

REVIEW

Pathophysiology of isoprostanes in the cardiovascular system: implications of isoprostane-mediated thromboxane A₂ receptor activation

Jochen Bauer¹, Anne Ripperger², Stefan Frantz^{3,4}, Süleyman Ergün¹, Edzard Schwedhelm⁵ and Ralf A Benndorf²

¹Institute of Anatomy and Cell Biology, University of Würzburg, Würzburg, Germany,

²Department of Clinical Pharmacy and Pharmacotherapy, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Halle, Germany, ³Department of Internal Medicine I, University Hospital Würzburg and Comprehensive Heart Failure Center, University of Würzburg, Würzburg, Germany, ⁴Comprehensive Heart Failure Center, University Hospital Würzburg, Würzburg, Germany, and ⁵Institute of Clinical Pharmacology and Toxicology and German Center for Cardiovascular Research, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence

Ralf A Benndorf, Department of Clinical Pharmacy and Pharmacotherapy, Institute of Pharmacy, Martin Luther University Halle-Wittenberg, Wolfgang-Langenbeck-Str. 4, D-06120 Halle (Saale), Germany. E-mail: ralf.benndorf@pharmazie.uni-halle.de

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Isoprostanes are free radical-catalysed PG-like products of unsaturated fatty acids, such as arachidonic acid, which are widely recognized as reliable markers of systemic lipid peroxidation and oxidative stress *in vivo*. Moreover, activation of enzymes, such as COX-2, may contribute to isoprostane formation. Indeed, formation of isoprostanes is considerably increased in various diseases which have been linked to oxidative stress, such as cardiovascular disease (CVD), and may predict the atherosclerotic burden and the risk of cardiovascular complications in the latter patients. In addition, several isoprostanes may directly contribute to the functional consequences of oxidant stress via activation of the TxA₂ prostanoid receptor (TP), for example, by affecting endothelial cell function and regeneration, vascular tone, haemostasis and ischaemia/reperfusion injury. In this context, experimental and clinical data suggest that selected isoprostanes may represent important alternative activators of the TP receptor when endogenous TxA₂ levels are low, for example, in aspirin-treated individuals with CVD. In this review, we will summarize the current understanding of isoprostane formation, biochemistry and (patho) physiology in the cardiovascular context.

Abbreviations

CVD, cardiovascular disease; ECs, endothelial cells; LDL, low-density lipoproteins; ROS, reactive oxygen species; VSMC, vascular smooth muscle cell

Introduction

Oxidative stress in biological systems is defined as an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defence mechanisms (Dalle-Donne *et al.*, 2006; Giustarini *et al.*, 2009). In contrast to the physiological condition, during which ROS are only present at moderate levels and play an important role as messengers in redox signalling, in pathological conditions, when the physiological redox state of cells is disturbed, ROS can severely affect cellular signalling and function. Indeed, ROS which are not neutralized or scavenged by antioxidant molecules, such as GSH or superoxide dismutase, may react with nucleic acids and proteins and thereby alter the biochemical and physical properties of these important cellular components. Moreover, exposure of lipids to free radicals induces a non-enzymatic reaction cascade resulting in an increased formation of bioactive molecules named isoprostanes. Consequently, systemic isoprostane formation is significantly increased in a variety of pathological processes associated with oxidative stress, for example, cancer as well as, cardiovascular, metabolic and neurodegenerative diseases, and isoprostanes are increasingly recognized not only as markers of oxidative stress but also as mediators of disease progression (Praticò *et al.*, 1997; Reilly *et al.*, 1998; Davì *et al.*, 1999; Minuz *et al.*, 2002; Vassalle *et al.*, 2003; Schwedhelm *et al.*, 2004, Xia *et al.*, 2005; Montuschi *et al.*, 2007; Schwedhelm *et al.*, 2010; Barocas *et al.*, 2011; Davies and Roberts, 2011; Khadem-Ansari *et al.*, 2011; Montine *et al.*, 2011; Sbardella *et al.*, 2013).

Isoprostanes are PG-like compounds derived from lipid peroxidation of esterified unsaturated fatty acids, for example, arachidonic acid, which are primarily generated in a free radical-dependent and non-enzymatic fashion (Figure 1; nomenclature follows Alexander *et al.*, 2013a). First being described in 1976 as a product of the autooxidation of polyunsaturated fatty acids (PUFA; Pryor *et al.*, 1976), free radical-induced formation of isoprostanes under conditions of oxidative stress has been demonstrated in the 1990s *in vitro* as well as *in vivo* (Morrow *et al.*, 1990a,b; 1992). In the following years, the biological activities of isoprostanes have been intensely studied, demonstrating, that is, an isoprostane-mediated modification of platelet aggregation and vascular tone (Yin *et al.*, 1994; Kromer and Tippins, 1996; Möbert *et al.*, 1997; Minuz *et al.*, 1998). These isoprostane-induced effects are mediated via the prostanoid TP receptor, thus pointing to a predominant role of this receptor in isoprostane signal transduction (receptor nomenclature follows Alexander *et al.*, 2013b). Moreover, the close isoprostane-TP receptor interaction may explain why isoprostane levels have been shown to correlate in clinical trials with the extent and severity of, for example, cardiovascular disease (CVD), and why isoprostanes may directly affect prognosis of various pathological processes (Vassalle *et al.*, 2003; Schwedhelm *et al.*, 2004; Di Minno *et al.*, 2012). In this review, we will summarize the current understanding of isoprostane formation, biochemistry and (patho) physiology. In addition, we will give an overview of the TP receptor receptor as the main target of isoprostane-mediated signalling in the cardiovascular system.

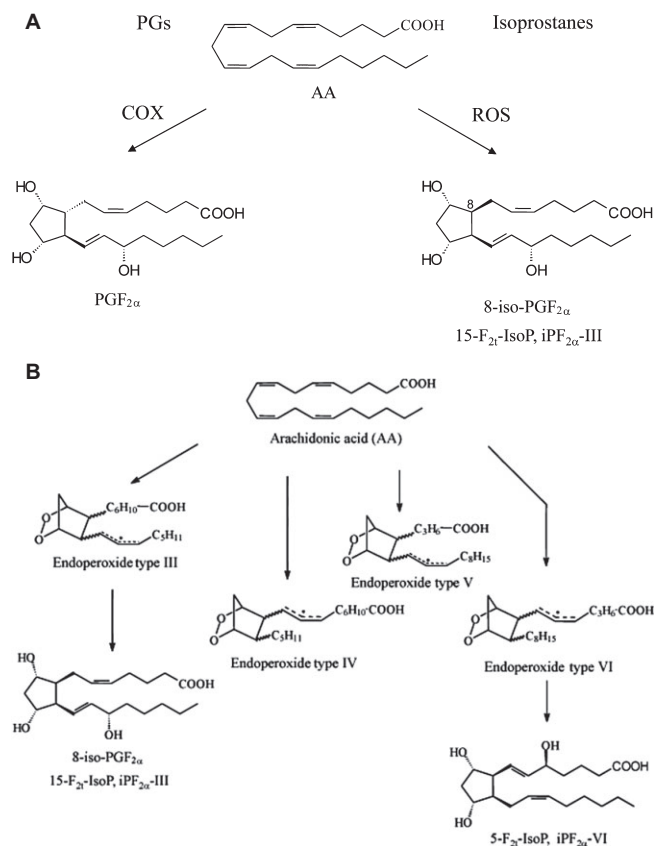


Figure 1

(A) Enzymatic and non-enzymatic formation of PGs and isoprostanes. (B) Non-enzymatic formation of isoprostanes exemplified for 8-iso-PGF_{2α} (15-F_{2t}-IsoP, iPF_{2α}-III) and 5-F_{2t}-IsoP (iPF_{2α}-IV). Arachidonic acid (AA) is released from phospholipids by PLA₂ and subsequently converted to PGs by COX. Esterified AA is converted non-enzymatically by ROS to phospholipid-bound isoprostanes. The latter being subsequently released as non-esterified congeners.

Formation of isoprostanes

In contrast to enzymatically formed prostanoids, such as PGE₂ or TxA₂, isoprostanes are generated *in vitro* as well as *in vivo*, primarily independent of COX via free radical-induced peroxidation of unsaturated fatty acids (Figure 1; Morrow *et al.*, 1990a). Under physiological conditions, these PG-like compounds can only be detected as esterified at very low concentrations in the nanomolar range or as free compounds in the picomolar range in biological fluids, for example, plasma and urine (Morrow *et al.*, 1990b). In conditions of oxidative stress, the burst of free radical formation leads to a significant increase in isoprostane levels (Morrow *et al.*, 1990b; 1992). For instance, systemic application of CCl₄, a strong inducer of free radical formation, in a rat model of hepatic failure, resulted in a more than 100-fold increase in hepatic isoprostane formation as compared with untreated animals (Morrow *et al.*, 1992). This effect was even more pronounced in rats treated simultaneously with diquat and CCl₄ (Morrow *et al.*, 1990b). In contrast, non-selective COX inhibitors do not significantly alter plasma isoprostane levels,

thereby supporting the notion of isoprostanes being primarily a product of non-enzymatic lipid modifications (Morrow *et al.*, 1990b). In addition, simple storage of plasma from normal volunteers resulted in a time-dependent *ex vivo* formation of isoprostanes, underlining the predominant role of enzyme-independent lipid peroxidation in the formation of isoprostanes (Morrow *et al.*, 1990a). Nevertheless, as described afterwards in more detail, COX, in particular the inducible isoform COX-2, may contribute to the generation of isoprostanes in monocytes and in vascular cells in the pulmonary circulation during pathological situations (Praticó and FitzGerald, 1996; Delannoy *et al.*, 2010). In contrast to F₂-isoprostanes, D₂/E₂-isoprostanes are hardly detectable under physiological conditions (Morrow *et al.*, 1994), whereas after induction of lipid peroxidation, the concentrations of D₂/E₂-isoprostane are increased in the circulation. These data emphasize the role of free radicals and oxidative stress in the formation of isoprostanes and indicate that lipid peroxidation is able to give rise to a variety of heterogeneous yet biologically active isoprostanes (Morrow *et al.*, 1994).

In 1976, Pryor *et al.* first described the formation of PG-like compounds during autooxidation of PUFA (Pryor *et al.*, 1976). In the 1990s, Morrow *et al.* could show the formation of isoprostanes from unstable endoperoxide intermediates *in vitro* as well as *in vivo* (Morrow *et al.*, 1990a; 1992). As shown in Figure 1, formation of F₂-isoprostanes, a group of 64 compounds isomeric to COX-derived PGF₂α, was described through intermediates, which undergo endo-cyclization to yield PGG₂-like bicyclic endoperoxides, which are then further reduced to form F-ring isoprostanes (Morrow *et al.*, 1990a). The rearrangement of endoperoxide intermediates results in the formation of D₂/E₂-isoprostanes (Morrow *et al.*, 1994; Chen *et al.*, 1999a). D₂/E₂-isoprostanes are formed in competition to F₂-isoprostanes and the depletion of reducing agents such as α-tocopherol and ascorbic acid favours the formation of D₂/E₂-isoprostanes over that of F₂-isoprostanes (Montine *et al.*, 2003). D₂/E₂-isoprostanes can undergo further rearrangements generating A/J-isoprostanes, which are known as cyclopentenone isoprostanes (Chen *et al.*, 1999a,b; Brooks *et al.*, 2008; Hardy *et al.*, 2011). Interestingly, degradation of A-isoprostane derivatives has been shown to occur during physiological conditions and has been demonstrated to give rise to biologically active intermediates (Benndorf *et al.*, 2008). Furthermore, the reduction of endoperoxide intermediates from docosahexanoic acid leads to the formation of so-called neuroprostanes in the nervous system (Roberts *et al.*, 1998).

In general, isoprostanes are formed by two routes of lipid peroxidation consisting of the endoperoxide and the dioxetane/endoperoxide mechanism. However, the contribution of the latter route to the generation of isoprostanes *in vivo* remains unclear (Montuschi *et al.*, 2007). The isoprostane pathway leading to the generation of F₂-isoprostanes starts with the formation of three arachidonoyl radicals followed by the formation of four peroxy radical isomers which subsequently undergo endo cyclization (Morrow, 2006). Four bicycloendoperoxide regioisomers are then reduced to generate F₂-isoprostanes (Morrow, 2006). In the dioxetane/endoperoxide route, the formation of the same regioisomers can be observed, but in this cascade, the second and not the first oxygen molecule is incorporated into the PGF ring

(Lawson *et al.*, 1999). In contrast to PGs, bioactive compounds generated by COX, isoprostanes have *cis*- or *trans*-stereochemistry at the five-membered ring junction as compared to the exclusive *trans*-ring junction in PGs (Figure 1). Unlike PGs, which are generated from free arachidonic acid, isoprostanes are formed *in situ* on arachidonoyl-containing lipids and then subsequently released in free form into the circulation via an enzyme-dependent mechanism (Morrow *et al.*, 1992; 1994). This process is dependent on the activity of PLA₂ because an incubation of lipid extracts with this enzyme leads to a release of free F₂-isoprostanes (Morrow *et al.*, 1992). Furthermore, both plasma and intracellular platelet-activating factor (PAF) acetylhydrolase are able to hydrolyse phospholipids to release esterified F₂-isoprostanes increasing free F₂-isoprostane concentrations (Stafforini *et al.*, 2006). However, little is known regarding the mechanisms responsible for the extrusion or liberation of isoprostanes from the intracellular space to the extracellular milieu, a process that is likely to significantly affect auto-, para- and endocrine activities of intracellularly formed isoprostanes. Having reached the systemic circulation, isoprostanes, such as 8-*iso*-PGF₂α, are partly metabolized by mechanisms involving, for example, peroxisomal β-oxidation (Schwedhelm *et al.*, 2000). Moreover, direct conjugation to GSH has been described for cyclopentenone isoprostanes, such as 15-A₂-isoprostane, in HepG2 cells indicating that phase II metabolism may also play a role in isoprostane metabolism (Milne *et al.*, 2004). Finally, isoprostanes and isoprostane metabolites are freely filtered in the glomerular apparatus of the kidneys and excreted in urine.

Contribution of enzymatic processes to isoprostane formation

As mentioned previously, enzymatic activity appears to at least partially contribute to the generation of isoprostanes. In human monocytes, LPS induced the formation of PGE₂ and the isoprostane 8-*iso*-PGF₂α in a time- and dose-dependent manner, accompanied with the induction of COX-2 (PGHS-2). Incubation with the selective inhibitor of COX-2, L-745,337, decreased the production of PGE₂ and 8-*iso*-PGF₂α, indicating that the induction of COX-2 in monocytes is associated with an increased production of isoprostanes (Patrignani *et al.*, 1996). The role of COX-2 in the formation of isoprostanes has been confirmed also by other groups. Stimulation of human monocytes with LPS induced the expression of this enzyme and was accompanied by an increased formation of PGE₂, TxB₂ and 8-*iso*-PGF₂α (Praticó and FitzGerald, 1996). Furthermore, inhibition of COX-2 as well as pretreatment with superoxide dismutase suppressed the formation of 8-*iso*-PGF₂α, indicating that monocytes may form bioactive 8-*iso*-PGF₂α in an enzyme- and free radical-catalysed pathway (Praticó and FitzGerald, 1996). As mentioned above, COX is the first enzyme catalysing the formation of traditional PGs from arachidonic acid. While COX-1 is expressed constitutively in a variety of different cell types, expression of COX-2 is primarily induced via inflammatory stimuli (Grosser *et al.*, 2010). Human vascular endothelial cells (ECs) treated with pro-inflammatory

cytokines, such as IL-1 β or TNF- α , showed a significantly increased release of 8-*iso*-PGF $_2\alpha$, which was blocked by COX-1 and COX-2 inhibitors (Jourdan *et al.*, 1999). In addition, superoxide-producing enzyme xanthine oxidase elevated the release of isoprostanes in these cells, thereby emphasizing the role of oxygen-derived radicals in isoprostane formation (Jourdan *et al.*, 1999). Under hypoxic conditions, an up-regulation of COX-2 in murine pulmonary arteries has been described, which was accompanied by an increase in 8-*iso*-PGF $_2\alpha$ release, suggesting a putative role of COX-2 in the generation of isoprostanes under hypoxic conditions (Delannoy *et al.*, 2010). Furthermore, in a model of renal ischaemia reperfusion injury, accumulation of 8-*iso*-PGF $_2\alpha$ was successfully blocked by administration of acetylsalicylic acid, thereby indicating that COX-dependent generation of isoprostanes may play a role under ischemic conditions (Favreau *et al.*, 2004). In human platelets, a minor role for COX-1 in the production of 8-*iso*-PGF $_2\alpha$ has been shown (Pratico *et al.*, 1995; Pignatelli *et al.*, 2011). In this context, it is proposed that platelet 8-*iso*-PGF $_2\alpha$ formation is mainly associated with NADPH oxidase-dependent superoxide release and only to a minor extent derives from COX-1 activation (Pratico *et al.*, 1995; Pignatelli *et al.*, 2011). In addition, NOS pathways may be involved in the generation and release of isoprostanes (Jourdan *et al.*, 1997). Interestingly, GSH, one of the most important and abundant intracellular antioxidants, has been shown to promote the formation of oxidative stress markers like malondialdehyde and 8-*iso*-PGF $_2\alpha$ from arachidonic acid in a COX-dependent way, indicating that antioxidants may have a paradoxical role in the generation of isoprostanes (Tsikas *et al.*, 2012). In summary, isoprostanes are predominantly generated in the free radical-dependent process of lipid peroxidation, but enzymatic processes may also contribute to the formation of these lipid mediators especially in the context of hypoxia or oxidative burst. Nevertheless, enzyme-dependent isoprostane generation should be analysed in more detail to help to fully elucidate the complex process of isoprostane formation.

Nomenclature of isoprostanes

Currently, two nomenclature systems for isoprostanes are used (Rokach *et al.*, 1997; Taber *et al.*, 1997). The Taber/Roberts nomenclature system has been approved by the IUPAC and Eicosanoid Nomenclature Committee and follows the normal PG conventions. In this system, the different regioisomers are designated by the carbon number of the side chain where the hydroxyl is located, with the carboxyl carbon designated as C-1. Based on this nomenclature, four isoprostane regioisomer classes derived from arachidonic acid are then denoted as either 5, 8, 12 or 15 series (Taber *et al.*, 1997). The abbreviation 2t in the prominent isoprostane (IsoP) 15-F $_{2t}$ -IsoP refers to the number of double bonds (two) and the *trans*-orientation of the side chains at the five-membered ring. 15-F $_{2t}$ -IsoP is also called 8-*iso*-PGF $_2\alpha$ because the chemical structure of this molecule differs from COX-derived PGF $_2\alpha$ only in the stereochemistry of the carbon atom 8. The second nomenclature system was evolved by Rokach *et al.* creating different regioisomer classes based on the ω -carbon being attacked to form the arachidonoyl radical (Rokach *et al.*,

1997). Free radical attack at carbon ω -8, -11 and -14 leads to the formation of regioisomers type III, IV and VI respectively. The four classes of F $_2$ -isoprostanes are designated as type III, IV, V and VI, whereas the oxidation of ω -3 lipids induces the formation of compounds starting with type I.

TP receptors as important mediators of isoprostane-induced signal transduction

As mentioned previously, isoprostanes most likely exert their effects exclusively via activation of the TP receptor (Minuz *et al.*, 1998; Huber *et al.*, 2003; Tang *et al.*, 2005; Benndorf *et al.*, 2008). Thus, the aim of this section is to give an overview of the TP receptor and its main ligand, TxA $_2$, and to briefly outline the role of the TP receptor in the pathogenesis of CVD.

TxA $_2$ is a PG derivative with chemical characteristics of prostanoids but structural differences especially in the ring structure (heterocyclic oxane ring structure vs. 5-carbon ring). It is a short-lived but highly bioactive molecule that mediates its effects via activation of the heptahelical G-protein-coupled TP receptor. TxA $_2$ acts as an autacoid in autocrine or paracrine systems and is involved in a wide variety of physiological and pathophysiological processes, such as vasospasm, hypertension, thrombosis, angiogenesis, inflammation, atherogenesis and myocardial infarction (Palmer *et al.*, 1970; Needleman *et al.*, 1976; Wilson *et al.*, 2005; Nakahata, 2008). Being an unstable intermediate in arachidonate metabolism with a chemical half-life of about 30 s, TxA $_2$ was detected in the conversion of PGG $_2$ into inactive TxB $_2$ in platelets (Hamberg *et al.*, 1975). In a first biosynthetic step, PLA $_2$ catalyses the release of arachidonic acid from membrane phospholipids, which is further converted via COX into the PG endoperoxides PGG $_2$ and PGH $_2$ (Daniel *et al.*, 1999; Nakahata, 2008). Via Tx synthase, an enzyme abundantly expressed in a wide variety of different tissues (Sun *et al.*, 1977), these endoperoxides are then further converted into TxA $_2$ and subsequently non-enzymatically degrade into biologically inactive TxB $_2$ (Needleman *et al.*, 1976).

The TP receptor gene is located at 19p13.3 of human chromosome, spans over 15 kb and contains three exons divided by two introns (Nüsing *et al.*, 1993). The TP receptor protein is widely expressed in different organs and localized on both cell membranes and intracellular structures (Armstrong *et al.*, 1983; Hedberg *et al.*, 1989; Borg *et al.*, 1994; Bowling *et al.*, 1994; Raychowdhury *et al.*, 1994; Fennekohl *et al.*, 1999; Muja *et al.*, 2001). Based on the sequence of the purified protein from human platelets, a GPCR human cDNA was cloned from human placenta, consisting of seven transmembrane spanning regions, three extracellular and three intracellular loops (Ushikubi *et al.*, 1989; Hirata *et al.*, 1991). In mice and rats, TP receptor analogues have been described that are similar to the human TP receptor from placenta (TP- α ; Namba *et al.*, 1992). In addition to the TP receptor- α isoform, a second isoform has been described in ECs, called TP receptor- β (Raychowdhury *et al.*, 1994). TP receptor- β results from alternative splicing of the cytoplasmic carboxyl tail (Raychowdhury *et al.*, 1994). In most cells and tissues,

both TP receptor isoforms are expressed, for instance, in vascular smooth muscle cells. However, in most cells, the TP receptor- α dominates over TP receptor- β expression, probably through pronounced constitutive and agonist-induced endocytosis of the TP receptor- β and increased subsequent degradation of this TP receptor isoform by the proteasome (Miggin and Kinsella, 1998; Sasaki *et al.*, 2007). In HUVECs approximately sixfold greater mRNA levels of TP receptor- α than TP receptor- β has been found (Miggin and Kinsella, 1998). Both TP receptor isoforms are able to form homo- and heterodimers via the formation of disulfide bonds (Laroche *et al.*, 2005). Interestingly, formation of (hetero) oligomers of TP receptors is an agonist-independent process regulating both TP receptor- α internalization and TP receptor-mediated signalling (Laroche *et al.*, 2005; Sasaki *et al.*, 2006). In TP receptors, as in many other members of the eicosanoid receptor family, the seventh transmembrane domain is highly critical for ligand binding (Funk *et al.*, 1993). Point mutations in this domain inhibited the binding of the TP receptor antagonist SQ29548 ([1S-[1 α ,2 β (5Z),3 β ,4 α]-7-[3-[[2-[(phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) to the receptor supporting the role of this structure in ligand binding (Funk *et al.*, 1993). Furthermore, residues in the transmembrane domains 4, 5 and 6 are implicated in ligand binding (Dorn *et al.*, 1997). Various mutational analyses identified critical residues in the first, second and third extracellular loop regulating ligand-TP interaction partially by forming hydrogen bonds (Chiang *et al.*, 1996; D'Angelo *et al.*, 1996; Turek *et al.*, 2002; So *et al.*, 2003). The N-terminal region of TP receptors contains two consensus glycosylation sites, which are supposed to be critical for ligand binding. Inhibition of these N-glycosylations reduced

the binding of SQ29548 to TP receptors and affected receptor signalling and efficient transmembrane expression (Walsh *et al.*, 1998). A study from Ruan *et al.* demonstrated that TP receptor agonists and antagonists share the ligand-binding pocket in general, but the configuration of this binding pocket for the agonist and antagonist are quite different (Ruan *et al.*, 2009). They proposed a model, in which antagonist binding to TP receptors induces an increase in β -sheet and a decrease in α -helical content inducing an unfavourable conformation for G-protein coupling (Ruan *et al.*, 2009). Additional critical residues for antagonist binding to TP receptors have been identified (Khasawneh *et al.*, 2006). The first and third intracellular domains of TP receptors have been shown to mediate the coupling to G-proteins through charge contact and therefore regulating intracellular signalling (D'Angelo *et al.*, 1996; Chung *et al.*, 1999; Geng *et al.*, 2004).

TP receptor signalling

Early studies demonstrated that TP receptor agonists, such as U44069 (9, 11-dideoxy-9 α , 11 α -epoxymethano-prosta-5Z, 13E-dien-1-oic acid) induced GTPase activity in platelet membranes accompanied by a stimulation of inositol phospholipid metabolism (Houslay *et al.*, 1986). Later it has been shown that TP receptors functionally couple to the G_q -family members $G_{q/11}$, G_{15} and G_{16} (Figure 2; Offermanns and Simon, 1995; Kinsella *et al.*, 1997). After TP receptor stimulation, these G-proteins mediate the activation of PLC- β , catalysing the conversion from PI-4,5-biphosphate to inositol-1,4,5-trisphosphate and DAG resulting in the release of intracellular Ca^{2+} stores and activation of PKC (Offermanns and Simon,

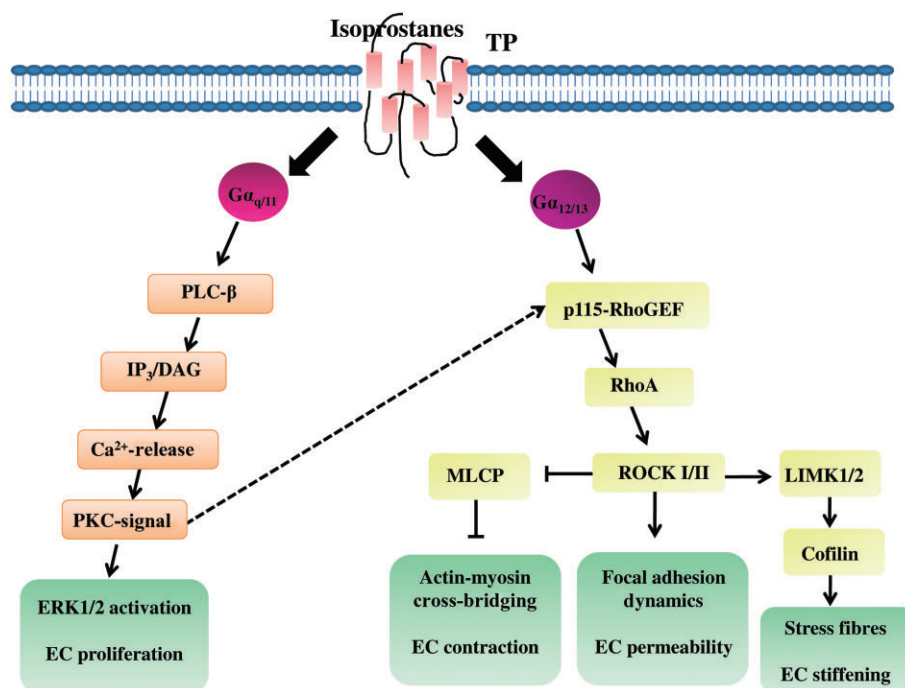


Figure 2

Isoprostane-mediated signalling via TP receptor activation in ECs associated with endothelial function and homeostasis.

1995; Kinsella *et al.*, 1997). In addition to $G_{q/11}$ -proteins, members of the G_{12} -family are involved in TP receptor-mediated cell signalling (Offermanns *et al.*, 1994; Moers *et al.*, 2003; Miyosawa *et al.*, 2006). A lack of $G\alpha_{13}$ reduced the TP receptor-mediated activation of RhoA, significantly decreasing the ability of TxA_2 to induce platelet shape changes and aggregation *in vivo* resulting in severe defects in primary haemostasis and an almost complete protection against arterial thrombosis (Moers *et al.*, 2003). Under pathological conditions, a modulation of TP receptor-coupled G-proteins can be observed. Hypoxia induces actin polymerization of pulmonary arteries independently of RhoA, reflecting a decreased association with $G_{12/13}$ in favour of G_q (Fediuk *et al.*, 2012).

Stimulation of TP receptor- α and - β result in differential activation of downstream signalling pathways. Agonist activation of TP receptor- α induced, via the dimeric G-protein G_{hi} , a stimulation of PLC-mediated inositol phosphate production, whereas agonist activation of TP receptor- β had no effect (Vezza *et al.*, 1999). Interestingly, the TP receptor isoforms are differentially regulated in response to the vasorelaxant molecules prostacyclin and NO (Reid and Kinsella, 2003; Wikström *et al.*, 2008). Whereas TP receptor- α undergoes both NO- and prostacyclin-mediated desensitization involving direct PKA and PKG phosphorylation within the C-terminal domain, signalling by TP receptor- β is unaffected by either NO or prostacyclin (Reid and Kinsella, 2003). Furthermore, in human aortic smooth muscle cells, both TP receptor isoforms independently regulate RhoA activation (Wikström *et al.*, 2008). But, although TP receptor- α -mediated RhoA signalling was directly impaired by prostacyclin and NO, TP receptor- β -mediated RhoA signalling was not affected (Wikström *et al.*, 2008).

Relevance of TP receptors in CVD

The role of TxA_2 and TP receptors in atherosclerosis and CVD has been investigated in a wide range of experimental and clinical studies. For instance, increased TxA_2 biosynthesis has been described in atherosclerosis (Mehta *et al.*, 1988). The initiation and progression of this chronic inflammatory disease and its complications is promoted by TxA_2 most likely via regulation of platelet activation, endothelial integrity and leukocyte-EC interaction (Kobayashi *et al.*, 2004). Moreover, the abundance and expression level of TxA_2 and TP receptors, respectively, increase during progression of atherogenesis, thereby indicating that TP receptor-dependent signalling pathways become increasingly important in patients with advanced atherosclerotic disease (Cyrus *et al.*, 2010). For instance, in patients with coronary artery disease, an increase in TP receptor expression level was observed in diseased vessels correlating with progression accompanying the increase in endogenous TxA_2 levels in CVD (Katugampola and Davenport, 2001; Katugampola *et al.*, 2002). Interestingly, pharmacological inhibition of TP receptors by its antagonist S18886 rather than systemic depletion of TxA_2 levels, was effective in reducing the development of atherosclerotic plaques in the ApoE knockout mouse model (Cayatte *et al.*, 2000). These findings indicate that TP receptor agonists other than TxA_2 , for example,

isoprostanes, may be important in the initiation and progression of atherosclerosis.

Moreover, clinical and experimental data point to a critical role of the TP receptor in ischaemia and myocardial infarction. Inhibition of TP receptors by receptor antagonists, for example, SQ29548 or AH-23848 ((4Z)-7-[(rel-1S, 2S,5R)-5-((1,1-biphenyl-4-yl)methoxy)-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid), prevented the extension of ischaemic damage in myocardial ischaemia and improved early survival following permanent coronary artery ligation (Brezinski *et al.*, 1985; 1987; Hock *et al.*, 1986). Furthermore, the application of the TP receptor agonist BAY u3405 ((3R)-3-[[[4-(fluorophenyl)sulfonyl]amino]-1,2,3,4-tetrahydro-9H-carbazole-9-propanoic acid), reduced myocardial infarct size as well as myocardial leukocyte accumulation, underlining the relevance of TP receptors and also indicating a role of immune cells in myocardial infarction and ischaemia-reperfusion injury, which has been confirmed by several further studies (Crawford *et al.*, 1988; Squadrito *et al.*, 1993; Vinten-Johansen, 2004).

On platelets from patients with acute myocardial infarction as well as stable and unstable angina pectoris, respectively, an increase in TP receptor expression level has been detected, correlating with the duration of chest pain (Dorn *et al.*, 1990; Modesti *et al.*, 1995). Furthermore, an increase in the maximal velocity of U46619-induced platelet aggregation has been observed in these patients, indicating a significant role of induced TP receptor expression in thrombogenesis (Dorn *et al.*, 1990). Indeed, the role of TxA_2 as an important activator of platelet aggregation especially in the context of endothelial dysfunction and CVD has been clearly demonstrated (Ally and Horrobin, 1980; Dorn and DeJesus, 1991). TxA_2 , an important member of the second-wave agonists of platelet aggregation, is able to alter platelet shape, to amplify integrin activation on adherent platelets and to mediate thrombus growth by recruiting additional platelets via the activation of TP receptor-coupled G-proteins (Moers *et al.*, 2003; Stegner and Nieswandt, 2011). Moreover, the clinical effectiveness of aspirin in preventing thrombotic events in patients with cardiovascular or cerebrovascular disease strongly emphasizes the biological relevance of platelet-derived TxA_2 in the pathological interaction of platelets and dysfunctional vascular ECs. Interestingly, during chronic hypoxia, platelet activation is enhanced indicating a correlation between hypoxia and TP receptor expression (Pidgeon *et al.*, 2004). In addition, hypoxia directly affects TP receptor localization, stability and avidity (Valentin *et al.*, 2004; Hinton *et al.*, 2006; 2007). Under normoxic conditions, the TP receptor- β is preferentially located intracellularly, presenting a significant ER-localized population (Valentin *et al.*, 2004). By inducing oxidative stress, an enhanced TP receptor translocation from the ER to the Golgi in COS-7 cells has been observed accompanied by an increased receptor stability and density at the membrane (Valentin *et al.*, 2004). These data indicate that oxidative stress induces maturation and intracellular translocation of TP receptors to increase its functional fraction in the cell membrane (Valentin *et al.*, 2004). In addition to TP receptor maturation and translocation, hypoxia induces an increase in TP receptor ligand binding and avidity (Hinton *et al.*, 2006; 2007). Modified receptor cycling as well as increased

G_q coupling seems to be critical in hypoxia-induced TxA₂ hypersensitivity in exposed myocytes (Hinton *et al.*, 2006; 2007; Fediuk *et al.*, 2012). In summary, the expression of TP receptors correlates with the extent and the severity of CVD. Conditions of oxidative stress seem to promote the expression, stability and avidity of TP receptors. These phenomena may enhance the biological relevance of endogenous TP receptor agonists, such as isoprostanes, in the context of cardiovascular pathologies.

Functional consequences of isoprostane–TP receptor interaction

Experimental data strongly suggest that isoprostane signalling is exclusively regulated via the interaction with TP receptors. In the context of isoprostane/TP signalling, an association of TP receptors with G-proteins such as G_q, G_i and G₁₁ has been described (Kinsella *et al.*, 1997; Acquaviva *et al.*, 2013). A co-transfection of TP receptor- α with G₁₁ produced greater mobilization of Ca²⁺ than did co-transfection of G_q in response to 8-*iso*-PGF_{2 α} stimulation, indicating a preferential association of TP receptors with G₁₁ in isoprostane/TP receptor signalling in human platelets (Kinsella *et al.*, 1997). Heterodimerization of TP receptor- α/β not only influences TxA₂ signalling, but stimulates isoprostane-mediated inositol phosphate generation thereby enhancing isoprostane-dependent signal transduction (Wilson *et al.*, 2007). Mutagenic analysis revealed that distinct amino acid residues of the TP receptor are responsible for isoprostane/TP receptor interactions (Khasawneh *et al.*, 2008). 8-*iso*-PGF_{2 α} interacts with two hydrophobic sites (Phe^{196/184}) and one hydrogen binding site (Asp¹⁹³) residing in transmembrane domain 5 and extracellular loop 2 of TP receptors (Khasawneh *et al.*, 2008). Experimental work of Khasawneh *et al.* additionally indicated that in human platelets, two separate 8-*iso*-PGF_{2 α} signalling pathways exist, being TP receptor-dependent and TP receptor-independent, possibly mediated via a so far unknown isoprostane receptor bearing close homology to TP receptors (Khasawneh *et al.*, 2008). Moreover, 8-*iso*-PGE₂, an isoprostane generated from the same endoperoxide intermediate as 8-*iso*-PGF_{2 α} , has been proven to be a partial agonist of the TP receptor (Longmire *et al.*, 1994; Audoly *et al.*, 2000; Benndorf *et al.*, 2008; Tables 1 and 2). These results from *in vitro* studies indicate that isoprostanes are partial agonists at TP receptors and that the biological activity of isoprostanes may be additionally mediated via an isoprostane-specific receptor. However, so far, no molecular evidence has been found for the existence of such an isoprostane-specific receptor. Furthermore, results from our and other groups strongly support the concept that isoprostanes mediate their biological functions exclusively via activation of TP receptors (Audoly *et al.*, 2000; Benndorf *et al.*, 2008).

Role of isoprostanes as modulators of platelet activation

Isoprostanes participate in oxidative injury by modulating platelet activation and adhesion and by reducing the

Table 1

Overview of TP receptor agonists including ligand-binding capacity and potencies

TP agonists		
	Ligand binding (K _d)/potency (pD ₂ = –log EC50)	References
U-46619	K _d : 6.19–16 nM	Saussy <i>et al.</i> , 1991
	K _d : 3.6–18.7 nM	Hedberg <i>et al.</i> , 1988
	pD ₂ : 8.34–8.79	Hou <i>et al.</i> , 2000
U-46609	K _d : 4.4–7 nM	Hedberg <i>et al.</i> , 1988
I-BOP	K _d : 5.5 nM	D'Angelo <i>et al.</i> , 1994
	K _d : 0.322–7.9 nM	Saussy <i>et al.</i> , 1991
SQ 26655	K _d : 1.12–3 nM	Saussy <i>et al.</i> , 1991
PGF _{2α}	K _d : 17.4 nM	Balapure <i>et al.</i> , 1989
	pD ₂ : 7.18–7.3	King <i>et al.</i> , 1991; Hou <i>et al.</i> , 2000
PGD ₂		King <i>et al.</i> , 1991
8- <i>iso</i> -PGE ₁	pD ₂ : 5.5	Janssen <i>et al.</i> , 2001
	pD ₂ : 5.4	Oliveira <i>et al.</i> , 2000
8- <i>iso</i> -PGE ₂	pD ₂ : 6.7	Sametz <i>et al.</i> , 2000; Janssen <i>et al.</i> , 2001
8- <i>iso</i> -PGF _{2α}	K _d : 31.8 nM	Yura <i>et al.</i> , 1999
	pD ₂ : 7.41–7.75	Hou <i>et al.</i> , 2000

Table 2

Overview of TP receptor antagonists including ligand-binding capacity

TP receptor antagonists		
	Ligand binding (K _d)	References
SQ 29548	K _d : 1.2–12 nM	Raychowdhury <i>et al.</i> , 1994
	K _d : 9.8–20.9 nM	Saussy <i>et al.</i> , 1991
BM 13505	K _d : 24.3–184 nM	Saussy <i>et al.</i> , 1991
I-PTA-OH	K _d : 14.5–384 nM	Saussy <i>et al.</i> , 1991
S-145	K _d : 1.2–3.3 nM	Hirata <i>et al.</i> , 1991; Namba <i>et al.</i> , 1992

antiplatelet activity of NO (Minuz *et al.*, 1998). A treatment of platelets with 8-*iso*-PGF_{2 α} in the concentration range from 10–1000 nmol·L^{–1} enhances platelet adhesion to fibrinogen by increasing the functionality of the adhesion molecule glycoprotein IIb/IIIa (Minuz *et al.*, 1998). Furthermore, the anti-aggregatory effect of NO, released by ECs, is reduced by 8-*iso*-PGF_{2 α} (Minuz *et al.*, 1998). All these effects were prevented by the TP receptor antagonist GR32191 ((1R-[1 α (Z),2 β ,3 β ,5 α]]-(+)-7-[5-([1,1-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl) cyclopentyl]-4-heptonic acid),

underlining the importance of isoprostane/TP receptor interaction in platelet activation (Minuz *et al.*, 1998). Indeed, 8-*iso*-PGF₂α acts as a partial agonist of TP receptors on platelets (Yin *et al.*, 1994). Whereas 8-*iso*-PGF₂α can cause platelet shape change itself, in the presence of full TP receptor agonists, such as U46619 and I-BOP ([1S- [1α, 2α(Z), 3β(1E, 3S), 4α]]-7-[3-[3-hydroxy-4- (4-iodophenoxy)- 1- butenyl]- 7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid), it exhibits anti-aggregatory effects. In contrast, 8-*iso*-PGF₂α may promote ADP-dependent platelet aggregation in a TP receptor-dependent fashion (Yin *et al.*, 1994; Audoly *et al.*, 2000; Schwedhelm *et al.*, 2010).

Isoprostanes regulate immune and EC interaction

In addition to altered platelet behaviour, CVD is characterized by an enhanced interaction of ECs with immune cells such as monocytes and neutrophils (Aukrust *et al.*, 2010). 8-*iso*-PGE₂ and 8-*iso*-PGF₂α may promote atherosclerosis by enhancing the interaction of monocytes with ECs (Leitinger *et al.*, 2001; Huber *et al.*, 2003). This process was mediated via a TP receptor-dependent activation of PKA and p38 (Leitinger *et al.*, 2001; Huber *et al.*, 2003). Interestingly, the effects of 8-*iso*-PGF₂α on the monocyte–endothelial interaction are dependent on the origin of the vascular beds. While inhibiting dose dependently the adhesion to human dermal microvascular ECs in a TP receptor-dependent manner, 8-*iso*-PGF₂α stimulates the binding of monocytes to HUVECs (Kumar *et al.*, 2005). In addition to monocytes, the binding of neutrophils to ECs is regulated by isoprostanes. Whereas 8-*iso*-PGE₂ has no effect on the adhesion of neutrophils, 8-*iso*-PGF₂α enhances adhesion in a TP receptor-dependent as well as a TP receptor-independent manner (Zahler and Becker, 1999; Fontana *et al.*, 2001; 2002; Huber *et al.*, 2003). The capacity of isoprostanes to regulate endothelial/immune cell interaction and thereby affecting the process of atherosclerosis has been confirmed *in vivo*. Peritoneal injection of 1 µg·kg⁻¹ body weight 8-*iso*-PGF₂α displayed a TP receptor-dependent significant increase in macrophage density and atherosclerotic burden in aortic root sections of mice (Tang *et al.*, 2005). This increased adhesion of macrophages to aortic ECs was accompanied by increased expression of sICAM-1 and CCL2, the latter being a chemokine, crucial in recruiting immune cells to the site of inflammation (Tang *et al.*, 2005). Furthermore, treatment of human macrophages with 8-*iso*-PGF₂α resulted in a NF-κB-independent increase in the expression of the pro-atherogenic molecule IL-8 (Scholz *et al.*, 2003). This underlines the importance of isoprostanes in atherosclerosis and other inflammatory disorders. The effect of isoprostanes on immune cells is not restricted to a modulation of their adhesive properties. Furthermore, 8-*iso*-PGF₂α can induce the activation of CD11b/CD18 and CD11c/CD18 in neutrophils resulting in the activation of NADPH oxidase (Fontana *et al.*, 2001). NADPH oxidase and its catalytic subunit gp91 play an important role in the generation of platelet-derived 8-*iso*-PGF₂α (Pignatelli *et al.*, 2011). Furthermore, it has been demonstrated that functionally active NADPH oxidase in microglial cells generates ROS

during inflammation in the CNS, thus exacerbating cerebral injury (Green *et al.*, 2001). These data indicate that isoprostanes can enhance their own generation by activating NADPH oxidase in immune cells. In addition to an increased interaction between immune and ECs, the oxidation of low-density lipoproteins (LDL) plays an important role in the formation of atherosclerotic lesions (Tomkin and Owens, 2001). The incubation of LDL with Cu²⁺ resulted in a decrease in esterified F₂-isoprostane levels and a significant increase in free isoprostane levels (Lynch *et al.*, 1994). This indicates that LDL is a critical source for local isoprostane generation and liberation in the process of atherogenesis.

Isoprostanes act as vasoconstrictors

An increase in vascular tone plays an important role in a variety of pathological processes, such as hypertension and ischaemia. Interestingly, a significant vasoconstrictory potential of isoprostanes has been described. In isolated guinea pig hearts, 8-*iso*-PGF₂α and 8-*iso*-PGE₂ caused a sustained and concentration-dependent coronary vasoconstriction with EC₅₀ values in the range of 10⁻⁵ M resulting in a decrease of coronary flow by as much as 50% (Möbert *et al.*, 1997). Simultaneous administration of SQ29548 abolished the vasoconstrictor effect of both isoprostanes indicating a TP receptor-dependent mechanism (Möbert *et al.*, 1997). This pro-vasoconstrictor potential of 8-*iso*-PGF₂α and 8-*iso*-PGE₂ has been confirmed in a wide range of different blood vessels, from human umbilical arteries, chicken embryo ductus arteriosus, pulmonary artery, femoral artery and porcine arteries to bovine coronary arteries, demonstrating the general vasoconstrictor potential of isoprostanes (Kromer and Tippins, 1996; van der Sterren and Villamor, 2011; Sakariassen *et al.*, 2012). Interestingly, no vasoconstriction was induced in bovine coronary arteries indicating a putative species-dependent functionality of 8-*iso*-PGF₂α (Kromer and Tippins, 1996). The vasoconstrictor effect of isoprostanes, such as 8-*iso*-PGF₂α and 8-*iso*-PGE₂, is mainly mediated via TP receptors leading to the release of internally sequestered Ca²⁺ and activation of the RhoA/Rho kinase 1 and 2 signalling pathway (Kromer and Tippins, 1996; Möbert *et al.*, 1997; Mueed *et al.*, 2008; Sakariassen *et al.*, 2012). On the other hand, bovine aortic ECs possess two distinct binding sites for isoprostanes, indicating that the vasoconstrictor effect of these PG-like compounds may also be mediated by a so far not identified TP receptor-related isoprostane receptor (Yura *et al.*, 1999; van der Sterren and Villamor, 2011). The vasoconstriction of pulmonary vasculature and intestine epithelium mediated via 8-*iso*-PGE₂ was mediated via TP receptors and the PGE receptor, indicating that receptors other than TP receptors may be involved in the mediation of the vasoconstrictor capacity of isoprostanes (Elmhurst *et al.*, 1997; Janssen and Tazzeo, 2002). The modulation of the vascular tone by isoprostanes can also occur in an indirect way. 8-*iso*-PGF₂α concentrations in the range of 10⁻⁷ M, stimulate, probably through transcriptional regulation, the production of endothelin-1, a mitogen for ECs with vasoconstrictor potential, thereby inducing strong vasoconstriction (Yura *et al.*, 1999).

Angiogenesis is affected by isoprostanes

Angiogenesis is a central process in several pathological disorders, such as cancer and diabetes (Folkman, 2002; Martin *et al.*, 2003) and is a key event in cardiovascular homeostasis and regeneration, a process often impaired in CVD patients (Griffioen and Molema, 2000; Khurana *et al.*, 2005). Isoprostanes regulate the formation of new blood vessels from pre-existing ones by various mechanisms. First, our group demonstrated that 8-*iso*-PGF₂α and 8-*iso*-PGA₂ synergistically and dose-dependently inhibit the migration and tubule formation of ECs *in vitro* via the activation of TP receptors (Benndorf *et al.*, 2008). In these studies, we additionally observed that 8-*iso*-PGA₂ can decompose into two biologically active compounds indicating that unstable isoprostanes may exert synergistic effects with endogenous isoprostanes affecting angiogenesis (Benndorf *et al.*, 2008). Moreover, in *ex vivo* and *in vivo* assays, the anti-angiogenic effect of 8-*iso*-PGF₂α was confirmed, being again mediated via TP receptors (Benndorf *et al.*, 2008). Interestingly, 8-*iso*-PGF₂α in the presence of VEGF-A induced an enhanced and persistent activation of the small GTPase RhoA in ECs as compared to the transient effect of VEGF-A on RhoA activity in absence of 8-*iso*-PGF₂α (Benndorf *et al.*, 2008). This may be crucial for isoprostane-mediated inhibition of angiogenesis as blockade of RhoA downstream effector Rho kinase completely reversed isoprostane-mediated anti-angiogenic effects *in vitro*. Indeed, persistent RhoA/Rho kinase activation may inhibit important steps in angiogenesis, such as EC movement and sprouting, via perturbation of cytoskeletal dynamics and focal adhesion turnover and reduces VEGF-induced EC sprouting (Kroll *et al.*, 2009). Moreover, isoprostanes affect biology of further vascular cell types, such as vascular smooth muscle cells (VSMC) and vascular fibroblasts, which have been implicated in vascular maturation and stiffness (Nehls *et al.*, 1994). Isoprostanes induce the proliferation of these cells, an effect that may affect the process of angiogenesis via modified VSMC–endothelial interaction and signalling (Takahashi *et al.*, 1992; Kunapuli *et al.*, 1997). These data indicate that isoprostanes inhibit new blood vessel formation and promote vascular stabilization via activation of TP receptors. However, in this context, it has to be mentioned that the role of TxA₂ and TxA₂ mimetics in angiogenesis, especially tumour-associated angiogenesis, is not fully elucidated. Synthetic TxA₂ mimetics inhibit fibroblast growth factor 2 – and VEGF-induced angiogenesis *in vitro* as well as *in vivo* (Ashton and Ware, 2004; Ashton *et al.*, 2004; Pal *et al.*, 2006; Benndorf *et al.*, 2008). In contrast, several studies particularly focusing on tumour-associated angiogenesis demonstrated that TxA₂ may also act as a pro-angiogenic factor (Daniel *et al.*, 1999; Nie *et al.*, 2000; Wei *et al.*, 2010). These conflicting results could be interpreted as showing that TP receptor isoforms may contribute, to different extents, to the process of angiogenesis and that TP receptor activation in cancer cells may induce production and release of pro-angiogenic molecules, which induce angiogenesis in a paracrine fashion. Taken together, isoprostanes modulate the process of angiogenesis via TP receptor activation and are likely to affect endothelial homeostasis and regeneration via this route. Nevertheless, further studies are

needed to clarify the role of TP receptors and TP receptor agonists especially in the context of tumour-associated angiogenesis.

Role of isoprostanes in cell cycle regulation and cardiac ion channel dysfunction

Under hypoxic conditions, an increase in the generation and release of 8-*iso*-PGF₂α has been observed (Hart *et al.*, 1998). In pulmonary artery ECs, this increase in isoprostane concentration was accompanied by monolayer dysfunction, which was in contrast to the isoprostane-induced apoptosis in ECs, not induced via cell death (Hart *et al.*, 1998; Benndorf *et al.*, 2008). Furthermore, a role of A/J isoprostanes in cell cycle regulation has been shown. These compounds can be incorporated into cells and accumulate in the nucleus, inducing a G1 cell cycle arrest (Chen *et al.*, 1999a; Brooks *et al.*, 2008).

ROS, generated during the process of ischaemia in the mitochondria of cardiomyocytes (Becker *et al.*, 1999) may affect the function of cardiac channel proteins. E₂-isoketals, highly reactive products of the isoprostane pathway, are associated with cardiac Na⁺ channel dysfunction indicating a role of isoprostanes in ischaemia-related conduction abnormalities and arrhythmias (Fukuda *et al.*, 2005). In summary, isoprostanes modulate platelet activation, the initiation of inflammatory processes, vasoconstriction, the disturbance of the vascular endothelial barrier, angiogenesis and EC cell death, indicating a mechanistically relevant role of these oxidative stress markers in the pathogenesis and progression of CVDs.

Relevance of isoprostanes in CVD

Based on the biological activities of isoprostanes discussed above, a role of these compounds in CVD seems to be obvious. Isoprostane-mediated effects with potential relevance for the pathogenesis of CVD are summarized in Figure 3. In apolipoprotein E-deficient mice, overexpression of the hydroperoxide scavenger GSH peroxidase-4 significantly reduced aortic F₂-isoprostane levels accompanied by a significant decrease in atherosclerotic lesions sizes (Guo *et al.*, 2008). Furthermore, in patients with coronary heart disease, those with advanced atherosclerotic plaque formation exhibit significantly higher extent of 8-*iso*-PGF₂α accumulation in close proximity to the atherosclerotic lesions (Mehrabi *et al.*, 1999). Levels of isoprostanes correlated with the number of risk factors for coronary artery disease present in patients and significantly increased with the number of diseased vessels thereby confirming the role of oxidative stress in the atherosclerotic process (Schwedhelm *et al.*, 2004; Basarici *et al.*, 2007; 2008). In several studies involving patients with coronary artery disease, up to 2–3-fold higher plasma and urinary levels of 8-*iso*-PGF₂α have been detected as compared with age- and sex-matched healthy individuals, additionally correlating with the extent and the severity of

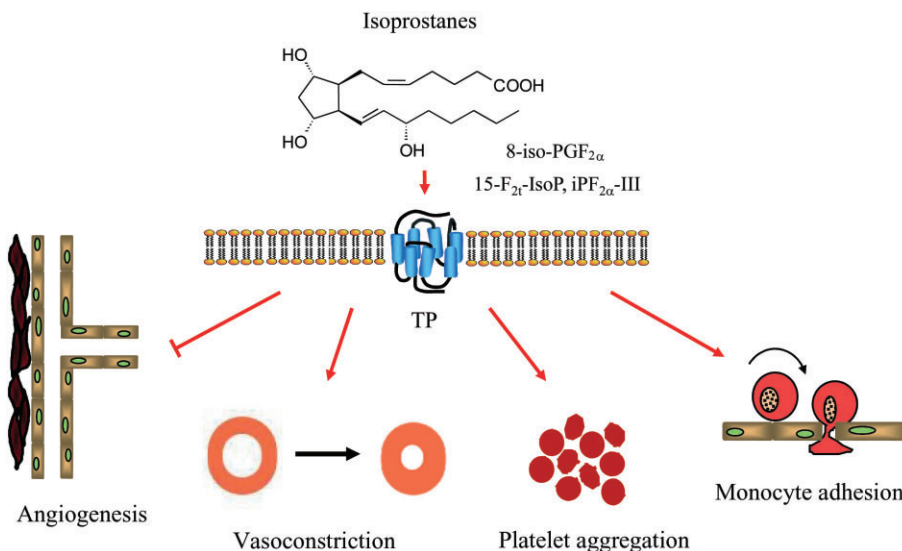


Figure 3

Proposed effects of isoprostanes in the cardiovascular system. Isoprostanes act as partial agonists of the TP receptor and may represent important alternative activators of the TP receptor especially in the context of oxidative stress.

the disease (Schwedhelm *et al.*, 2000; 2004; Vassalle *et al.*, 2003; Wang *et al.*, 2006; Radovanovic *et al.*, 2008; Roest *et al.*, 2008; Di Minno *et al.*, 2012). These studies indicate that isoprostanes are cumulative and independent risk markers in coronary artery diseases.

Coronary endothelial dysfunction in humans is characterized by local enhancement of oxidative stress without a decrease in basal NO release (Lavi *et al.*, 2008). Interestingly, isoprostane concentrations measured in the coronary sinus were 29% higher in patients with endothelial dysfunction, emphasizing the role of 8-iso-PGF_{2α} as a marker of regional endothelial dysfunction in humans (Lavi *et al.*, 2008). Furthermore, by investigating changes in coronary artery diameter and coronary flow, a more important role of isoprostanes in epicardial than in microcirculatory endothelial dysfunction has been described (Lavi *et al.*, 2008).

In the effluents of isolated and perfused rat hearts, an increase in 8-iso-PGF_{2α} concentration from only a few pg·mL⁻¹ up to nearly 100 pg·mL⁻¹ during ischaemia has been observed (Xia *et al.*, 2003; 2005). This elevation in isoprostane levels was accompanied by an increased myocardial infarct size and exacerbated post-ischaemic myocardial dysfunction, probably mediated via a stimulated production and release of endothelin-1 during ischaemia (Xia *et al.*, 2005). In a canine model of coronary thrombolysis and in patients with acute myocardial infarction, an increase in urinary 8-iso-PGF_{2α} concentrations of approximately 28 and 300%, respectively, was observed, indicating that coronary reperfusion is associated with an increased generation of isoprostanes, which is likely to reflect oxidant stress *in vivo* (Delanty *et al.*, 1997). Furthermore, in patients undergoing coronary artery bypass surgery or acute revascularization in the context of myocardial infarction, a 2–3-fold increase in plasma and urinary 8-iso-PGF_{2α} levels has been detected, confirming the association between ischaemia/reperfusion and isoprostane generation (Reilly *et al.*, 1997; Ansley *et al.*, 2003). The gen-

eration of isoprostanes resulting from lipid peroxidation seems to occur immediately after reperfusion because no further increase in the isoprostane concentration could be observed in subsequent post-operative period (Ansley *et al.*, 2003; Ulus *et al.*, 2003). In contrast, in clinical ischaemia/reperfusion injury, no increase of 8-iso-PGF_{2α} levels in plasma and urine during early reperfusion of the ischaemic kidney or heart has been described, indicating a highly complex and sensitive process of isoprostane formation under ischaemic conditions (de Vries *et al.*, 2013). Furthermore, in patients with ischaemic chronic heart failure, levels of 8-iso-PGF_{2α} correlated significantly with indices of remodelling (Radovanovic *et al.*, 2008). Here, the authors demonstrated that markers of oxidative stress, such as isoprostanes, are unlikely to play an important role in early stages of chronic heart failure, but might become important in the course of this disease (Radovanovic *et al.*, 2008). In this stage, urinary 8-iso-PGF_{2α} could be used as a reliable indicator of symptomatic chronic heart failure (Radovanovic *et al.*, 2008). Generally, a correlation between oxidative stress, elevated isoprostane concentrations and the severity and outcome of CVD has been demonstrated in animal and human studies. Therefore, a targeted inhibition of isoprostane generation or its interaction with TP receptors could help to improve outcome in patients suffering from CVD.

Outlook

Several cardiovascular pathologies are characterized by elevated isoprostane formation and excretion (Cracowski *et al.*, 2001; Cracowski and Durand, 2006; Schwedhelm *et al.*, 2007). Moreover, isoprostanes are involved in the pathophysiology of CVD by activating the TP receptor (Galano *et al.*, 2013). The inhibition of isoprostane formation or TP receptor activation may therefore represent a valuable clinical

strategy in patients at a high cardiovascular risk. Considering a causative role of isoprostanes in CVD, detection of isoprostane concentrations in plasma or further body fluids could help to identify patients at high risk of developing cardiovascular complications. Formation of isoprostanes may then be suppressed by several therapeutic strategies such as up-regulation of antioxidant enzymes, such as SOD and pharmacological inhibition of ROS formation by novel low MW NADPH oxidase inhibitors. Moreover, pharmacological antagonism of TP receptors could represent an alternative therapeutic strategy in patients with extensive isoprostane formation. Several TP receptor antagonists have been developed and used in pre- and clinical testing (Davi *et al.*, 2012), but their clinical impact is still negligible today. In this regard, preclinical and clinical development of TP receptor antagonists may have suffered from insufficient specificity and efficacy or unexpected side effects of drug candidates. Design of more specific TP receptor antagonists and identification of patients who may clearly benefit from additional TP receptor blockade could thus be a rewarding challenge in the near future. So far, the TP receptor has not been crystallized and structural information is still incomplete. Therefore, fully elucidating the molecular structure of the TP receptor may foster the development of more specific and effective antagonists of this receptor, which may help to further reduce cardiovascular complications in high-risk patients.

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Conflict of interest

The authors declare that there is no conflict of interest.

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