAM-1. From ref.²⁸.

Figure 4.

S18886 decreases atherosclerosis in the apolipoprotein E deficient mouse. Apolipoprotein E deficient mice were fed normal mouse chow and treated with either S18886 or aspirin from 10 to 21 weeks of age. Atherosclerotic lesions at the base of the aorta were assessed by cross sections of the aortic root (upper left). The lesion was circumscribed (upper right) and the area measured. S18886 caused a significant 25% decrease in lesion area, whereas aspirin had no significant effect. From ref. ²⁶.

Figure 5.

S18886 prevents the acceleration of atherosclerosis caused by diabetes. Apolipoprotein E deficient mice made diabetic with streptozotocin from 9 to 15 weeks of age and some were treated with S18886. Lipid laden atherosclerotic plaques were stained with Sudan IV and photographed (left). Lesions were visible on the aortic arch and at spinal branch points. Diabetic apolipoprotein E deficient mice experienced a 3-fold increase in lesions, but the increase was entirely prevented by treatment with S18886. From ref. ¹⁹.