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REVIEW

Myocardial ischemia: Current concepts and future perspectives

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Summary Ischemic heart disease is the leading cause of morbidity and mortality in a worldwide epidemic. Myocardial ischemia is characterized by an imbalance between myocardial oxygen supply and demand, causing cardiac dysfunction, arrhythmias, myocardial infarction, and sudden death. Various clinical ischemic manifestations are caused by obstruction of coronary blood flow by coronary plaques, thrombosis, and/or hyperconstriction/vasospasm of epicardial and microvascular coronary arteries, in which gender difference also is involved due in part to estrogen hormonal state. The coronary circulation matches blood flow with oxygen requirements by coordinating the resistances within microvasculature, where the endothelium plays an important role by liberating several vasodilator substances. The impaired endothelial regulation is involved in the pathogenesis of a wide variety of cardiovascular diseases and therefore is an important therapeutic target. Activation of Rho-kinase pathway is involved in the pathogenesis of both endothelial dysfunction and vascular smooth muscle hypercontraction and also should be an important therapeutic target.

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Introduction

Ischemic heart disease is the leading cause of morbidity and mortality in a worldwide epidemic. Myocardial ischemia is characterized by an imbalance between myocardial oxygen supply and demand, causing cardiac dysfunction, arrhythmias, myocardial infarction, and sudden death. Various clinical ischemic manifestations are caused by obstruction of coronary blood flow by coronary stenosis, thrombosis, and/or hyperconstriction (vasospasm) of epicardial and microvascular coronary arteries.

The coronary circulation matches blood flow with myocardial oxygen demand by coordinating the vascular resistances within microvasculature, where the endothelium plays an important role [1,2]. The endothelium also regulates the tone of the underlying vascular smooth muscle cells (VSMC) by releasing several endothelium-derived relaxing factors, such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) [1,2]. The cells also release several vasoconstricting factors, such as endothelin, superoxide anions (O_2^-), and thromboxane, under certain pathological conditions [1,2]. Endothelial dysfunction is regarded as a clinical syndrome that exhibits systemic manifestation of atherosclerosis and resultant myocardial ischemia, and is associated with significant morbidity and mortality [1,2].

In this review article, we will briefly review the current concepts and future perspectives on myocardial ischemia, with a special reference to endothelial dysfunction, the Rho-kinase pathway, and microvascular angina.

Myocardial ischemia and its assessment

Myocardial ischemia is defined as an imbalance between myocardial oxygen demand and supply [3]. In patients with ischemic heart disease, the presence of myocardial ischemia is an important determinant of prognosis [4] and several diagnostic

methods are currently used to detect myocardial ischemia in the clinical setting (Table 1).

Myocardial ischemia is clinically indicated by transient ST-segment electrocardiogram (ECG) changes on exercise or pharmacological stress test and reversible perfusion defects on stress myocardial scintigraphy. Metabolic changes, including myocardial lactate production, coronary sinus oxygen desaturation, and pH reduction in the coronary sinus, are also important objective proof of myocardial ischemia. Myocardial release of lipid peroxide products in the coronary circulation is a marker of myocardial ischemia with a high sensitivity even for brief and/or mild myocardial ischemia [5]. Myocardial phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy is another sensitive method to identify myocardial ischemia by measuring myocardial high-energy phosphates phosphocreatine and adenosine triphosphate [6]. In addition to those ischemic metabolites, left ventricular wall motion abnormalities, detected by two-dimensional stress echocardiography, is a useful diagnostic method [7].

Measurement of coronary blood flow is useful, but only provides information associated with myocardial ischemia. Positron-emission tomography (PET) allows the quantitative calculation of coronary blood flow [8]. Magnetic resonance imaging (MRI) with intravenous infusion of contrast media can also be used for the quantification of myocardial blood flow [9]. Coronary flow reserve is expressed by the ratio of blood flow during maximal hyperemia (e.g. adenosine or papaverine) to that at rest. Coronary flow reserve can be measured invasively by the thermodilution or Doppler technique [10] and is considered abnormal when it is less than 2.0. Trans-thoracic color Doppler echocardiography enables noninvasive assessment of coronary flow/velocity reserve, especially in the territory of the left anterior descending coronary artery [11,12]. Since flow resistance is mainly determined at the microvascular level, especially in patients with angiographically normal arteries, the reduction in coronary flow reserve reflects coronary microvascular dysfunction.

Table 1 Diagnostic tools for myocardial ischemia and coronary blood flow

Myocardial ischemia	
ECG	Exercise stress test, Holter ECG
Scintigraphy	Thallium-201, Technetium-99m
Metabolic markers	Myocardial lactate production, coronary sinus O ₂ desaturation, pH reduction, lipid peroxides
³¹ P NMR	High-energy phosphates phosphocreatine, adenosine triphosphate
Coronary blood flow	
PET	Oxygen-15, nitrogen-13, rubidium-82
MRI	Perfusion and diffusion imaging
Coronary flow reserve	Thermodilution method, Doppler method (intracoronary, transthoracic)

ECG, electrocardiography; ³¹P NMR, myocardial phosphorus-31 nuclear magnetic resonance; PET, positron-emission tomography; MRI, magnetic resonance imaging.

Relevance of microvascular dysfunction in the pathogenesis of myocardial ischemia

Recently, it has become increasingly apparent that clinical manifestations of myocardial ischemia are associated not only with epicardial coronary flow, but also with downstream microcirculatory flow at the level of coronary microvessels [13,14]. The recognition of microvascular dysfunction could cause a paradigm shift in clinical practice. For instance, in patients with acute myocardial infarction, coronary microvascular dysfunction is responsible for the so-called “no-reflow” phenomenon, which is associated with a worse outcome as compared with those without it [15,16]. Therefore, in patients with acute myocardial infarction undergoing reperfusion therapy, careful attention should be paid not only to achieve epicardial coronary artery patency, but also to improve microvascular perfusion status [17]. It is also noted that, even in the absence of epicardial coronary artery disease, myocardial perfusion abnormality could develop due to microvascular dysfunction in patients with hypercholesterolemia, hypertension, and diabetes mellitus [18].

Endothelium-dependent modulation of coronary tone

In pathological conditions, the balance between endothelium-dependent relaxation and direct VSMC constriction plays an important determinant role in vascular tone [1,2]. Among the endothelium-derived relaxing factors, NO was originally found in the relaxation of isolated rabbit aorta in response to acetylcholine (ACh) [19]. NO binds to guanylyl cyclase and increases cyclic guanosine monophos-

phate (cGMP), resulting in VSMC relaxation. When the endothelium is removed, vasodilatation to ACh is converted to vasoconstriction, reflecting the effect of muscarinic VSMC contraction. Importantly, endothelial cells also play an important role in modulation of vascular tone of coronary microvessels. However, the response to physical forces (e.g. shear stress) and paracrine mediators varies depending on the vessel size [1,2,20]. Indeed, endothelial cells are substantially involved in regulating both epicardial and resistance coronary arteries.

Endothelium-derived NO

NO is formed in endothelial cells from L-arginine to citrulline by constitutive endothelial NO-synthase (eNOS) [21,22]. This reaction is controlled by calcium and calmodulin and is dependent on molecular oxygen, nicotinamide adenine dinucleotide phosphate (NADH) and its reduced form (NADPH), tetrahydrobiopterin (BH₄), adenosine diphosphate (ADP), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMD). NO diffuses to VSMC and causes relaxation mainly by stimulating soluble guanylate cyclase, which catalyzes the production of cGMP. NO mediates vascular relaxation of relatively large, conduit arteries (i.e. aorta and epicardial coronary arteries), which is enhanced by cyclical or pulsatile changes in coronary shear stress (Figs. 1 and 2) [23]. NO-mediated vasodilatation is impaired in patients with risk factors for coronary artery disease due to reduced NO production and/or enhanced inactivation of NO [21,22].

Prostacyclin

Metabolism of arachidonic acid via cyclooxygenase can produce prostacyclin, which causes relaxation of certain VSMC by activating adenylate cyclase and increasing the production of cyclic 3',5'-adenosine

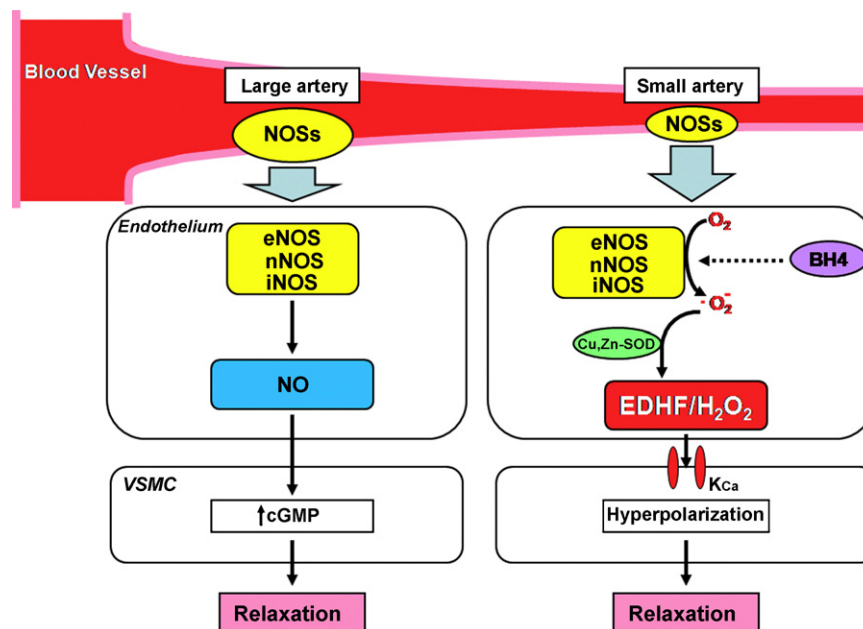


Figure 1 Different roles of endothelial NO synthases system depending on vessel size. Endothelial NO synthases system plays different roles depending on the vessel size, mainly NO generation in the conduit arteries and EDHF generation in microvessels. eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; BH₄, tetrahydrobiopterin; SOD, superoxide dismutase; EDHF, endothelium-derived hyperpolarization factor; cGMP, cyclic guanosine monophosphate. (Reproduced from Ref. [23] with permission.)

monophosphate (cAMP). In most resistance vessels, the contribution of prostacyclin to endothelium-dependent relaxation is relatively minor [2] (Fig. 1). However, vasodilator prostaglandins are important

determinants of coronary collateral vessel tone, and inhibition of cyclooxygenase reduces collateral perfusion in dogs [24]. It is also important that prostacyclin acts synergistically with NO to cause vasodilatation [25].

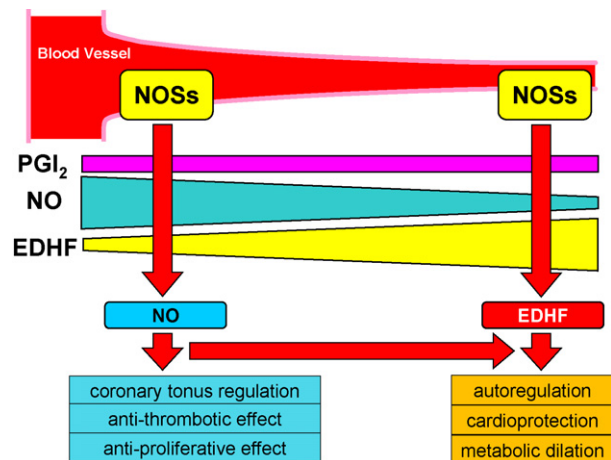


Figure 2 Different roles of endothelial NO synthases system in the coronary circulation in vivo. Endothelial NO synthases system plays an important role in modulation of vascular tone in the epicardial coronary artery as NO-generating system, whereas in coronary microcirculation, it exerts several protective effects as EDHF-generating system in collaboration with NO from the epicardial coronary artery, including coronary autoregulation, protection against myocardial ischemia/reperfusion injury, and metabolic coronary dilatation. PGI₂, prostaglandin I₂.

EDHF

Feletou and Vanhoutte [26] and Chen et al. [27] independently demonstrated that diffusible substance released by the endothelium causes hyperpolarization of underlying VSMC, thus proposing the existence of EDHF. Several substances/mechanisms have been proposed for the nature of EDHF, including epoxyeicosatrienoic acids (metabolites of arachidonic P450 epoxygenase pathway) [28,29], K ions [30,31], and electrical communications through myoendothelial gap junctions [32,33]. We have recently demonstrated that endothelium-derived hydrogen peroxide (H₂O₂) is an EDHF in mouse [34] and human mesenteric arteries [35], and in porcine [36] and canine coronary microvessels [37]. Furthermore, endothelial Cu, Zn-superoxide dismutase (Cu,Zn-SOD) plays an important role for the synthesis of EDHF/H₂O₂ [38]. EDHF modulates vascular tone in small, resistance arteries in vitro [39], and in human forearm microcirculation in vivo [40] (Fig. 1). As in the case with NO, EDHF-mediated relaxations also are attenu-

ated by several atherosclerotic risk factors [41,42]. Importantly, we were able to demonstrate that endogenous EDHF/H₂O₂ plays important cardioprotective roles in coronary microcirculation in vivo, including autoregulation [43], protection against ischemia/reperfusion [44], and metabolic coronary dilatation (Fig. 2) [45]. We also have recently demonstrated that in mice lacking all three NOS isoforms (triple NOS^{-/-}), EDHF-mediated responses are absent in addition to NO-mediated responses [23] and that myocardial infarction occurs spontaneously associated with metabolic syndrome manifestations [46], indicating that endothelial NOS system plays a pivotal role in maintaining cardiovascular homeostasis (Fig. 2).

Clinical implications of endothelial dysfunction

The clinical implications of endothelial dysfunction are well established. Risk factors, such as smoking, aging, hypercholesterolemia, hypertension, hyperglycemia, and a family history, are all associated with an attenuation or loss of endothelium-dependent vasodilatation [2,47,48]. It is also noted that markers of systemic inflammation are associated with endothelial dysfunction, including increased levels of C-reactive protein, and, as recently recognized, obesity and metabolic syndrome [49–51]. More importantly, recent studies demonstrated that the severity of endothelial dysfunction relates to cardiovascular events, including cardiac death, myocardial infarction, and need for revascularization [52,53] and that future events were poorly predicted by the degree of angiographic coronary stenosis alone [54].

As a surrogate for coronary circulation with less invasive fashion, endothelial function of forearm resistance vessels can be assessed by intra-arterial infusion of ACh. In their prospective follow-up study with patients with coronary artery diseases, Heitzer et al. showed that forearm blood flow response to intra-arterial ACh was an independent predictor of cardiovascular events and that a concomitant infusion of ascorbic acid improved endothelial function, probably due to its antioxidant effects [55]. The study with high-resolution ultrasound for the assessment of flow-mediated vasodilatation also demonstrated that endothelial dysfunction can identify patients at increased risk for cardiovascular events [56]. Thus, endothelial dysfunction is an important systemic process that could be identified in vascular beds other than the coronary or cerebral circulations.

Studies on endothelial progenitor cells (EPCs) have demonstrated the novel aspect of the important role of the endothelium. Indeed, the degree of endothelial dysfunction is correlated with the number of EPCs [57] and the number of circulating EPCs also predicts the occurrence of cardiovascular events and death from cardiovascular diseases [52,58]. Recently, it has been reported that patients with cardiac syndrome X (microvascular angina) have a significantly increased number of circulating EPCs, suggesting endothelial dysfunction as an underlying mechanism in this disorder [59].

Microvascular angina (cardiac syndrome X)

Up to 20–30% of patients with angina-like chest pain who undergo coronary angiography have no flow-limiting epicardial coronary stenosis or spasm [60,61]. These patients are often defined as cardiac syndrome X [62] or microvascular angina [63], which is an important clinical entity. The cause(s) of this syndrome appears to be heterogeneous, in which coronary microvascular dysfunction appears to be involved, reflecting an inadequate coronary vasodilator capacity and/or enhanced coronary vasoconstrictor responses [64].

In patients with microvascular angina, limited microvascular vasodilator reserve to various types of physiological and pharmacological stimuli has been repeatedly observed, including exercise, adenosine, dipryridamole, and atrial pacing [65–68]. Myocardial ischemia in those patients can be detected by pacing-induced myocardial lactate production [69] or regional myocardial perfusion defects on single photon emission computed tomography or PET imaging [70,71], for which inadequate increase in coronary blood flow appears to be involved.

As an underlying mechanism of the impaired microvascular vasodilator reserve in microvascular angina, several lines of evidence suggest the involvement of blunted NO-dependent microvascular dilatation [68]. Indeed, long-term (4 weeks) oral supplementation with L-arginine improved exercise tolerance in those patients with the disorder [72]. It has been recently suggested that an increased synthesis of asymmetric dimethylarginine, which is known to reduce the bioavailability of L-arginine for NO synthase, contributes to the impaired NO activity in those patients [73]. Although it is highly possible that impaired EDHF responses also are involved in the pathogenesis of microvascular angina based on experimental findings [43–45], this

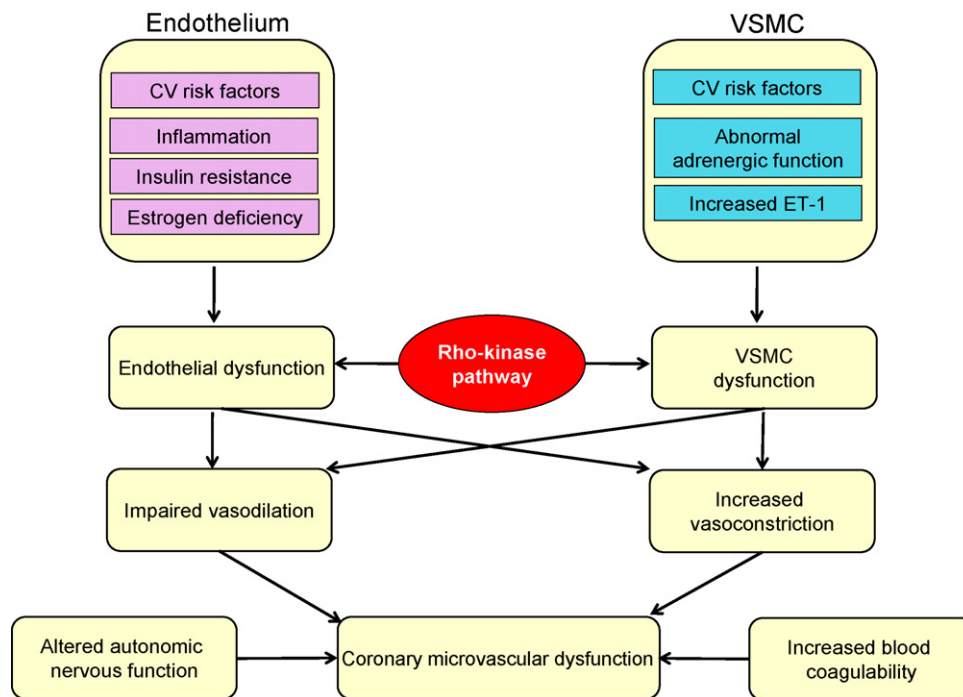


Figure 3 Pathogenetic mechanisms of coronary microvascular dysfunction. The pathogenetic mechanisms of coronary microvascular dysfunction may be heterogeneous, and many confounding factors cause both endothelial dysfunction and VSMC hyperconstriction, where activated Rho-kinase pathway may play an important role. CV, cardiovascular; ET-1, endothelin-1.

issue remains to be confirmed in patients with the disorder.

Several pathogenetic mechanisms and functional abnormalities may be involved in the pathogenesis of coronary microvascular dysfunction (Fig. 3). Increased plasma levels of endothelin-1 (ET-1) were reported in patients with microvascular angina [74–76]. Moreover, ET-1 levels have been reported to increase in the coronary circulation in response to atrial tachypacing in patients with the disorder [77].

Microvascular spasm and Rho-kinase

Enhanced Rho-kinase activity plays an important role in the pathogenesis of not only epicardial coronary spasm, but also microvascular spasm [78,79] (Fig. 3). Rho-kinase has been identified as one of the effectors of the small GTP-binding protein Rho. As a pharmacological inhibitor of Rho-kinase, fasudil [80] and hydroxyfasudil [81] have been developed. Intracoronary administration of fasudil or hydroxyfasudil markedly inhibits epicardial coronary spasm in porcine models with various inflammatory stimuli *in vivo* [82–85]. Indeed, the inhibition of Rho-kinase with fasudil/hydroxyfasudil is associated with the sup-

pression of enhanced myosin light chain (MLC) phosphorylations (both MLC monophosphorylations and diphosphorylations) at the spastic coronary segments in those models (Figs. 3 and 4) [81,83]. Furthermore, activated Rho-kinase down-regulates endothelial NO synthase, causing endothelial dysfunction [86]. Thus, Rho-kinase activation is the central mechanism for vascular dysfunction with endothelial dysfunction and VSMC hypercontraction (Fig. 3).

We have previously demonstrated that in patients with rest angina, ischemic ECG changes and myocardial lactate production can be induced by intracoronary ACh without large epicardial stenosis or spasm (Fig. 5) [87]. Hasdai et al. also demonstrated that coronary blood flow, when evaluated with the Doppler flow guidewire system, was acutely decreased by intracoronary ACh without large epicardial coronary spasm [71]. Microvascular spasm is the underlying cause of myocardial necrosis in the cardiomyopathic Syrian hamster [88]. In patients with microvascular angina/spasm, pretreatment with intracoronary infusion of fasudil effectively prevented ACh-induced angina and myocardial ischemia (myocardial lactate production), indicating that Rho-kinase activation plays an important role in the pathogenesis of this disorder [89].

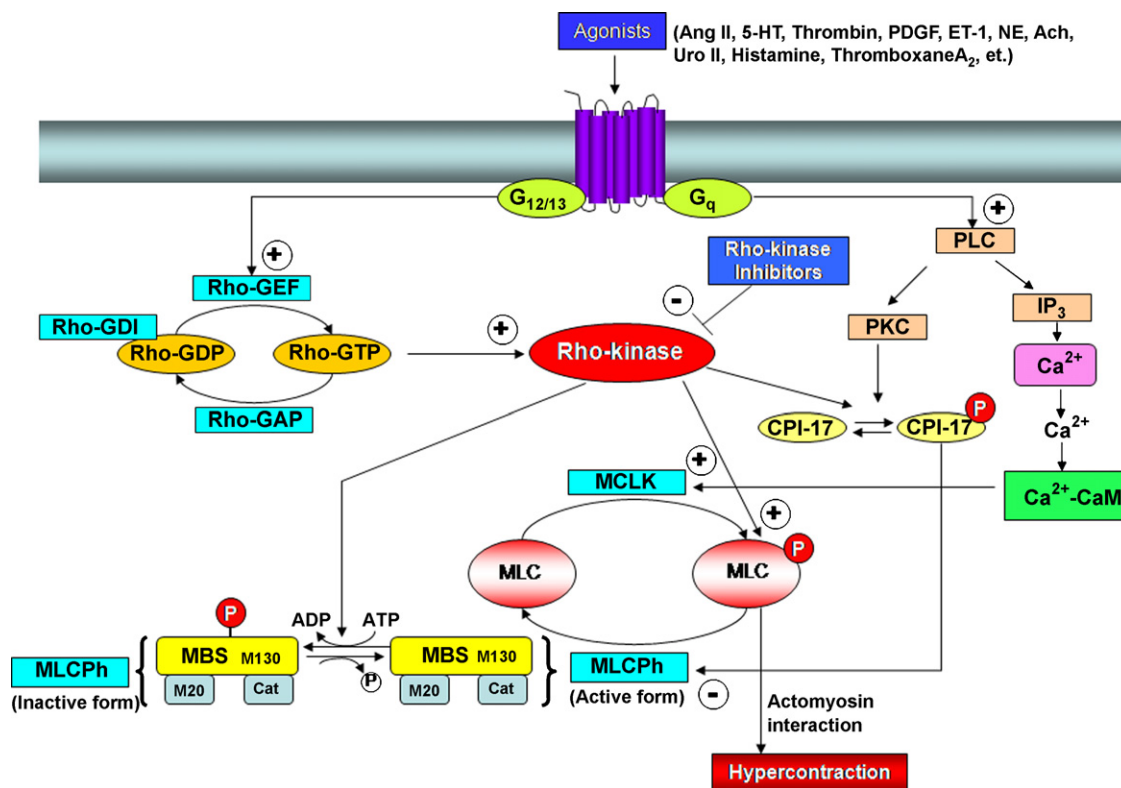


Figure 4 Role of Rho/Rho-kinase signaling pathway in VSMC hyperconstriction. Contraction is induced by the increased phosphorylation of MLC. The agonist-induced activation of G-protein-coupled receptors leads to the stimulation of MCLK through an increase in intracellular Ca²⁺ concentration, and inhibition of MLCPh. Following stimulation by various agonists, the Rho/Rho-kinase-mediated pathway is activated, resulting in the inhibition of MLCPh (through phosphorylation of its MBS), with a resultant increase in MLC phosphorylation. This Rho-kinase-mediated contraction of VSMC can occur independently of intracellular Ca²⁺ levels and is known as “calcium sensitization”. Rho-kinase can also increase MLC phosphorylation and contractility by inactivating MLCPh after phosphorylation of CPI-17 or by direct phosphorylation of MLC. Ach, acetylcholine; Ang II, angiotensin II; Cat, catalytic subunit; ET-1, endothelin-1; IP₃, inositol (1,4,5)-trisphosphate; M20, 20-kDa subunit; NE, norepinephrine; PLC, phospholipase C; PDGF, platelet-derived growth factor; Uro II, urotensin II. Stimulation is denoted by +; inhibition is denoted by –. (Reproduced from Ref. [110] with permission.)

Potential involvement of estrogen in the gender difference of microvascular dysfunction

Since most patients (approximately 70%) with microvascular angina are women during or after menopause [90–93], it has been suggested that estrogen deficiency plays a pathogenic role in this disorder [94,95]. During menopause, estrogen levels are reduced to ~10% of pre-menopausal levels [96]. Estrogen receptors are widely expressed in the cardiovascular system [97] and modulate endothelial function [98]. Indeed, acute administration of exogenous estradiol increases peripheral blood flow [99] and improves endothelial function in menopausal women with microvascular angina [100,101]. Furthermore, it was demon-

strated that short-term supplementation with 17 β -estradiol reduced the frequency of angina episodes in post-menopausal women with the disorder [102]. However, to date, there is no direct evidence that estrogen supplementation causes sustained improvement in coronary microvascular responses in those patients. These findings indicate the complexity of gender-related cardiovascular diseases and heterogeneity in the pathogenesis of microvascular angina. For example, women with the disorder have higher levels of anxiety or stress than those with coronary artery disease or healthy age-matched women [103]. Post-menopausal women also have many vascular risk factors (e.g., diabetes mellitus, obesity, hypertension, mental stress), which cluster more frequently in women than in men [104]. We have previously demonstrated that estrogen inhibits and nicotine

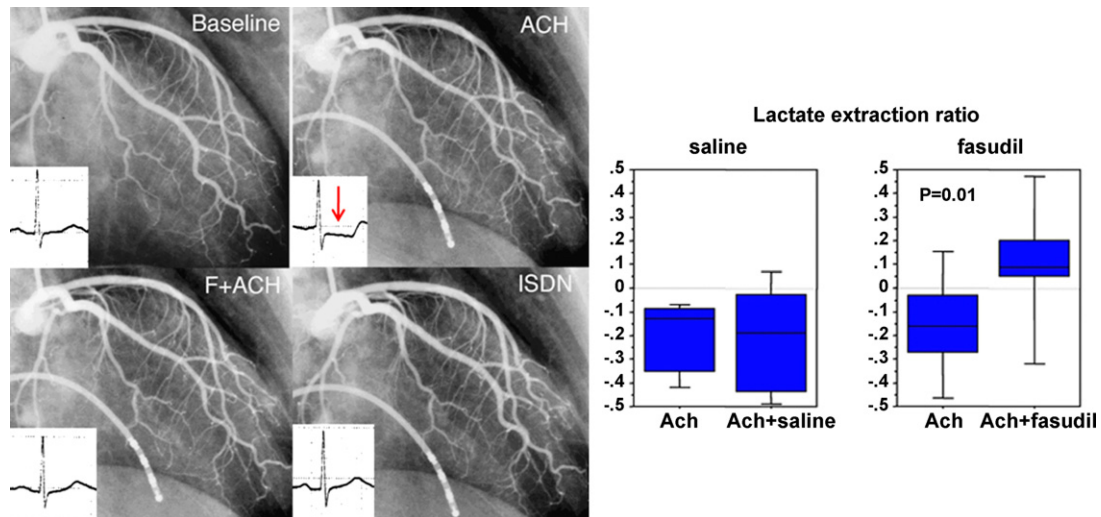


Figure 5 Clinical findings in a patient with microvascular angina. Representative coronary angiography and ECG recordings (left) and group data comparison of the lactate extraction ratio during acetylcholine (ACh) infusion with ($n = 13$, fasudil group) and without pre-treatment of fasudil ($n = 5$, saline group) (right). Intracoronary administration of ACh caused no appreciable vasoconstriction of epicardial coronary arteries, whereas ECG changes and myocardial lactate production indicated the occurrence of myocardial ischemia. Intracoronary pre-treatment with fasudil abolished the ACh-induced myocardial ischemia. F, fasudil; ISDN, isosorbide dinitrate. (Reproduced from Ref. [89] with permission.)

enhances the expression of Rho-kinase in human coronary VSMC in vitro [105] and that Rho-kinase is up-regulated in coronary VSMC in a porcine model of mental stress in vivo [106]. These results may explain, at least in part, why microvascular angina is frequent in women who are post-menopausal and/or under mental stress conditions.

Future strategies to improve vascular dysfunction

In this review, we introduced Rho-kinase inhibition and hormone (estrogen) replacement as a potential therapy to improve vascular dysfunction in specific clinical conditions. Although there

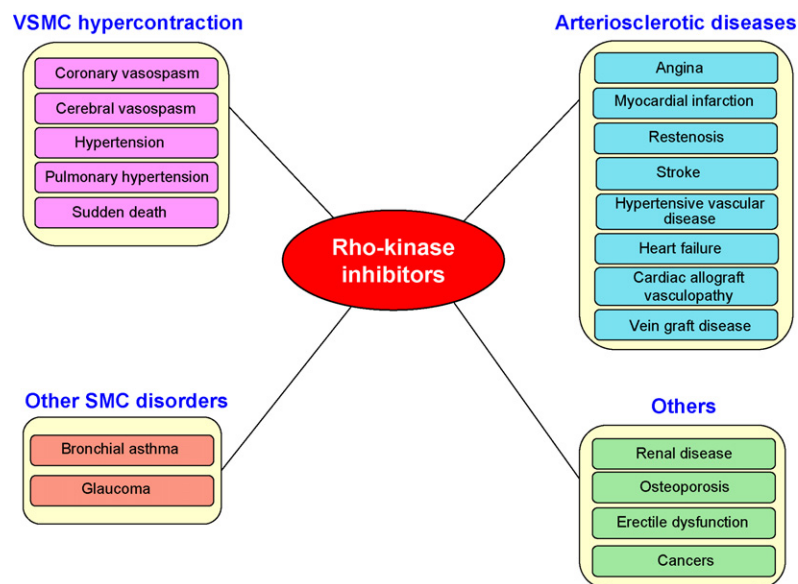


Figure 6 Possible indications of Rho-kinase inhibitors. Rho-kinase inhibitors may be useful for the treatment of a wide variety of cardiovascular diseases with various etiologies, including VSMC hypercontraction, arteriosclerosis, other smooth muscle cell (SMC) disorders, and others. (Reproduced from Ref. [110] with permission.)

is currently no gold standard treatment, the key pharmacological agents for endothelial dysfunction include statins, eicosapentaenoic acid (EPA), and angiotensin-converting enzyme (ACE) inhibitors. These agents are well known to reduce cardiac events with less direct anti-ischemic/anginal effects, underscoring the role of endothelial and VSMC functions in cardiovascular events.

While reduction in serum cholesterol levels is likely the major mechanism by which statins improve endothelial function, *in vitro* studies suggest that so-called pleiotropic effects of statins may also be involved. Statins directly enhance expression, phosphorylation state, and activity of eNOS [107,108]. Angiotensin II increases NAD(P)H oxidase activity, leading to increased production of reactive oxygen species and inactivation of NO. Angiotensin II generation also causes increased production of ET-1 and oxygen free radicals [109]. ACE inhibitors not only inhibit the generation of angiotensin II, but also inhibit the breakdown of bradykinin, a substance that stimulates NO/EDHF production. It remains to be fully elucidated whether angiotensin receptor blockers also could improve vascular functions and if so, what mechanisms are involved.

Accumulating evidence indicates that Rho-kinase inhibitors could cover the wide range of pharmacological effects of the above-mentioned conventional cardiovascular drugs [110]. The phase II trial in patients with stable angina pectoris has demonstrated that long-term oral treatment with fasudil is effective in ameliorating exercise tolerance with adequate safety profiles [111,112]. Indeed, Rho-kinase inhibitors may be effective in a wide range of diseases, including coronary and cerebral vasospasm, hypertension, pulmonary hypertension, stroke, and heart failure (Fig. 6).

Conclusions

Abnormal endothelial and VSMC functions impair coronary circulation and cause myocardial ischemia, not only in epicardial coronary arteries, but also in coronary microcirculation. Rho-kinase pathway is recognized as an important regulator of vascular function at both epicardial and microvascular coronary level and therefore emerges as a novel therapeutic target in cardiovascular medicine.

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