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Published in:
Pharmacology and Therapeutics

DOI:
10.1016/j.pharmthera.2010.04.013

2010

Cardiovascular Risk Factors Regulate the Expression of Vascular Endothelin Receptors

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Abstract

Cardiovascular disease remains as the leading cause of death in the developed world. However, there is limited knowledge about how cardiovascular risk factors actually cause vascular disease. Traditional cardiovascular risk factors include increased circulating levels of low-density lipoproteins, cigarette smoking and hypertension (both strongly related to arterial wall injury), inflammation and atherosclerosis.

The vascular endothelin receptors are a protein family that belongs to the larger family of G-protein coupled receptors. They mediate vascular smooth muscle contraction, proliferation and apoptosis, which are important events in the pathogenesis of atherosclerotic vascular disease. Recent investigations into intracellular signaling mechanisms suggest that the above risk factors increase the expression of endothelin receptors in vascular smooth muscle cells by activating intracellular mitogen-activated protein kinase pathways and downstream transcription factors such as nuclear factor-kappaB. Understanding the mechanisms involved in vascular endothelin receptor upregulation during cardiovascular disease may provide novel therapeutic approaches.

Key words: Cardiovascular risk factors; Endothelin; Receptor; MAPK signaling; Cardiovascular disease.

Abbreviations

CHD, coronary heart disease; CVD, cardiovascular disease; ECE, endothelin-converting enzymes; ET-1, endothelin-1; ET_A, endothelin type A receptors; ET_B, endothelin type B receptors; ERK1/2, extracellular signal-regulated kinase 1 and 2; LDL, low-density lipoprotein; LDL-c, low-density lipoprotein-cholesterol; MAPK, mitogen-activated protein kinase; NO, nitric oxide; NF-κB, nuclear factor-kappaB; oxLDL, oxidized LDL; PKC, protein kinase C; PGI_2, prostacyclin; VEC, vascular endothelial cells; VSMC, vascular smooth muscle cells.

Contents

1. Introduction

The overall mortality from cardiovascular disease (CVD) has decreased markedly over the last decade, but coronary heart disease (CHD) and cerebral ischemic stroke remain as the leading causes of mortality in the developed countries. It is well documented that lowering low-density lipoprotein-cholesterol (LDL-c) with statins reduces the risk of CVD, and yet about 50% of all CHD and strokes occur in individuals with normal cholesterol levels (Lewington et al., 2007). Thus, even if the target LDL-c level is achieved, there still remains a significant risk for CHD events (Davidson, 2005). Traditional risk factors for atherosclerotic lesions include hypertension, diabetes, cigarette smoking and obesity, all of which are associated with CHD and stroke. In recent years, studies have shown that there is an incease in the incidence of CVD in subjects with high plasma levels of systemic inflammatory markers such as C-reactive protein (Engstrom et al., 2009; Libby & Ridker, 2004; Tanne et al., 2006). However, the knowledge about how the risk factors lead to CVD is limited. Drugs targeting the molecular mechanisms that mediate the development of CVD still need to be discovered.

Endothelin-1 (ET-1) is the most potent vasoconstrictor (Li et al., 2007) and a strong growth factor (Janakidevi et al., 1992). Elevated plasma levels of ET-1 are seen after exposure to the risk factor cigarette smoke (Haak et al., 1994), suggesting that ET-1 contributes to the pathogenesis of CVD. Thus, pharmacological manipulation of the endothelin system might be a promising therapeutic tool for CVD. Indeed, clinical trials with endothelin receptor antagonists for treating CVD have already resulted in

the use of the mixed endothelin receptor antagonist bosentan for the treatment of pulmonary arterial hypertension (Galie et al., 2008). However, little attention has been directed toward elucidating the underlying molecular mechanisms that are responsible for the regulation of endothelin receptor expression.

The development of CVD is multifactorial and includes both environmental and genomic risk factors. There are many cardiovascular risk factors and the numbers are growing. Moreover, genomics and proteomics have recently discovered novel genes, gene expression patterns, proteins and single nucleotide polymorphisms as risk predictors of CVD. The present review will be mainly focussed on the role of low-density lipoproteins (LDL), cigarette smoke and high blood pressure in the development of CVD, because they are the most common and strongest cardiovascular risk factors. Hence, we will consider recent data on plausible mechanisms explaining how the above cardiovascular risk factors are associated with vascular ET-1 and endothelin receptor expression and function; this association suggests novel therapeutic targets for the treatment of CVD.

2. Endothelins and endothelin receptors

The vascular endothelium is a physical barrier between the blood and the vessel wall structures. It is also the source of enzymes that activate and deactivate cardiovascular hormones and can produce relaxing and contracting factors, growth factors, growth inhibitors and inflammatory mediators (Luscher et al., 1993). These endothelium-derived factors contribute to the finely tuned control of circulation, hemostasis and inflammation by endocrine, autocrine and paracrine activity (Morganti et al., 2002).

2.1. Endothelins

ET-1 was initially isolated and identified from conditioned medium of cultured porcine endothelial cells. It is a potent vasoconstrictor peptide that consists of 21 amino acids (Yanagisawa et al., 1988) and exerts an extremely diverse set of actions that influence homeostatic mechanisms throughout the body. The human endothelin family contains three 21-amino-acid-long isopeptides, namely, ET-1, endothelin-2 (ET-2), and endothelin-3 (ET-3), which are encoded by separate, unique genes (Inoue et al., 1989). The discovery and biological significance of the endothelins has stimulated interest in their biosynthesis and structure-activity relationships.

The biosynthesis of endothelins is initiated by the transcription of endothelin genes in vascular endothelial cells (VEC) and their subsequent translation into prepro-endothelin (a synthetic precursor) and then big-endothelin (a biologically inactive intermediate), which is further cleaved by specific endothelin-converting enzymes (ECE) into the ET-1, -2 and -3 isoforms (Hynynen & Khalil, 2006). It is worth noting that the amino acid sequence of ECE was obtained after a cloning procedure due to the scarcity of ECE in tissues (Schmidt et al., 1994; Shimada et al., 1994). In the human cardiovascular system, ET-1 is the most important isoform of the endothelin family; it induces a long-lasting vasoconstriction (Li et al., 2007) and stimulates proliferation of vascular smooth muscle cells (VSMC) (Janakidevi et al., 1992). Endothelial cells are a major source of ET-1, making this peptide fairly ubiquitous, and the constitutive release of ET-1 from the endothelium may contribute to basal vascular tone (Gray et al., 2000). Interestingly, ET-1 can be produced by a

variety of other cell types, including those of the inner medullary collecting duct and most of the other nephron segments, neurons of the central nervous system, postganglionic sympathetic neurons and monocytes/macrophages. Under proinflammatory conditions, VSMC and pulmonary epithelial cells also produce ET-1 (Davenport & Maguire, 2006). ET-2 is predominantly expressed in the intestine, colon, ovary and uterus, but its expression has also been reported in the brain and kidney (Uchide et al., 2003). ET-3 is produced by monocytes/macrophages and by renal tubular cells, although in much smaller quantities than ET-1 (Wang et al., 1996). Activation of the vascular endothelium by cardiovascular risk factors such as high levels of LDL and cigarette smoke exposure cause an increased production and secretion of ET-1 (Haak et al., 1994; Lubrano et al., 2008). The elevated secretion of ET-1 in connection with heart disease, hypoxia and hypertension is partly due to enhanced activity of ECE (Schiffrin et al., 1997; Davenport & Maguire, 2006).

2.2. Endothelin receptors

An important discovery during the early stages of endothelin research was the demonstration of specific endothelin receptors. The existence of multiple endothelin receptor subtypes was first hinted by the biphasic blood pressure response to ET-1 (Yanagisawa et al., 1988). This response is characterized by an initial transient reduction in blood presure followed by a sustained vasopressor response (Sun et al., 1992). Receptor subtypes were also hinted at by the differing pressor profiles of the three endothelin isoforms (Inoue et al., 1989). Later, cloned cDNA sequences of two receptors for endothelin were published, and the receptors were designated as the

endothelin type A (ET_A) and endothelin type B (ET_B) receptors (Arai et al., 1990). Although they were transcribed from different genes, both receptors were shown to belong to the same family of heptahelical G-protein coupled receptors by analysis of their amino acid sequences. The ET_A receptor binds ET-1 and ET-2 with greater affinity than it does ET-3, whereas the ET_B receptor binds all three isoforms with equal affinity (Davenport, 2002). The two receptors are distributed in various tissues and cells, but are expressed at different levels, suggesting the presence of a multifunctional endothelin system. An additional receptor subtype, named the endothelin type C (ET_C) receptor, has been suggested by radioligand binding and pharmacological experiments, but has so far not been identified at the molecular level (Karne et al., 1993; Douglas et al., 1995). In humans, the ET_A receptor consists of 427 amino acids, and shows 64% sequence similarity to the 442 amino acid human ET_B receptor (Adachi et al., 1991). Endothelin receptors from several mammalian species display a fairly high degree of homology with the human ET_A and ET_B receptors (Davenport, 2002). Moreover, endothelin receptors are expressed in a wide variety of cells and tissues (Yasuda et al., 2005) and, undoubtedly, a complex array of signaling molecules are employed by the receptors in order to achieve the diverse effects of endothelins on their target cells (Shraga-Levine & Sokolovsky, 2000).

In the vasculature, ET_A receptors are located on VSMC and mediate vasoconstriction and VSMC proliferation (Janakidevi et al., 1992) involving activation of mitogen-activated protein kinase (MAPK) (Chen et al., 2009). In contrast, the ET_B receptors are primarily located on VEC (also named ET_{B1}) and

mediate vasodilatation and inhibition of VSMC proliferation through the release of nitric oxide (NO) and prostacyclin (PGI_2) (Marasciulo et al., 2006; Schneider et al., 2007). A subpopulation of contractile ET_B receptors (named ET_{B2}) also exists in the VSMC and mediates vasoconstriction (Adner et al., 1996; Gray et al., 2000) and proliferation (Janakidevi et al., 1992) (Fig. 1). The ET_A receptor activates the G proteins Gq/11 and G12/13. This results in contraction and proliferation of VSMC through activation of intracellular signaling molecules such as phospholipase C, intracellular Ca^{2+}, protein kinase C (PKC) and extracellular signal-regulated kinase 1 and 2 (ERK1/2). The ET_B receptor stimulates the Gi and the Gq/11 families of proteins in VSMC and VEC (Gohla et al., 2000; Cramer et al., 2001; Marasciulo et al., 2006). ET-1 binds tightly to the ET_A receptor and gives rise to a strong, long-lasting contraction with a low receptor turnover (Sudjarwo et al., 1994). This prolonged effect may be due to the localization of the receptors in caveolae with a low rate of internalization (Chun et al., 1994). The ET_B receptor, on the other hand, is rapidly internalized and inactivated through phosphorylation following activation (Cramer et al., 1997) and, thus, evokes a more transient response.

In CVD, ET_B receptors may switch their phenotype from relaxing (ET_{B1}) to contractile (ET_{B2}), as observed after an experimental stroke (Stenman et al., 2002). This change in receptor phenotype can be experimentally mimicked by organ culture of arteries in serum-free medium (Adner et al., 1996). Following organ culture of the arteries, there is a time-dependent increase in ET_{B2} receptor mRNA, which is followed by increased levels of ET_{B2} receptor protein and function (Zheng et al.,

2010). Cytokines like interleukin-1beta and tumor necrosis factor-alpha can enhance this upregulation of ET_{B2} receptors (Uddman et al., 1999). This receptor upregulation is influenced by both PKC and MAPK activities (in particular the MEK/ERK pathways) (Uddman et al., 2002; 2003).

Organ culture has, in our hands, been a convenient method for studying endothelin receptor alterations and the endothelin receptor phenotype changes *in vitro* (Adner et al., 1996). Initially, organ culture demonstrated an upregulation of ET_{B2} receptors in rat mesenteric VSMC after 1-5 days of culture (Adner et al., 1998b). This receptor upregulation also takes place in human coronary arteries after organ culture (Wackenfors et al., 2003) and in human ischemic heart disease (Wackenfors et al., 2004). Changes in the endothelin receptor phenotype seen during organ culture of rat cerebral and peripheral arteries are similar to the endothelin receptor phenotype changes observed in peripheral artery disease (Lind et al., 1999), in subarachnoidal hemorrhage (Hansen-Schwartz et al., 2003) and after cerebral ischemic stroke (Stenman et al., 2002). Thus, organ culture is a useful model for studying endothelin receptor alterations, delineating their regulation by different inflammatory mediators (Uddman et al., 1999) and determining the involvement of various intracellular signal transduction pathways (Xu et al., 2008a).

3. Cardiovascular risk factors

3.1. LDL

A high blood level of LDL-c is closely related to cardiovascular risks (Raitakari et al., 2003). Treatment with statins can reduce blood LDL-c levels and prevent

cardiovascular events (Komorovsky et al., 2010) as well as strokes (Glader et al., 2010). The level of total cholesterol is an obvious risk factor for CVD (Wilson et al., 1998). Circulating cholesterol is carried by high-density lipoproteins, LDL, very low density lipoproteins, intermediate-density lipoproteins and chylomicrons (Ip et al., 2009). LDL-c is unstable, and adheres to and penetrates into artery walls, where LDL can be oxidized.

Intracellular signaling pathways activated by oxidized LDL (oxLDL) in the vasculature include the MAPK family, which shows a time- and concentration-dependent phosphorylation of p42/p44 MAPK in VSMC that can be inhibited by PD98059 (an inhibitor of MEK1/2) in a concentration-dependent manner (Yang et al., 2001). In addition, oxLDL activates nuclear protein import, a critical element in regulating gene expression, transcription and, subsequently, cell proliferation and apoptosis. Exposure of VSMC to oxLDL, but not to native LDL, significantly increases nuclear protein import and the number of VSMC. These changes occur through an ERK1/2 MAPK-dependent mechanism (Chahine et al., 2009). Immunization with oxLDL generates antibodies to oxLDL that decrease atherosclerosis in both rabbit and mouse models (Palinski et al., 1995; Ameli et al., 1996; Freigang et al., 1998). These data suggest that LDL oxidation and intracellular MAPK signaling may play key roles in cardiovascular pathogenesis.

The vascular activity of endogenous ET-1 is enhanced in hypercholesterolemic patients, whereas their sensitivity to exogenous ET-1 remains unchanged. These findings suggest an increased production of ET-1, which may participate in the

pathophysiology of vascular disease characteristic of hypercholesterolemia (Cardillo et al., 2000). The increase in tissue ET-1 level seems more important than the elevated plasma level of ET-1 for the development of CVD. Recent studies have revealed that LDL may reduce endothelium-dependent relaxation (Zhang et al., 2008) and increase vascular ET_{B2} receptor expression, leading to enhanced vasoconstriction (Fig. 2A and 2B). The molecular mechanisms behind this may include LDL oxidation, activation of intracellular MAPK and downstream transcription factors. However, there is limited knowledge on how LDL induces endothelin receptor upregulation in CVD. Further studies on samples from cardiovascular patients, *in vivo* animal models of hyperlipidemia, as well as *in vitro* studies of LDL effects and oxidation, will help to delineate the underlying molecular mechanisms that are responsible for LDL-induced vascular endothelin receptor upregulation.

3.2. Cigarette smoking

Cigarette smoking and second hand smoking are well known risk factors for atherosclerosis, CHD, stroke, myocardial infarction, aortic aneurysm and peripheral vascular disease (Hawkins et al., 2002; Ezzati et al., 2005). There is an increase in aortic stiffness and blood pressure in smokers, and this effect persists longer in smokers with hypertension (Kim et al., 2005; Rhee et al., 2007). In stroke patients, cigarette smoking significantly increases the risk for acute ischemic attacks, and the risk is further increased in passive smokers (Bonita et al., 1999). A population-based cohort study in middle-aged adults concluded that both active and passive smoking are associated with a progression of atherosclerosis, particularly in patients with

diabetes and hypertension (Howard et al., 1998).

Lipid-soluble cigarette smoke particles in the circulation are transported by lipoproteins to the arterial wall (Shu & Bymun, 1983), where they can directly cause damage to the arterial wall via modification of lipoprotein properties (Xu et al., 1994). Exposure of nonsmoking subjects to second hand smoke also breaks down the serum antioxidant defense, leading to accelerated lipid peroxidation, LDL modification and an accumulation of LDL-c in human macrophages (Valkonen & Kuusi, 1998).

Exposure to lipid-soluble cigarette smoke particles increase the expression of vascular ET_A (Xu et al., 2008b) and ET_B (Xu et al., 2008a) receptors as well as thromboxane A_2 receptors (Zhang et al., 2008). The G-protein coupled receptor upregulation results in a strong contraction of arteries and may contribute to the development of vascular damage. This effect is seen at concentrations of lipid-soluble particles similar to those that occur in smokers, while water-soluble smoke particles or nicotine at physiological levels do not have such effects on receptor expression (Zhang et al., 2006; Xu et al., 2008a). These findings suggest that the lipid-soluble smoke particles induce upregulation of the vascular endothelin receptors.

3.3. Hypertension

Hypertension, a powerful independent contributor to cardiovascular morbidity and mortality, is a major burden on public health worldwide. Hypertensive patients are two to three times more likely to have CVD than normotensives (Kannel, 1996; Dieterle et al., 2010). Early organ damage in hypertensive CVD includes left ventricular hypertrophy, microalbuminuria and cognitive dysfunction; more dramatic

events such as stroke, heart attack, renal failure and dementia usually take place after a long period of uncontrolled hypertension (Messerli et al., 2007).

Hypertension is associated with an increased formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in the plasma (Griendling & Alexander, 1997; Lacy et al., 1998). In addition, plasma concentrations of angiotensin II are often elevated in patients with hypertension (Furuhashi et al., 1990). These substances unavoidably reduce the formation of endothelial NO and increase leukocyte adhesion and peripheral resistance (Vanhoutte & Boulanger, 1995).

Patients with hypertension have increased endothelin receptor activity and an increased vascular tone (Li et al., 2007), which predisposes them to target organ damage. In addition, enhanced ET_A and ET_{B2} receptor-mediated contractions with increased receptor expression were seen in the left internal mammary arteries from patients with hypertension compared to normotensive patients at both mRNA and protein levels (Nilsson et al., 2008a). This may be part of a molecular mechanism mediating hypertension. Blockade of endothelin receptors improves endothelium-dependent vasodilator function in hypertensive patients, thereby suggesting that an increased ET-1 activity may play a role in the pathophysiology of this abnormality (Cardillo et al., 2002). Endothelin receptor antagonists have been found to reduce blood pressure in hypertensive animals (Nishikibe et al., 1993) and in patients with essential hypertension (Krum et al., 1998; Cardillo et al., 1999). Both selective ET_A and ET_B receptor antagonists and non-selective endothelin receptor antagonists induce a greater vasodilatation in the forearm of hypertensive patients

than in normotensive subjects (Cardillo et al., 2002; Taddei et al., 1999). This indicates that endothelin receptors play a role in the pathogenesis of hypertension.

An investigation on the antihypertensive efficacy and safety of darusentan, a new selective ET_A receptor antagonist, demonstrated that this drug provides a minor additional reduction in blood pressure in patients who have not attained their treatment goals with three or more antihypertensive drugs (Nakov et al., 2002; Weber et al., 2009). However, darusentan has major adverse effects mainly related to fluid accumulation. Oedema or fluid retention occurred in 67 (27%) patients given darusentan compared with 19 (14%) given placebo (Weber et al., 2009).

On the other hand, the ET_{B1} receptors are located on the endothelium and mediate vasodilatation. Cardiovascular risk factors are known to induce attenuation of endothelium-dependent relaxation by decreasing the release of PGI_2, NO or endothelium-derived hyperpolarizing factor (Zhang et al., 2006). LDL (Zhang et al., 2008) and lipid-soluble smoking particles (Zhang et al., 2006) injure the endothelial function; this, in turn, results in an attenuation of the ET_{B1} receptor-mediated vasodilatation by decreasing the production of PGI_2, NO or endothelium-derived hyperpolarizing factor (Nilsson et al., 2008c). The decreased vasodilator functions of ET_{B1} receptors and/or the increased ET_A and ET_{B2} receptor-mediated contractions lead to elevated blood pressure, vascular spasm and ischemia in CVD.

4. Upregulation of vascular endothelin receptors

Upregulation of endothelin receptors has been demonstrated in patients with CVD (Wackenfors et al., 2004; Nilsson et al., 2008a) and in experimental animal models of

cerebral ischemia (Stenman et al., 2002). ET_{B2} receptor expression is increased in the VSMC of resistance arteries from patients with ischemic heart disease, while no alteration is seen in the expression of ET_A receptors. The level of upregulation of ET_{B2} receptors is associated with the degree of ischemic heart disease (Dimitrijevic et al., 2009). These data suggest that vascular hypoxia due to reduced blood flow by vascular spasm and/or obstruction in ischemic CVD may contribute to an alteration of endothelin receptors. Chronic intermittent hypoxia increased the expression of ET_B receptors in the carotid body (Rey et al., 2007). Increased ET_B receptor expression has also been seen in blood vessels of patients with ischemic heart disease (Wackenfors et al., 2004) and after experimental ischemic stroke in rats (Stenman et al., 2002). This suggests a role for hypoxia and re-oxygenation in the process of induced ET_B receptor expression *in vivo*. However, incubation of isolated rat pulmonary arteries under hypoxia decreased the ET_B receptor-mediated contraction (Wang et al., 2006), suggesting that re-oxygenation is required for the induced ET_B receptor expression.

Interestingly, there is a similar phenomenon of endothelin receptor upregulation in temporal arteries obtained from patients with giant cell arteritis (Dimitrijevic et al., 2010), suggesting that chronic inflammation in the arterial wall may mediate the endothelin receptor upregulation. Patients with hypertension have increased endothelin receptor activity and an increased vascular tone (Li et al., 2007). The atherosclerotic arteries exhibit a similar receptor upregulation (Dagassan et al., 1996; Pernow et al., 2000), suggesting that this receptor upregulation takes place during pathological conditions. In human atherosclerotic lesions, there is also an increase in

ET-1 and ET_B receptors and this increase is seen in the medial VSMC, just beneath the foam cells (Iwasa et al., 1999). Increased ET_B receptor-mediated responses have been demonstrated in human blood vessels of patients with ischaemic heart disease (Wackenfors et al., 2004) and after experimental ischemic stroke in rats (Stenman et al., 2002). These data suggest that the upregulation of endothelin receptors is an important molecular mechanism that mediates the development of CVD. However, the knowledge about how endothelin receptors are upregulated in CVD is limited.

The initial organ culture experiments were designed using the specific inhibitors to examine the roles of intracellular signaling pathways in the regulation of vascular endothelin receptor expression. Organ culture of rat cerebral (Henriksson et al., 2004) and mesenteric (Uddman et al., 2002) arteries as well as porcine coronary arteries (Nilsson et al., 2008b) induced an upregulation of ET_{B2} receptors, which was attenuated by PKC inhibitors. In addition, treatment with PKC inhibitors in experimental ischemic stroke and subarachnoidal hemorrhage decreased the infarction area and the neurological symptoms, as well as the associated ET_{B2} receptor upregulation (Beg et al., 2007; Henriksson et al., 2007). These results suggest that PKC may play an important role in regulating ET_{B2} receptor expression. In parallel, the MAPK MEK/ERK pathway was involved in endothelin receptor upregulation both *in vitro* (Uddman et al., 2003) and *in vivo* (Beg et al., 2006). The PKC and the MEK/ERK pathways appear to interact, as demonstrated by a Western blot study (Ansar & Edvinsson, 2008). This suggests the involvement of an intracellular signal cross-talk in the regulation of vascular endothelin receptor expression.

5. The role of MAPK in vascular endothelin receptor upregulation

The underlying molecular mechanisms of how cardiovascular risk factors lead to increased VSMC endothelin receptor upregulation are largely unknown. Intracellular MAPK signaling pathways have been recognized to play an important role in regulating vascular endothelin receptor expression (Xu et al., 2008a; Chen et al., 2009). The MAPK are a group of serine/threonine kinases that are evolutionary well conserved in all eukaryotes. Activation of MAPK phosphorylates various gene regulatory proteins which results in a range of cellular responses. There are three well characterized MAPK pathways, namely, ERK1/2, p38 and c-jun N-terminal kinase (Lewis et al., 1998). Both ERK1/2 and p38 play important roles in cerebral ischemia, and treatment with specific inhibitors of these MAPK in experimental models of cerebral ischemia is neuroprotective (Alessandrini et al., 1999; Barone et al., 2001; Namura et al., 2001).

In order to understand the role of MAPK in the upregulation of ET_B receptors, we have examined the effects of different protein kinase inhibitors in the organ culture model (Xu et al., 2008a). The inhibitors selected to target different kinases in the cascade leading to ERK1/2 activation are U0126 and SB386023. U0126, a noncompetitive inhibitor of mitogen-activated protein kinase 1 and 2 (MEK1/2) (Favata et al., 1998), blocks the enzymatic activity of MEK1/2 and subsequently inhibits the activation of ERK1/2. SB386023 inhibits the MAPK upstream of MEK, which is in the Raf family. Both U0126 and SB386023 decreased organ culture-induced ET_B receptor upregulation (Henriksson et al., 2004). Raf-1 (c-Raf) is

a MAPK required for Ras/MEK/ERK signal transduction, which is the first enzyme in the MAPK cascade consisting of the three protein kinases Raf-1, MEK and ERK (Chin et al., 2004). Inhibition of Raf-1 attenuated cigarette smoke-induced expression of endothelin receptors in mice (Lei et al., 2008).

A crucial role of the ERK1/2 MAPK in ET_{B2} receptor upregulation was demonstrated by Western blot in combination with specific inhibitors (Xu et al., 2008a). A similar ET_{B2} receptor upregulation was reported for experimental focal ischemia and subarachnoid hemorrhage, along with increased levels of ET_B receptor mRNA and protein and a contractile phenotype of the receptors (Stenman et al., 2002; Hansen-Schwartz et al., 2003). Other studies have also suggested that cardiovascular risk factors may induce VSMC endothelin receptor upregulation via activation of MAPK-mediated nuclear factor-kappaB (NF-κB) inflammatory signal pathways (Xu et al., 2008a). The lipid-soluble smoking particles can induce activation of ERK1/2, p38 and the downstream transcriptional factor NF-κB within 3 hrs, with a subsequent upregulation of VSMC ET_{B2} receptors after 6 hrs. Inhibition of ERK1/2, p38 or NF-κB activities by their specific inhibitors significantly attenuates the lipid-soluble smoking particle-induced upregulation of VSMC ET_{B2} receptor expression (Xu et al., 2008a). These studies on risk factors and MAPK suggest that the risk factors may, via activation of MAPK-dependent NF-κB-mediated intracellular inflammatory signal transduction pathways, enhance endothelin receptor expression and subsequently result in increased VSMC contraction and proliferation in CVD (Fig. 3). Thus, inhibition of MAPK may be a novel therapeutic target for the treatment of CVD.

6. MAPK as promising pharmacological targets in CVD

Undoubtedly, the most effective way to manage cardiovascular disease is to treat the underlying disorders, such as treating hypercholesterolemia with statins, hypertension with anti-hypertensive drugs, diabetes mellitus with blood glucose regulators, and smoking by smoke-cessation. Due to the unique pharmacological response (i.e. sustained contraction) elicited by ET-1 and the possible involvement of ET-1 in CVD, many studies have been performed on the endothelin system. Endothelin receptor antagonists appeared soon after the discovery of ET-1. At an early stage of endothelin research, a cyclic pentapeptide BE1857A found in the fermentation broth of the microbe *Streptomyces misakiensis* (Ihara et al., 1992) was shown to be an ET_A receptor antagonist. A potent ET_A receptor antagonist, cyclic pentapeptide BQ123, was subsequently derived from this substance (Ihara et al., 1992; Spatola & Crozet, 1996). Further extensive structure-activity relationship analysis of the carboxyl terminal of the endothelin peptide led to peptide antagonists that had affinity for both ET_A and ET_B receptors, such as PD142893 (Warner et al., 1993). Interestingly, most of the endothelin receptor antagonists found in nature are ET_A receptor antagonists. The first non-peptide endothelin receptor antagonist that remains effective following oral administration was found in sulfonamide derivatives that were first synthesized as part of an initiative to develop antidiabetic therapies. Modification of the lead compound resulted in the non-selective endothelin receptor antagonists Ro46-2005 (Clozel et al., 1993) and Ro47-0203 (also termed bosentan) (Clozel et al., 1994). Modification of the sulfonamide derivative produced another potent ET_A receptor

antagonist (Stein et al., 1994). Many endothelin receptor antagonists have since appeared, including some that are selective for ET_A receptors, some that are selective for ET_B receptors, and some that show similar affinities for both receptor subtypes. The co-incubation of the endothelin receptor blockers effectively antagonized both ET-1- and sarafotoxin 6c-induced responses, but did not modify the receptor upregulation by organ culture (Adner et al., 1998a) or following cerebral ischemia (Stenman et al., 2007).

Studies on the MAPK signal transduction cascade have revealed that activation of MAPK plays important roles in the enhanced expression of vascular endothelin receptors in CVD. U0126 directly inhibits the MAPK family members MEK-1 and MEK-2. MAPK signaling pathways are involved in cellular events such as growth, differentiation and stress responses. The ERK1/2 pathway inhibitors SB386023 and U0126 and the p38 protein kinase pathway inhibitor SB239063 have significant inhibitory effects on the enhanced contractile responses of rat mesenteric arteries to ET_{B2} receptor activation following organ culture (Uddman et al., 2003). *In vivo*, U0126 diminishes the upregulated contractile responses and ET_{B2} receptor expression in rat middle cerebral arteries after occlusion (Henriksson et al., 2007); this finding suggests that the MAPK-mediated upregulation of endothelin receptors may provide a new perspective on the pathophysiology and therapy of CVD. As MAPK is involved in a wide spectrum of pathophysiological processes, there is a caution that inhibition of MAPK may have potential side effects in pharmacological treatments.

Targeting endothelin receptors by using their antagonists such as bosentan

results in excessive levels of ET-1 in the circulation (Hiramoto et al., 2007). Theoretically, inhibition of the MAPK signal mechanisms will exert similar therapeutic effects as endothelin antagonists in that it also improves the endothelium function and artery remodeling; nevertheless, it would also avoid the accumulation of ET-1 in the circulation by decreasing ET-1 production and, moreover, inhibition of MAPK will inhibit VSMC proliferation. Thus, targeting MAPK signal mechanisms should be better tools than the endothelin receptor antagonists.

7. Summary and conclusions

CVD is the major cause of death and disability worldwide. However, the underlying molecular mechanisms that mediate the development of CVD are not fully understood. New therapeutic strategies targeting the molecular mechanisms still need to be discovered. In this review, we have discussed recent findings on how cardiovascular risk factors induce the MAPK-mediated upregulation of vascular endothelin receptors and reviewed the roles of MAPK signal mechanisms in the development of CVD. The MAPK-mediated upregulation of vascular endothelin receptors might be one of the important molecular mechanisms that lead to the development of CVD. Thus, it may provide novel pharmacological targets for the treatment of CVD.

Acknowledgments

This work was supported by Swedish Research Council (grant no 5958), the Swedish Heart-Lung Foundation and the Flight Attendant Medical Research Institute (FAMRI, U.S.A.).

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Figure legends

Figure 1.

Schematic diagram of endothelin receptor-mediated regulation of VSMC contraction and proliferation. Solid lines represent activation and stimulation. Broken lines indicate inhibition.

Figure 2.

(A) Contractile response to sarafotoxin 6c (S6c) in rat mesenteric arteries (without endothelium) after organ culture with LDL (50, 100, 200 mg/L) for 24 hrs. Data are presented as the percentage of contraction induced by 60 mM of K^+, which is not significantly altered by LDL. Each point is derived from 8 experiments and presented as mean ± S.E.M.

(B) Western blot experiments (n=3) show that LDL (200 mg/L) induces VSMC ET_{B2} receptor expression in rat mesenteric arteries after 6 hrs of organ culture. Ctrl = control (vehicle).

Figure 3.

Schematic diagram of the MAPK-mediated upregulation of vascular endothelin receptors. Cardiovascular risk factors (LDL, hypertension, cigarette smoke, etc) may induce VSMC endothelin receptor upregulation via activation of MAPK-mediated NF-κB signal pathways. The endothelin receptor upregulation may lead to increased VSMC proliferation and contraction and, subsequently, cause CVD. Large circle line represents cell membrane and small circle line indicates nuclear membrane.

Figure 1.

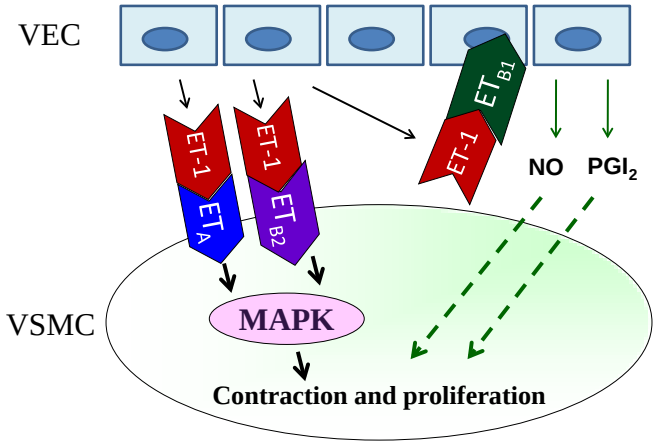

VEC
ET-1
ET-1
ET-1
ET$_{B1}$
NO
PGI$_2$
ET$_A$
ET$_{B2}$
MAPK
VSMC
Contraction and proliferation

Figure 2.

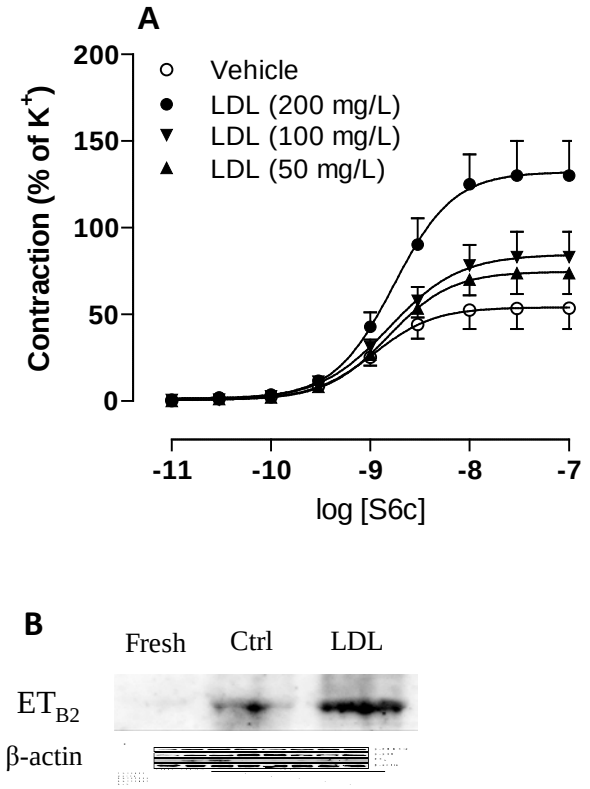
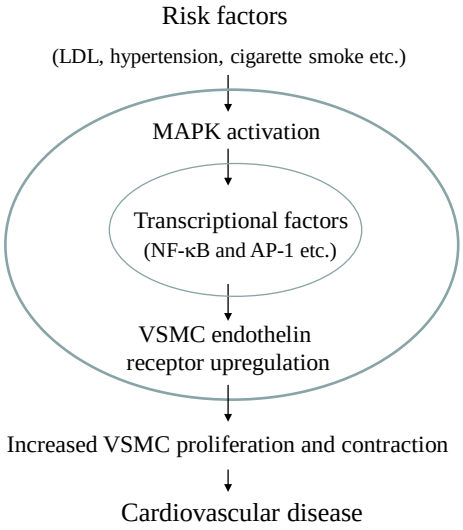

A
200
150
100
50
0
Contraction (% of K+)
Vehicle
LDL (200 mg/L)
LDL (100 mg/L)
LDL (50 mg/L)
-11
-10
-9
-8
-7
log [S6c]
B
Fresh
Ctrl
LDL
ET B2
β-actin

Figure 3.


Risk factors
(LDL, hypertension, cigarette smoke etc.)
MAPK activation
Transcriptional factors
(NF-κB and AP-1 etc.)
VSMC endothelin
receptor upregulation
Increased VSMC proliferation and contraction
Cardiovascular disease