Mechanisms of Coronary Artery Spasm

Kensuke Nishimiya, Jun Takahashi, Kazuma Oyama, Yasuharu Matsumoto, Satoshi Yasuda and Hiroaki Shimokawa

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Abstract

Recent clinical trials have highlighted that percutaneous coronary intervention in patients with stable angina provides limited additional benefits on top of optimal medical therapy. This has led to much more attention being paid to coronary vasomotion abnormalities regardless of obstructive or non-obstructive arterial segments. Coronary vasomotion is regulated by multiple mechanisms that include the endothelium, vascular smooth muscle cells (VSMCs), myocardial metabolic demand, autonomic nervous system and inflammation. Over the years, several animal models have been developed to explore the central mechanism of coronary artery spasm. This review summarises the landmark studies on the mechanisms of coronary vasospasm demonstrating the central role of Rho-kinase as a molecular switch of VSMC hypercontraction and the important role of coronary adventitial inflammation for Rho-kinase upregulation in VSMCs.

Keywords

Coronary artery spasm, Rho-kinase, vascular smooth muscle cells, coronary adventitia, inflammation, perivascular adipose tissue

The ISCHEMIA and REVIVED-BCIS2 trials have highlighted that percutaneous coronary intervention (PCI) in patients with stable coronary syndrome provides limited additional prognostic benefits when used on top of optimal medical therapy.[1,2] This has led to much attention being paid to coronary vasomotion abnormalities regardless of the presence or absence of obstructive coronary artery disease. Coronary vasomotion is regulated by multiple mechanisms that include the endothelium, vascular smooth muscle cells (VSMCs), myocardial metabolic demand, autonomic nervous system and inflammation.[3–5] Coronary vasomotion abnormalities, such as coronary artery spasm, play important roles in the pathogenesis of diverse cardiovascular diseases.

Maseri et al. have pointed out that coronary vasomotion abnormalities modulate residual coronary flow in patients with chronic stable, unstable and variant angina, and syndrome X.[6,7] Over the years, several animal models of the spasm have been developed to explore its central mechanism.[8–10]

It is deemed that atherosclerotic lesions are angiographically predisposed to coronary artery spasm. Based on this clinical observation, we first used an experimental porcine model with coronary atherosclerotic lesions induced by a combination of balloon endothelium removal and a high-cholesterol diet.[10–12] In this model, coronary artery spasm was repeatedly induced in response to intracoronary administration of serotonin or histamine in mild atherosclerotic lesions (*Figure 1*), providing the first experimental evidence for the close link between coronary artery spasm and coronary atherosclerosis.[11,12] Ten years later, this experimental finding

was confirmed in patients who had undergone coronary balloon angioplasty and later in those with vasospastic angina (VSA).[13,14] Importantly, Maseri and his group reported the enhanced local constrictor response to a variety of stimuli acting on different receptors of ergonovine and acetylcholine in patients with variant angina.[15,16] These alterations of local hyperreactivity are in the same coronary segment over weeks and months, although remaining non-spastic coronary segments are equally exposed to the drug.[17,18] Lines of evidence from our basic studies have confirmed that the local hyperreactivity of VSMC rather than an abnormal specific agonist–receptor interaction plays a key role in inducing coronary artery spasm regardless of stimuli in pigs *in vivo*.[9]

Seminal pathological studies have shown that spastic coronary segments have abundant adventitial inflammatory cells and perivascular nerve lesions.[19,20] To determine the roles of endothelial dysfunction and VSMC hypercontraction separately, we developed a second porcine model with chronic adventitial inflammation but without endothelium removal.[21–25] In this model, chronic inflammatory stimuli with adventitial application of interleukin-1β (IL-1β) caused mild arteriosclerotic coronary lesions, where coronary artery spasm was repeatedly induced in response to intracoronary serotonin or histamine but not at the control (non-atherosclerotic) lesion (*Figure 2*).[21] These results were consistent when animals were chronically treated with IL-1α and tumour necrosis factor-α for 4 weeks.[24] Importantly, in this porcine model, endothelial vasodilator functions were fully preserved as expected.[25] Thus, we concluded that VSMC hypercontraction associated with adventitial inflammatory changes plays a major role in the pathogenesis of the spasm.[9,21]

Figure 1: Coronary Artery Spasm in a Porcine Model with Coronary Atherosclerotic Lesions

A: In an experimental porcine model with coronary atherosclerotic lesions induced by a combination of balloon endothelium removal and a high-cholesterol diet, coronary artery spasm was repeatedly induced in response to intracoronary administration of serotonin or histamine. B: There was a close topological correlation between spastic sites and early atherosclerotic lesions. Source: A: Shimokawa et al. 1983.[11] Reproduced with permission from AAAS; B: Shimokawa et al. 2014.[9] Reproduced with permission from Oxford University Press.

Figure 2: Coronary Artery Spasm in a Porcine Model with Chronic Adventitial Inflammation but without Endothelium Removal
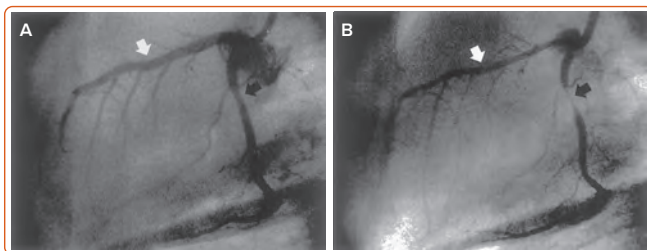
Chronic inflammatory stimuli with adventitial application of interleukin-1β but without endothelium removal caused mild arteriosclerotic lesion where coronary artery spasm was repeatedly induced in response to intracoronary serotonin but none at the healthy (non-atherosclerotic) control lesion. Source: Miyata et al. 1999.[25] Reproduced with permission from Wolters Kluwer.

Mechanistically, contractile responses of VSMC were augmented at the spastic coronary segment in the porcine models of coronary artery spasm, which was later confirmed in a patient with variant angina.[26–28] However, in the first porcine model with balloon endothelium removal and a high-cholesterol diet, the spastic segment showed no alteration of the VSMC vasocontractile response to incremental concentrations of calcium ions (Ca^{2+}), suggesting that the key mechanism for coronary spasm other than Ca^{2+} sensitivity of contractile proteins *per se* is present somewhere between receptors and contractile proteins in the signal transduction pathway for VSMC contraction (*Figure 3*).[26] Furthermore, in the second porcine model with chronic IL-1β treatment for 4 weeks, coronary hypercontraction is mediated by the 5-hydroxytryptamine (5-HT)2A serotonergic receptor, whereas the expression or functions of the 5-HT2A receptor are not significantly altered when compared with normal coronary artery segments.[29] These results suggested that the key mechanism for coronary artery spasm is present somewhere below the receptors and above the contractile proteins in the signal transduction pathway for VSMC contraction. Furthermore, direct activation of protein kinase C (PKC) causes coronary artery spasm and inhibition of PKC suppressed the spasm in both porcine models.[30,31] The inhibitory effects of PKC inhibitors were not evident in the coronary contractions in response to prostaglandin F2α.[30,31] These results indicated that the PKC-mediated pathway is substantially involved in the pathogenesis of coronary spasm (*Figure 3*). VSMC contraction is triggered by Ca^{2+}/calmodulin-activated

Figure 3: Vascular Smooth Muscle Contraction and Rho-kinase Pathway
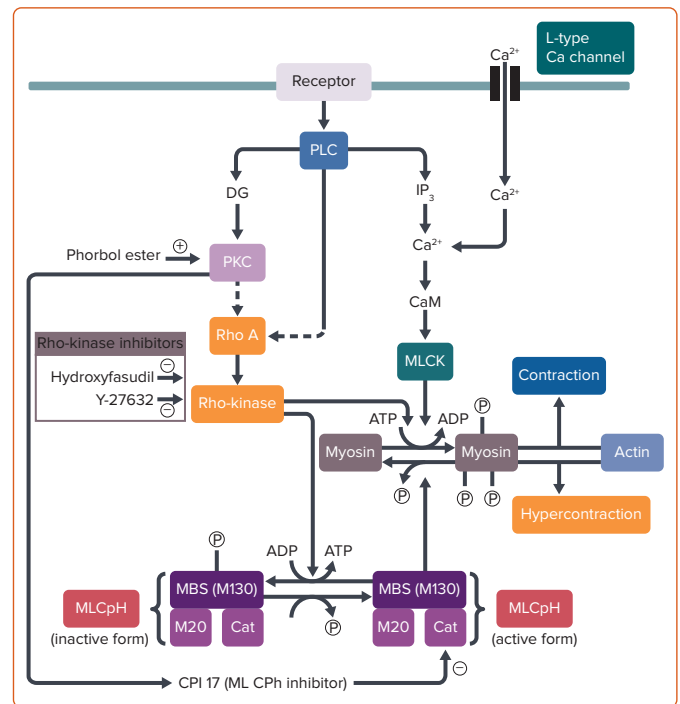
VSMC contraction is regulated by the balance between MLC phosphorylation by MLCK in a Ca^{2+}/CaM-dependent manner and MLC diphosphorylation by MLCPh. Rho-kinase causes potent and sustained MLC phosphorylation (di- and even triphosphorylations) through inhibition of MLCPh with resultant VSMC hypercontraction in a Ca^{2+}-independent manner. ADP = adenosine diphosphate; ATP = adenosine triphosphate; Ca = calcium; CaM = calmodulin; DG = diacylglycerol; IP_3 = inositol trisphosphate; MBS = myosin-binding subunit; MLCK = myosin light chain kinase; MLCPh = myosin light chain phosphatase; PKC = protein kinase C; PLC = phospholipase C; Source: Shimokawa et al. 1983.[11] Reproduced with permission from AAAS.

myosin light chain kinase (MLCK) with subsequent phosphorylation of the 20-kDa regulatory MLC (*Figure 3*).[32] Intriguingly, the porcine model with chronic IL-1β treatment revealed that MLC monophosphorylation was enhanced at the spastic coronary segment and that there was a positive correlation between the serotonin-induced coronary vasoconstrictions and MLC monophosphorylation and also diphosphorylation.[27] MLC diphosphorylation occurs only in the actively growing cells and the spastic coronary segment.[24,31] The increase in intracellular Ca^{2+} levels causes MLC

monophosphorylation alone, whereas inactivation of MLC phosphatase causes both mono- and diphosphorylation of MLC.[33,34] Thus, MLC phosphatase activity appeared to be essential for the induction of MLC diphosphorylation in VSMCs, which is known to induce potent and sustained VSMC contraction compared with MLC monophosphorylation.[32]

Rho, a small guanosine triphosphate, is involved in the GTP-enhanced Ca^{2+} sensitivity of VSMC contraction.[35] Rho regulates MLC phosphorylation through its target Rho-kinase and the myosin-binding sub-unit (MBS) of myosin phosphatase.[36] Activated Rho causes Rho-kinase activation and then activated Rho-kinase phosphorylates the MBS with resultant inactivation of myosin phosphatase. An active form of Rho-kinase enhances MLC phosphorylation and induces VSMC contraction (*Figure 3*).[35,37,38] We identified that fasudil, which is clinically used for the treatment of cerebral vasospasm after subarachnoid haemorrhage in Japan, is metabolised to hydroxyfasudil which specifically inhibits both isoforms of Rho-kinase – ROCK1 and ROCK2 – but not other kinases such as MLCK or PKC.[39–42] Indeed, hydroxyfasudil shows dose-dependent inhibition of serotonin-induced coronary spasm in the porcine model with chronic IL-1β treatment through suppression of MLC mono- and diphosphorylations.[43] These results indicate that the Rho/Rho-kinase pathway plays an important role in the enhanced MLC phosphorylations in the spastic coronary artery, suggesting that inhibition of the Rho-kinase pathway may be a novel therapeutic target of coronary artery spasm (*Figure 3*).

In our porcine model, real-time (RT)-polymerase chain reaction (PCR) analysis demonstrated that expressions of Rho-kinase and RhoA mRNA, as well as the extent of MBS phosphorylation, were significantly upregulated in the spastic segment compared with the control.[44] Similar to hydroxyfasudil, another Rho-kinase inhibitor of Y-27632 inhibited not only serotonin-induced hypercontractions *in vivo* and *in vitro* but also the increase in MBS phosphorylations.[45] Thus, we concluded that upregulated and activated Rho-kinase plays a key role in inducing VSMC hypercontraction by inhibiting MLC phosphatase through MBS phosphorylation in porcine models *in vivo* (*Figure 3*).[8–10,43,44]

Epicardial coronary spasm is the local phenomenon that is unique to a given site of the coronary artery, whereas endothelial dysfunction is rather systemic. Furthermore, vasodilating responses to endothelium-dependent vasodilators, such as bradykinin or substance P, are fairly preserved at the spastic coronary segment in patients with variant angina.[25] Indeed, endothelial dysfunction does not explain the high prevalence of coronary spasm from night to early morning, whereas the circadian variation of Rho-kinase activity (a molecular switch of VSMC contraction) has been well studied.[46] Furthermore, nitrates exert acute inhibitory effects on coronary spasm by VSMC relaxation but have no acute effect on endothelial dysfunction.[47] For these reasons, we consider that VSMC hypercontraction, rather than endothelial dysfunction, plays a primary role in the mechanism of coronary artery spasm.

We further reported that remnant lipoproteins from patients with sudden cardiac death enhance coronary vasospastic responses through upregulation of Rho-kinase in pigs *in vivo* and that sustained elevation of serum cortisol levels sensitises coronary vasoconstricting responses to restraint stress in pigs *in vivo*.[48,49] Inflammatory stimuli, such as angiotensin II and IL-1β, upregulate Rho-kinase expression and activity in human coronary vascular smooth muscle cells *in vitro*, for which oestrogen mediated by an oestrogen receptor and nicotine exert divergent inhibitory and stimulatory effects on Rho-kinase expression, respectively.[50,51] The

significant interaction between ageing – possibly due to the mechanisms of the menopause – and smoking for women with VSA was followed in a study by the Japanese Coronary Spasm Association (JCSA) using large-scale cohort data.[47,50,52–54] Clinical studies revealed that intracoronary fasudil exerts an inhibitory effect not only on epicardial coronary spasm but also microvascular spasm in patients *in vivo*.[55,56] Intracoronary fasudil is also effective in relieving intractable coronary spasm resistant to nitrates and Ca-channel blockers, and this effect is consistently observed for acetylcholine-induced coronary hyperconstricting responses immediately after PCI.[57–58] More recently, intracoronary fasudil significantly improved PCI-related MI expressed by corrected thrombolysis in MI (TIMI) frame count and TIMI flow grade.[59] The role of the Rho-kinase pathway is extensive in a wide variety of cardiovascular diseases, such as the development of coronary atherosclerosis and in-stent restenosis lesions after PCI, cardiac allograft vasculopathy, heart failure, essential hypertension, pulmonary arterial hypertension and aortic aneurysm.[60–70]

In their review, Maseri et al. mentioned that there is ethnic heterogeneity in the coronary artery vasomotor reactivity.[71] In this paper, Japanese patients with variant angina appeared to have diffuse and multivessel spasm as compared with white patients. Furthermore, Japanese patients with a recent MI had a higher incidence of spasm induced by provocative testing with acetylcholine, ergonovine, methylergonovine or serotonin, than white patients. This observation was later confirmed by the same group by conducting the same spasm provocative tests with acetylcholine in Japanese and Italian patients with recent MI.[72] However, contrary to these findings, several European groups have recently reported a relatively higher incidence of inducible coronary artery spasm in larger cohorts of white patients who underwent coronary angiography and were found to have unobstructed coronary arteries.[73,74] Likewise, the most recent study from an international and prospective cohort for microvascular angina by the COVADIS group has underlined that there was no difference in prognosis for different ethnic groups.[75] Therefore, ethnic differences in coronary vasomotion abnormalities may be less relevant than we considered previously.

## Rho-kinase Activity in Circulating Leukocytes as a Marker of Vasospastic Angina

In addition to the relevant clinical implication of selective Rho-kinase inhibitors,[39–42] Rho-kinase activity in circulating neutrophils holds promise as its potential to predict disease activity and long-term prognosis of VSA. Rho-kinase activity is determined by the extent of phosphorylation of MBS, a substrate of Rho-kinase and then Rho-kinase activity in circulating neutrophils is expressed as the ratio of phosphorylated MBS (p-MBS) to total MBS (t-MBS) (*Figure 4A*).[76] A p-MBS ratio of 1.18 was identified as the best cut-off level to predict the diagnosis of VSA (*Figure 4B*).[76] Findings were followed by lines of evidence that Rho-kinase activity in circulating neutrophils in VSA patients was temporally enhanced associated with disaster-related mental stress during the 2011 earthquake and tsunami in Asia, and that the Rho-kinase activity corresponds to distinct circadian variation of VSA, peaking in the early morning and is associated with altered coronary vasomotor responses and autonomic activity.[46,77] Maseri and his group suggested that parasympathetic withdrawal may be a part of the mechanism of spontaneous coronary artery spasm.[5] Rho-kinase activity of VSA patients in the early morning was significantly correlated with parasympathetic nervous activity from midnight to early morning.[46] Furthermore, patients with VSA with higher Rho-kinase activity (≥1.20) had a poor long-term prognosis (*Figure 4C*). In the study, a p-MBS ratio of 1.24 was identified as the best cut-off level to predict future cardiac events in VSA patients.[78] Of note, a combination of Rho-kinase activity with the

Figure 4: Rho-kinase Activity in Circulating Leukocytes as a Marker of Vasospastic Angina
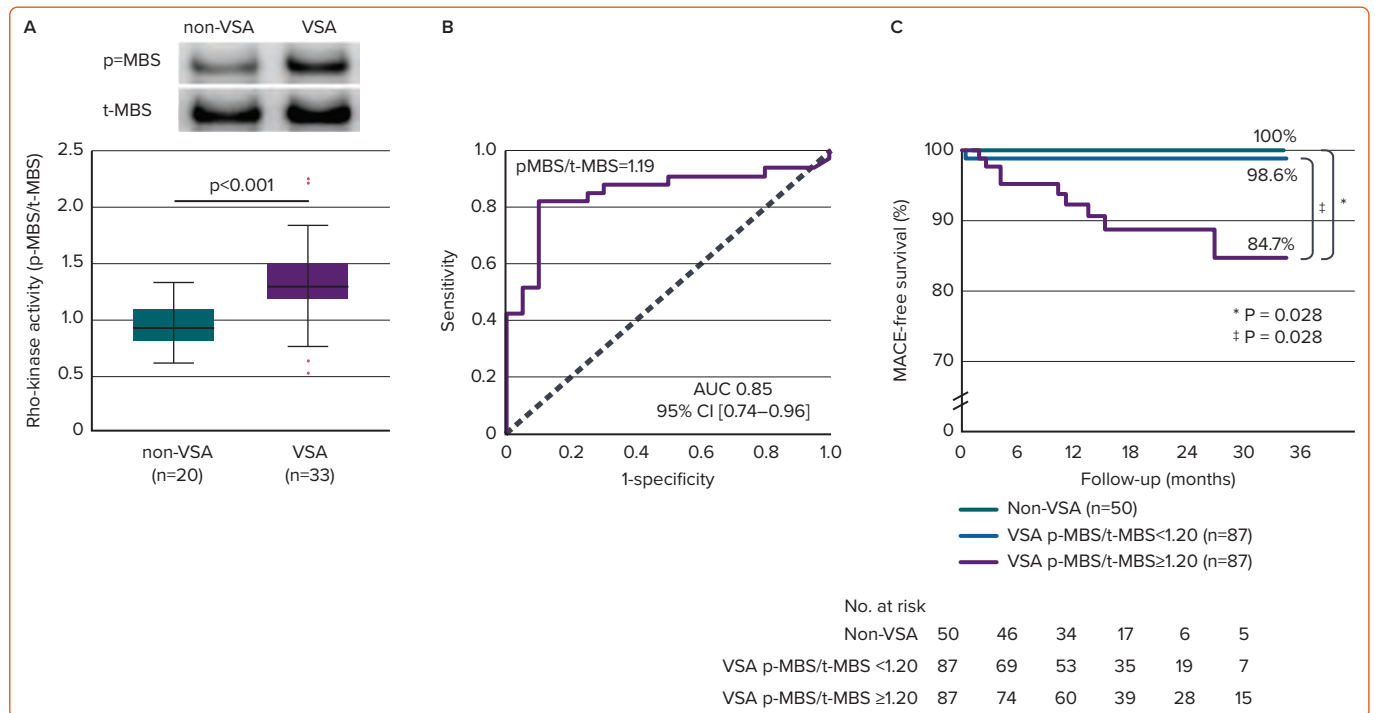
A: Rho-kinase activity in circulating leukocytes is determined by the extent of phosphorylation of MBS (a substrate of Rho-kinase) and is expressed as the ratio of phosphorylated MBS (p-MBS) to total MBS (t-MBS). B: ROC curve analysis confirmed that a p-MBS ratio of 1.18 was the best cut-off level for the diagnosis of VSA. C: The Kaplan-Meier curve analysis showed that the prevalence of cardiac events (cardiac death, non-fatal MI and hospitalisation for unstable angina) was higher in VSA patients with high Rho-kinase activity than those with low Rho-kinase activity or non-VSA patients. AUC = area under the curve; MACE = major adverse cardiovascular events; MBS = myosin-binding subunit; ROC = Receiver-operating characteristic; VSA = vasospastic angina. Source: A and B: Kikuchi et al. 2011.[77] Reproduced with permission from Elsevier; Source: C: Nihei et al. 2018.[78] Reproduced with permission from Oxford University Press.

Figure 5: Involvement of Coronary Adventitial Inflammation in the Pathogenesis of Coronary Artery Spasm
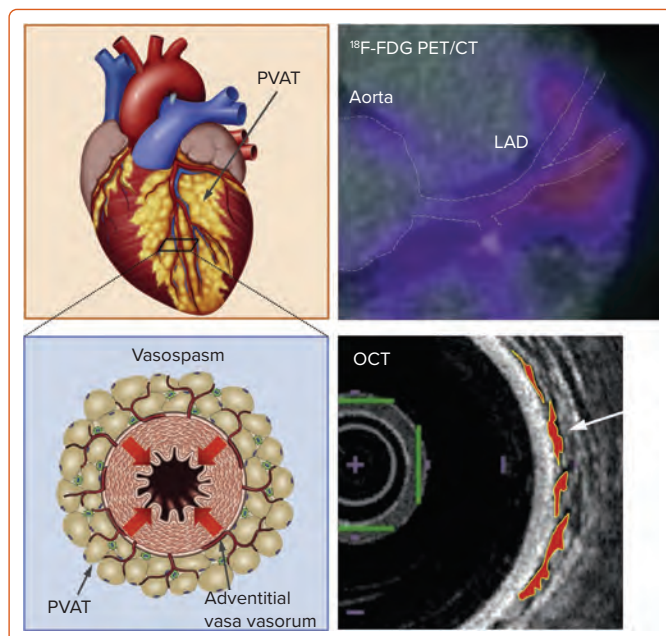
A: A schema illustrates locations of PVAT. B: Representative ^{18}F-FDG PET image of a coronary segment in a VSA patient indicates that PVAT inflammation is markedly increased at the spastic segments of the LAD coronary artery of the VSA patient. C: A schema illustrates the cross-sectional image of the spastic coronary segment surrounded by PVAT and adventitial vasa vasorum. Red arrows denote 'outside-in' inflammatory changes. D: OCT-delineated adventitial vasa vasorum formation (yellow arrows) is significantly enhanced at the spastic segment of the VSA patient. [18]FDG-PET = 18F-fluorodeoxyglucose positron emission tomography; LAD = left anterior descending coronary artery; OCT = optical coherence tomography; PVAT = perivascular adipose tissue; VSA = vasospastic angina. Source: Ohyama et al. 2018.[100] Adapted with permission from Elsevier.

JCSA risk score saw a pronounced improvement of the prognostic value with either alone.[47,78]

Coronary microvascular dysfunction (CMD) has been emerging as an exacerbating factor of long-term prognosis of cardiovascular patients.[79,80] The first international study from the COVADIS group has demonstrated that major cardiovascular adverse events (MACE), including cardiovascular death, non-fatal MI, non-fatal stroke and hospitalisation due to heart failure or unstable angina, occurred irrespective of sex or ethnicity.[75,81] In the context of a novel biomarker for VSA and comorbid CMD, we have demonstrated that CMD determined by the increased index of microcirculatory resistance (IMR) >18 heightened the MACE rate of VSA patients.[82] In such patients, intracoronary administration of fasudil significantly ameliorated IMR, which suggests a possible role of the Rho-kinase pathway in CMD.[82] Another landmark study has reported that the measured plasma concentration of serotonin from the coronary artery and coronary sinus was highest in VSA patients with CMD, while there was no difference in the VSA without CMD versus non-VSA groups.[83] Fractional flow reserve (FFR), as known as a marker for evaluating the degree of flow-limiting coronary stenotic region, successfully extracted the high-risk population among VSA patients, particularly with significant organic stenosis.[84] Current guidelines and expert consensus recommends spasm provocation tests with additional interventional approaches even when no organic stenosis is found angiographically.[85,86] The approaches that we will summarise are a promising avenue to fully elucidate the pathogenesis of coronary artery spasm in patients with VSA.

Roles of Adventitial Inflammatory Changes in Enhanced Coronary Vasoconstricting Responses

Ample evidence has been accumulated to elucidate the detailed mechanisms of coronary artery spasm *in vivo*. Using the porcine models

Figure 6: Coronary Artery Morphologies and Prognosis for Ischaemia with No Obstructive Coronary Artery Disease
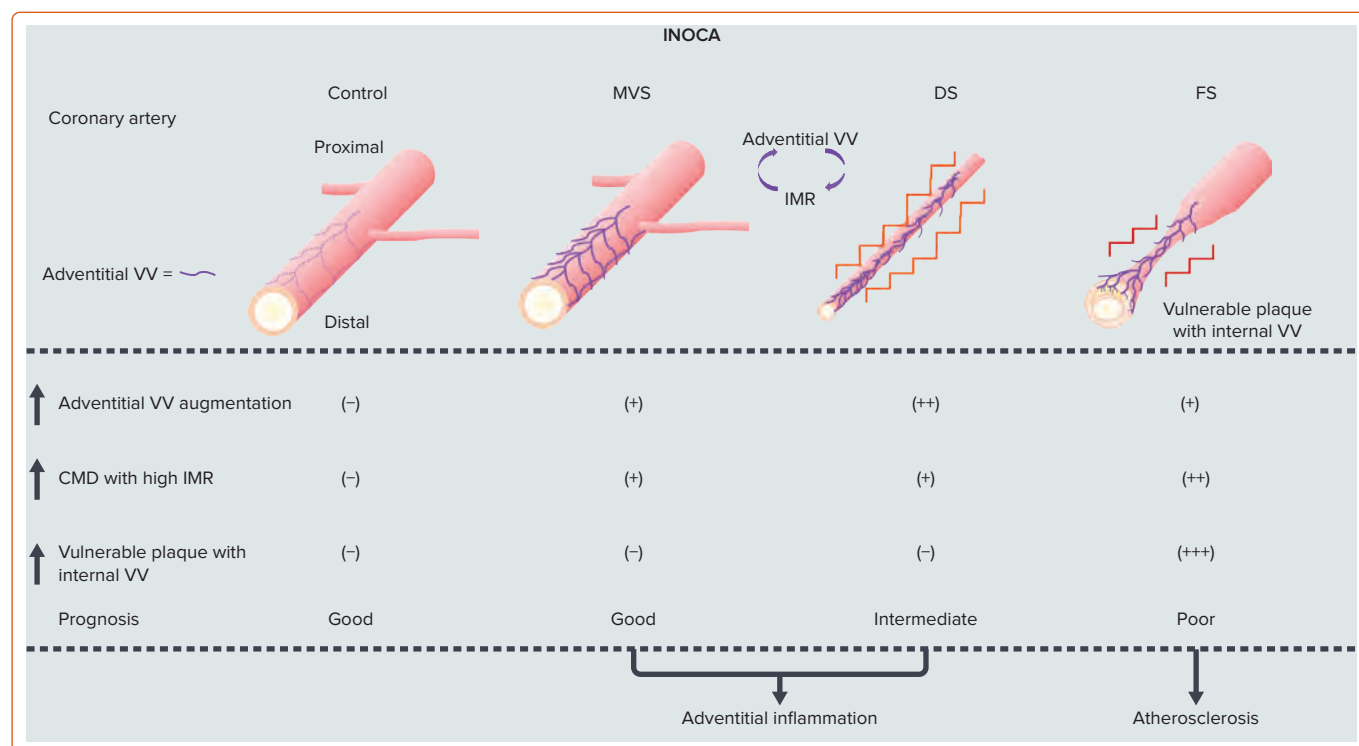
The present study examined the heterogeneity in coronary morphological changes and prognosis for INOCA. OCT-derived adventitial VV formation was profoundly augmented in MVS, DS and FS, but none in the controls. Patients with high IMR, a marker for CMD, were distributed to MVS, DS and FS with a gradual increase, while none in the controls. In particular, adventitial VV augmentation was associated with the degree of IMR in MVS and DS. The majority of coronary plaques delineated by OCT were categorised as mild lesions with intimal thickening in the controls, MVS and DS, whereas lipid-rich fibroatheroma with vulnerable features of internal VV, cholesterol crystals or macrophage infiltration was prominent in FS. DS showed an intermediate prognosis which was independently contributed by adventitial VV. Meanwhile, FS showed the worst prognosis in INOCA, which was strongly correlated with OCT-delineated intra-plaque neovessels. CMD = coronary microvascular dysfunction; DS = diffuse spasm; FS = focal spasm; IMR = index of microcirculatory resistance; INOCA = ischaemia with no obstructive coronary artery disease; MVS = microvascular spasm; OCT = optical coherence tomography; VV = vasa vasorum. Source: Nishimiya et al. 2021.[101] Adapted with permission from Elsevier.

with inducible coronary artery spasm, we found the role of coronary adventitial inflammatory changes – known as the 'outside-in' pathway – in the pathogenesis of coronary artery spasm and extensively in coronary atherosclerosis.[21,44,60] In brief, coronary adventitia harbours a wide range of cellular and subcellular components, such as nutrient blood vessels (termed vasa vasorum), perivascular adipose tissue (PVAT), autonomic nervous system, and lymphatic vessels that drain adventitial inflammatory cells.[87–94] We have demonstrated that these adventitial residents have interactions with each other, all of which are involved in coronary hyperconstricting responses after drug-eluting stent (DES) implantation in pigs in vivo (Figure 5).[87–93]

The first observation was that inflammatory changes were potent in the histology of coronary adventitia isolated from the porcine coronary segment with first-generation DES implantation compared with bare-metal stent implantation.[95,96] Adventitial inflammation was significantly reduced when implanting newer-generation biocompatible DES in pigs.[87,97] Experimental micro-CT revealed that adventitial vasa vasorum formation was augmented around the coronary segment implanted with first-generation DES (Figure 5) as compared with the segment with biocompatible DES.[87] The extent of vasa vasorum formation was proportional to that of M1-type macrophage and mast cell infiltrations, indicating the important role of vasa vasorum as an inflammatory conduit.[87] Furthermore, these adventitial inflammatory changes were associated with DES-induced coronary hyperconstricting responses in vivo.[87,97] PVAT surrounds the coronary arterial wall and is thought to be an origin of various inflammatory cytokines, such as adipokines.[91] We then

demonstrated that ^{18}F-fluorodeoxyglucose positron emission tomography ([18]FDG-PET) is useful for evaluating the magnitude of coronary PVAT inflammation in pigs after DES implantation ex vivo and in vivo (Figure 5).[90] In the novel approach with [18]FDG-PET, the extent of PVAT inflammation is expressed as a target-to-background ratio, the standardised uptake value (SUV) corrected for blood activity by dividing the average blood SUV estimated from the ascending aorta.[90] Importantly, [18]FDG-PET-derived PVAT inflammation was strongly associated with DES-induced coronary hyperconstricting responses in vivo.[90] We further confirmed that the autonomic nervous system existing in the coronary adventitia was associated with exacerbated inflammation and enhanced vasoconstriction via the kidney-brain-heart axis in the same porcine model.[92] More recent studies underline that impairment of cardiac lymphatic drainage function after DES implantation contributes to sustained adventitial inflammation, medial VSMC hypercontraction and resultant coronary hyperconstriction through activation of Rho-kinase in pigs in vivo.[93,94]

We further examined the role of coronary adventitial inflammation in the clinical settings by using a multimodality imaging approach with optical coherence tomography (OCT) and [18]FDG-PET.[98–101] OCT is capable of visualising coronary adventitial vasa vasorum in pigs and humans and OCT-delineated adventitial vasa vasorum (VV) formation was significantly enhanced at the spastic segments of VSA patients as compared with those of the control subjects (Figure 5).[98–101] Maseri et al. suggested that focal coronary artery spasm is a distinct condition characterised by focal coronary constriction sufficient to cause transient total or sub-total coronary occlusion.[6] Indeed, when grouping 335 patients with ischaemia

and no obstructive coronary artery (INOCA) into the group of non-cardiac controls, microvascular spasm, diffuse epicardial spasm and focal epicardial spasm, the group of focal epicardial spasm had the worst prognosis followed by the group with diffuse epicardial spasm (*Figure 6*).[101] The extent of adventitial vasa vasorum formation visualised by OCT was increased in patients with microvascular spasm as compared with controls, and peaked in those with diffuse spasm. The augmentation of adventitial vasa vasorum was positively correlated with the level of IMR, indicating the possible link between coronary adventitial inflammation and microcirculatory resistance.[101] In particular, the vasa vasorum that links to intramural layers, termed as intra-plaque neovessels, showed a relevant prognostic value among patients with focal coronary artery spasm.[101] Also, [18]FDG-PET-derived coronary PVAT inflammatory changes were more extensive at the spastic coronary segments of VSA patients compared with those of control subjects (*Figure 5*).[100] The lines of basic and clinical studies strongly support the notion that coronary adventitial inflammation could be a novel therapeutic target for coronary artery spasm.

When treating coronary adventitial inflammation, it is ideal to directly administer anti-angiogenic and/or anti-inflammatory agents to patients. However, systemic use of these drugs may often cause serious side effects.[102,103] The anti-angiogenic strategy for vasa vasorum may cause intramural haemorrhage as a consequence of increased vascular permeability.[104] We then introduced that catheter-based renal sympathetic denervation can effectively suppress DES-induced coronary hyperconstricting responses via the kidney-brain-heart axis in pigs *in*

vivo.[92] However, this approach requires multiple invasive steps. Thus, we focused on the low-intensity pulsed ultrasound (LIPUS) therapy as a novel non-invasive therapeutic option for coronary adventitial inflammation and coronary artery spasm.[105–112] Since the intensity of ultrasound used in the LIPUS therapy is below the upper limit of acoustic output standards for diagnostic devices, it causes no compression, heat, or discomfort.[105–112] Our recent study has demonstrated that the LIPUS therapy suppresses DES-induced coronary hyperconstricting responses in pigs *in vivo* by suppressing Rho-kinase activity.[94] The detailed molecular mechanisms of the LIPUS are that it enhances cardiac lymphatic vessel formation associated with endothelial nitric oxide synthase upregulation with no effects on the vasa vasorum, and that the enhancement of lymphatic vessel formation improves inflammatory drainage function, helping abrogate adventitial inflammation and Rho-kinase activity.[94] In addition to coronary artery spasm, PVAT inflammation has been demonstrated in human coronary atherosclerotic lesions *in vivo*.[113] Taken together, the LIPUS therapy could be a novel and safe therapeutic approach for a broad spectrum of cardiovascular disease in clinical practice.[114]

Conclusion

Recent clinical trials have highlighted that PCI in patients with stable angina provides limited additional prognostic benefits on top of optimal medical therapy.[1,2] In the era after ISCHEMIA and REVIVED-BCIS2, coronary vasomotion abnormalities, regardless of obstructive or non-obstructive arterial segments, will increase in clinical significance year by year. Unveiling the mechanism of coronary artery spasm, particularly in the clinical setting, will be a next step to mitigate this important disorder. ❑

1. Maron DJ, Hochman JS, Reynolds HR, et al. Initial invasive or conservative strategy for stable coronary disease. *N Engl J Med* 2020;382:1395–407. https://doi.org/10.1056/NEJMoa1915922; PMID: 32227755.
2. Perera D, Clayton T, O'Kane PD, et al. Percutaneous revascularization for ischemic left ventricular dysfunction. *N Engl J Med* 2022;387:1351–60. https://doi.org/10.1056/NEJMoa2206606; PMID: 36027563.
3. Canty JM Jr. Coronary blood flow and myocardial ischemia. In: Bonow RO, Mann DL, Zipes DP, et al., eds. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine*. Philadelphia, PA: Saunders, 2012;1049–75.
4. Lanza GA, Pedrotti P, Pasceri V, et al. Autonomic changes associated with spontaneous coronary spasm in patients with variant angina. *J Am Coll Cardiol* 1996;28:1249–56. https://doi.org/10.1016/S0735-1097(96)00309-9; PMID: 8890823.
5. Li J, Zhang H, Zhang C. Role of inflammation in the regulation of coronary blood flow in ischemia and reperfusion: mechanisms and therapeutic implications. *J Mol Cell Cardiol* 2012;52:865–72. https://doi.org/10.1016/j.yjmcc.2011.08.027; PMID: 21924274.
6. Maseri A, Newman C, Davies G. Coronary vasomotor tone: a heterogeneous entity. *Eur Heart J* 1989;10(Suppl F):2–5. https://doi.org/10.1093/eurheartj/10.suppl_f.2; PMID: 2695334.
7. Maseri A. Abnormal coronary vasomotion in ischemic heart disease. *J Cardiovasc Pharmacol* 1992;20(Suppl 7):S30–1. https://doi.org/10.1097/00005344-199200207-00007; PMID: 1284154.
8. Shimokawa H, Sunamura S, Satoh K. RhoA/Rho-kinase in the cardiovascular system. *Circ Res* 2016;118:352–66. https://doi.org/10.1161/CIRCRESAHA.115.306532; PMID: 26838319.
9. Shimokawa H. 2014 Williams Harvey Lecture: importance of coronary vasomotion abnormalities-from bench to bedside. *Eur Heart J* 2014;35:3180–93. https://doi.org/10.1093/eurheartj/ehu427; PMID: 25354517.
10. Shimokawa H, Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 2005;25:1767–75. https://doi.org/10.1161/01.ATV.0000176193.83629.c8; PMID: 16002741.
11. Shimokawa H, Tomoike H, Nabeyama S, et al. Coronary artery spasm induced in atherosclerotic miniature swine. *Science* 1983;221:560–2. https://doi.org/10.1126/science.6408736; PMID: 6408736.
12. Shimokawa H, Tomoike H, Nabeyama S, et al. Coronary artery spasm induced in miniature swine: angiographic

evidence and relation to coronary atherosclerosis. *Am Heart J* 1985;110:300–10. https://doi.org/10.1016/0002-8703(85)90148-6; PMID: 4025107.
13. McFadden EP, Bauters C, Lablanche JM, et al. Response of human coronary arteries to serotonin after injury by coronary angioplasty. *Circulation* 1993;88:2076–85. https://doi.org/10.1161/01.cir.88.5.2076; PMID: 8222101.
14. Ozaki Y, Keane D, Serruys PW. Progression and regression of coronary stenosis in the long-term follow-up of vasospastic angina. *Circulation* 1995;92:2446–56. https://doi.org/10.1161/01.cir.92.9.2446; PMID: 7586344.
15. Kaski JC, Crea F, Meran D, et al. Local coronary supersensitivity to diverse vasoconstrictive stimuli in patients with variant angina. *Circulation* 1986;74:1255–65. https://doi.org/10.1161/01.cir.74.6.1255; PMID: 3779913.
16. Kaski JC, Maseri A, Vejar M, et al. Spontaneous coronary artery spasm in variant angina is caused by a local hyperreactivity to a generalized constrictor stimulus. *J Am Coll Cardiol* 1989;14:1456–63. https://doi.org/10.1016/0735-1097(89)90382-3; PMID: 2809004.
17. Maseri A, Davies G, Hackett D, Kaski JC. Coronary artery spasm and vasoconstriction. The case for a distinction. *Circulation* 1990;81:1983–91. https://doi.org/10.1161/01.cir.81.6.1983; PMID: 2188757.
18. Maseri A, Beltrame JF, Shimokawa H. Role of coronary vasoconstriction in ischemic heart disease and search for novel therapeutic targets. *Circ J* 2009;73:394–403. https://doi.org/10.1253/circj.cj-09-0033; PMID: 19202303.
19. Forman MB, Oates JA, Robertson D, et al. Increased adventitial mast cells in a patient with coronary spasm. *N Engl J Med* 1985;313:1138–41. https://doi.org/10.1056/NEJM198510313131807; PMID: 2413358.
20. Jougasaki M, Yasue H, Takahashi K. Perivascular nerve lesion of the coronary artery involved in spasm in a patient with variant angina. *Pathology* 1989;21:304–7. https://doi.org/10.3109/00313028909061079; PMID: 2633119.
21. Shimokawa H, Ito A, Fukumoto Y, et al. Chronic treatment with interleukin-1β induces coronary intimal lesions and vasospastic responses in pigs *in vivo*. The role of platelet-derived growth factor. *J Clin Invest* 1996;97:769–76. https://doi.org/10.1172/JCI118476; PMID: 8609234.
22. Ito A, Shimokawa H, Kadokami T, et al. Tyrosine kinase inhibitor suppresses coronary arteriosclerotic changes and vasospastic responses induced by chronic treatment with interleukin-1β in pigs in vivo. *J Clin Invest* 1995;96:1288–94. https://doi.org/10.1172/JCI118163; PMID: 7657803.
23. Ito A, Shimokawa H, Fukumoto Y, et al. The role of fibroblast

growth factor-2 in the vascular effects of interleukin-1β in porcine coronary arteries *in vivo*. *Cardiovasc Res* 1996;32:570–9. https://doi.org/10.1016/S0008-6363(96)00120-4; PMID: 8881517.
24. Fukumoto Y, Shimokawa H, Ito A, et al. Inflammatory cytokines cause coronary arteriosclerosis-like changes and alterations in the smooth muscle phenotypes in pigs. *J Cardiovasc Pharmacol* 1997;29:222–31. https://doi.org/10.1097/00005344-199702000-00011; PMID: 9057072.
25. Miyata K, Shimokawa H, Yamawaki T, et al. Endothelial vasodilator function is preserved at the spastic/inflammatory coronary lesions in pigs. *Circulation* 1999;100:1432–7. https://doi.org/10.1161/01.cir.100.13.1432; PMID: 10500045.
26. Satoh S, Tomoike H, Mitsuoka W, et al. Smooth muscles from spastic coronary artery segment show hyperreactivity to histamine. *Am J Physiol* 1990;259:H9–13. https://doi.org/10.1152/ajpheart.1990.259.1.H9; PMID: 2375416.
27. Katsumata N, Shimokawa H, Seto M, et al. Enhanced myosin light chain phosphorylations as a central mechanism for coronary artery spasm in a swine model with interleukin-1β. *Circulation* 1997;96:4357–63. https://doi.org/10.1161/01.cir.96.12.4357; PMID: 9416904.
28. Yokoyama M, Akita H, Hirata K, et al. Supersensitivity of isolated coronary artery to ergonovine in a patient with variant angina. *Am J Med* 1990;89:507–15. https://doi.org/10.1016/0002-9343(90)90383-o; PMID: 2220883.
29. Miyata K, Shimokawa H, Higo T, et al. Sarpogralate, a selective 5-HT2A serotonergic receptor antagonist, inhibits serotonin-induced coronary artery spasm in a porcine model. *J Cardiovasc Pharmacol* 2000;35:294–301. https://doi.org/10.1097/00005344-200002000-00018; PMID: 10672864.
30. Ito A, Shimokawa H, Nakaike R, et al. Role of protein kinase C-mediated pathway in the pathogenesis of coronary artery spasm in a swine model. *Circulation* 1994;90:2425–31. https://doi.org/10.1161/01.cir.90.5.2425; PMID: 7525109.
31. Kadokami T, Shimokawa H, Fukumoto Y, et al. Coronary artery spasm does not depend on the intracellular calcium store but is substantially mediated by the protein kinase C-mediated pathway in a swine model with interleukin-1β. *Circulation* 1996;94:190–6. https://doi.org/10.1161/01.cir.94.2.190; PMID: 8674178.
32. Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 1994;372:231–6. https://doi.org/10.1038/372231a0; PMID: 7969467.
33. Seto M, Sakurada K, Kamm KE, et al. Myosin light chain diphosphorylation is enhanced by growth promotion of cultured smooth muscle cells. *Pflugers Arch* 1996;432:7–13.

https://doi.org/10.1007/s004240050099; PMID: 8662262.

34. Noda M, Yasuda-Fukazawa C, Moriishi K, et al. Involvement of Rho in GTP-γS-induced enhancement of phosphorylation of 20-kDa myosin light chain vascular smooth muscle cells: inhibition of phosphatase activity. FEBS Lett 1995;367:246–50. https://doi.org/10.1016/0014-5793(95)00573-r; PMID: 7607316.

35. Hirata K, Kikuchi A, Sasaki T, et al. Involvement of Rho p21 in the GTP-enhanced calcium ion sensitivity of smooth muscle contraction. J Biol Chem 1992;267:8719–22. https://doi.org/10.1016/S0021-9258(19)50337-4; PMID: 1577714.

36. Kimura K, Ito M, Amano M, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 1996;273:245–8. https://doi.org/10.1126/science.273.5272.245; PMID: 8662509.

37. Chihara K, Amano M, Nakamura N, et al. Cytoskeletal rearrangements and transcriptional activation of c-fos serum response element by Rho-kinase. J Biol Chem 1997;272:25121–7. https://doi.org/10.1074/jbc.272.40.25121; PMID: 9312122.

38. Kureishi Y, Kobayashi S, Amano M, et al. Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. J Biol Chem 1997;272:12257–60. https://doi.org/10.1074/jbc.272.19.12257; PMID: 9139666.

39. Shimokawa H. Rho-kinase as a novel therapeutic target in treatment of cardiovascular diseases. J Cardiovasc Pharmacol 2002;39:319–27. https://doi.org/10.1097/00005344-200203000-00001; PMID: 11862109.

40. Higashi M, Shimokawa H, Hattori T, et al. Long-term inhibition of Rho-kinase suppresses angiotensin II-induced cardiovascular hypertrophy in rats in vivo: effect on endothelial NAD(P)H oxidase system. Circ Res 2003;93:767–75. https://doi.org/10.1161/01.RES.0000096650.91688.28; PMID: 14500337.

41. Inokuchi K, Ito A, Fukumoto Y, et al. Usefulness of fasudil, a Rho-kinase inhibitor, to treat intractable severe coronary spasm after coronary artery bypass surgery. J Cardiovasc Pharmacol 2004;44:275–7. https://doi.org/10.1097/01.fjc.0000134775.76636.3f; PMID: 15475822.

42. Shimokawa H, Satoh K. 2015 ATVB Plenary Lecture: translational research on Rho-kinase in cardiovascular medicine. Arterioscler Thromb Vasc Biol 2015;35:1756–69. https://doi.org/10.1161/ATVBAHA.115.305353; PMID: 26069233.

43. Shimokawa H, Seto M, Katsumata N, et al. Rho-kinase-mediated pathway induces enhanced myosin light chain phosphorylations in a swine model of coronary artery spasm. Cardiovasc Res 1999;43:1029–39. https://doi.org/10.1016/s0008-6363(99)00144-3; PMID: 10615430.

44. Kandabashi T, Shimokawa H, Miyata K, et al. Inhibition of myosin phosphatase by upregulated Rho-kinase plays a key role for coronary artery spasm in a porcine model with interleukin-1β. Circulation 2000;101:1319–23. https://doi.org/10.1161/01.cir.101.11.1319; PMID: 10725293.

45. Uehata M, Ishizaki T, Satoh H, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. Nature 1997;389:990–4. https://doi.org/10.1038/40187; PMID: 9353125.

46. Nihei T, Takahashi J, Tsuburaya R, et al. Circadian variation of Rho-kinase activity in circulating leukocytes of patients with vasospastic angina. Circ J 2014;78:1183–90. https://doi.org/10.1253/circj.cj-13-1458; PMID: 24670923.

47. Takahashi J, Nihei T, Takagi Y, et al. Prognostic impact of chronic nitrate therapy in patients with vasospastic angina: multicentre registry study of the Japanese coronary spasm association. Eur Heart J 2015;36:228–37. https://doi.org/10.1093/eurheartj/ehu313; PMID: 25189599.

48. Oi K, Shimokawa H, Hiroki J, et al. Remnant lipoproteins from patients with sudden cardiac death enhance coronary vasospastic activity through upregulation of Rho-kinase. Arterioscler Thromb Vasc Biol 2004;24:918–22. https://doi.org/10.1161/01.ATV.0000126678.93747.80; PMID: 15044207.

49. Hizume T, Morikawa K, Takaki A, et al. Sustained elevation of serum cortisol level causes sensitization of coronary vasoconstricting responses in pigs in vivo: a possible link between stress and coronary vasospasm. Circ Res 2006;99:767–75. https://doi.org/10.1161/01.RES.0000244093.69985.2f; PMID: 16960099.

50. Hiroki J, Shimokawa H, Higashi M, et al. Inflammatory stimuli upregulate Rho-kinase in human coronary vascular smooth muscle cells. J Mol Cell Cardiol 2004;37:537–46. https://doi.org/10.1016/j.yjmcc.2004.05.008; PMID: 15276023.

51. Hiroki J, Shimokawa H, Mukai Y, et al. Divergent effects of estrogen and nicotine on Rho-kinase expression in human coronary vascular smooth muscle cells. Biochem Biophys Res Commun 2005;326:154–9. https://doi.org/10.1016/j.bbrc.2004.11.011; PMID: 15567165.

52. Kawana A, Takahashi J, Takagi Y, et al. Gender differences

in the clinical characteristics and outcomes of patients with vasospastic angina – a report from the Japanese Coronary Spasm Association. Circ J 2013;77:1267–74. https://doi.org/10.1253/circj.cj-12-1486; PMID: 23363662.

53. Takagi Y, Yasuda S, Takahashi J, et al. Clinical implications of provocation tests for coronary artery spasm: safety, arrhythmic complications, and prognostic impact: multicentre registry study of the Japanese Coronary Spasm Association. Eur Heart J 2013;34:258–67. https://doi.org/10.1093/eurheartj/ehs199; PMID: 22782943.

54. Takagi Y, Takahashi J, Yasuda S, et al. Prognostic stratification of patients with vasospastic angina: a comprehensive clinical risk score developed by the Japanese Coronary Spasm Association. J Am Coll Cardiol 2013;62:1144–53. https://doi.org/10.1016/j.jacc.2013.07.018; PMID: 23916938.

55. Masumoto A, Mohri M, Shimokawa H, et al. Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. Circulation 2002;105:1545–7. https://doi.org/10.1161/hc1002.105938; PMID: 11927519.

56. Mohri M, Shimokawa H, Hirakawa Y, et al. Rho-kinase inhibition with intracoronary fasudil prevents myocardial ischemia in patients with coronary microvascular spasm. J Am Coll Cardiol 2003;41:15–9. https://doi.org/10.1016/s0735-1097(02)02632-3; PMID: 12570938.

57. Aizawa K, Yasuda S, Takahashi J, et al. Involvement of Rho-kinase activation in the pathogenesis of coronary hyperconstricting responses induced by drug-eluting stents in patients with coronary artery disease. Circ J 2012;76:2552–60. https://doi.org/10.1253/circj.cj-12-0662; PMID: 22813839.

58. Tsuburaya R, Takahashi J, Nakamura A, et al. Beneficial effects of long-acting nifedipine on coronary vasomotion abnormalities after drug-eluting stent implantation: the NOVEL study. Eur Heart J 2016;37:2713–21. https://doi.org/10.1093/eurheartj/ehw256; PMID: 27354043.

59. Kikuchi Y, Takahashi J, Hao K, et al. Usefulness of intracoronary administration of fasudil, a selective Rho-kinase inhibitor, for PCI-related refractory myocardial ischemia. Int J Cardiol 2019;297:8–13. https://doi.org/10.1016/j.ijcard.2019.09.057; PMID: 31611086.

60. Miyata K, Shimokawa H, Kandabashi T, et al. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. Arterioscler Thromb Vasc Biol 2000;20:2351–8. https://doi.org/10.1161/01.atv.20.11.2351; PMID: 11073837.

61. Matsumoto Y, Uwatoku T, Oi K, et al. Long-term inhibition of Rho-kinase suppresses neointimal formation after stent implantation in porcine coronary arteries: involvement of multiple mechanisms. Arterioscler Thromb Vasc Biol 2004;24:181–6. https://doi.org/10.1161/01.ATV.0000105053.46994.5B; PMID: 14592842.

62. Hattori T, Shimokawa H, Higashi M, et al. Long-term treatment with a specific Rho-kinase inhibitor suppresses cardiac allograft vasculopathy in mice. Circ Res 2004;94:46–52. https://doi.org/10.1161/01.RES.0000107196.21335.2B; PMID: 14615290.

63. Do e Z, Fukumoto Y, Sugimura K, et al. Rho-kinase activation in patients with heart failure. Circ J 2013;77:2542–50. https://doi.org/10.1253/circj.cj-13-0397; PMID: 23883874.

64. Ikeda S, Satoh K, Kikuchi N, et al. Crucial role of Rho-kinase in pressure overload-induced right ventricular hypertrophy and dysfunction in mice. Arterioscler Thromb Vasc Biol 2014;34:1260–71. https://doi.org/10.1161/ATVBAHA.114.303320; PMID: 24675663.

65. Mukai Y, Shimokawa H, Matoba T, et al. Involvement of Rho-kinase in hypertensive vascular disease: a novel therapeutic target in hypertension. FASEB J 2001;15:1062–4. https://doi.org/10.1096/fj.00-0735fje; PMID: 11292668.

66. Ito K, Hirooka Y, Kishi T, et al. Rho/Rho-kinase pathway in the brainstem contributes to hypertension caused by chronic nitric oxide synthase inhibition. Hypertension 2004;43:156–62. https://doi.org/10.1161/01.HYP.0000114602.82140.a4; PMID: 14732730.

67. Abe K, Shimokawa H, Morikawa K, et al. Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. Circ Res 2004;94:385–93. https://doi.org/10.1161/01.RES.0000111804.34509.94; PMID: 14670839.

68. Shimizu T, Fukumoto Y, Tanaka S, et al. Crucial role of ROCK2 in vascular smooth muscle cells for hypoxia-induced pulmonary hypertension in mice. Arterioscler Thromb Vasc Biol 2013;33:2780–91. https://doi.org/10.1161/ATVBAHA.113.301357; PMID: 24135024.

69. Tanaka S, Fukumoto Y, Nochioka K, et al. Statins exert the pleiotropic effects through small GTP-binding protein dissociation stimulator upregulation with a resultant Rac1 degradation. Arterioscler Thromb Vasc Biol 2013;33:1591–600. https://doi.org/10.1161/ATVBAHA.112.300922; PMID: 23640485.

70. Nogi M, Satoh K, Sunamura S, et al. Small GTP-binding protein GDP dissociation stimulator prevents thoracic aortic aneurysm formation and rupture by phenotypic preservation of aortic smooth muscle cells. Circulation 2018;138:2413–33. https://doi.org/10.1161/CIRCULATIONAHA.118.035648; PMID: 29921611.

71. Beltrame JF, Sasayama S, Maseri A. Racial heterogeneity in coronary artery vasomotor reactivity: differences between Japanese and Caucasian patients. J Am Coll Cardiol 1999;33:1442–52. https://doi.org/10.1016/s0735-1097(99)00073-x; PMID: 10334407.

72. Pristipino C, Beltrame JF, Finocchiaro ML, et al. Major racial differences in coronary constrictor response between Japanese and Caucasians with recent myocardial infarction. Circulation 2000;101:1102–8. https://doi.org/10.1161/01.cir.101.10.1102; PMID: 10715255.

73. Ong P, Athanasiadis A, Borgulya G, et al. Clinical usefulness, angiographic characteristics, and safety evaluation of intracoronary acetylcholine provocation testing among 921 consecutive white patients with unobstructed coronary arteries. Circulation 2014;129:1723–30. https://doi.org/10.1161/CIRCULATIONAHA.113.004096; PMID: 24573349.

74. Ford TJ, Yii E, Sidik N, et al. Ischemia and no obstructive coronary artery disease: prevalence and correlates of coronary vasomotion disorders. Circ Cardiovasc Interv 2019;12:e008126. https://doi.org/10.1161/CIRCINTERVENTIONS.119.008126; PMID: 31833416.

75. Shimokawa H, Suda A, Takahashi J, et al. Clinical characteristics and prognosis of patients with microvascular angina: an international and prospective cohort study by the Coronary Vasomotor Disorders International Study (COVADIS) Group. Eur Heart J 2021;42:4592–600. https://doi.org/10.1093/eurheartj/ehab282; PMID: 34038937.

76. Kikuchi Y, Yasuda S, Aizawa K, et al. Enhanced Rho-kinase activity in circulating neutrophils of patients with vasospastic angina: a possible biomarker for diagnosis and disease activity assessment. J Am Coll Cardiol 2011;58:1231–7. https://doi.org/10.1016/j.jacc.2011.05.046; PMID: 21903056.

77. Nihei T, Takahashi J, Kikuchi Y, et al. Enhanced Rho-kinase activity in patients with vasospastic angina after the Great East Japan Earthquake. Circ J 2012;76:2892–4. https://doi.org/10.1253/circj.cj-12-1238; PMID: 23131720.

78. Nihei T, Takahashi J, Hao K, et al. Prognostic impacts of Rho-kinase activity in circulating leukocytes in patients with vasospastic angina. Eur Heart J 2018;39:952–9. https://doi.org/10.1093/eurheartj/ehx657; PMID: 29165549.

79. Ong P, Camici PG, Beltrame JF, et al. International standardization of diagnostic criteria for microvascular angina. Int J Cardiol 2018;250:16–20. https://doi.org/10.1016/j.ijcard.2017.08.068; PMID: 29031990.

80. Bairey Merz CN, Pepine CJ, Walsh MN, Fleg JL. Ischemia and no obstructive coronary artery disease (INOCA). Developing evidence-based therapies and research agenda for the next decade. Circulation 2017;135:1075–92. https://doi.org/10.1161/CIRCULATIONAHA.116.024534; PMID: 28289007.

81. Beltrame JF, Crea F, Kaski JC, et al. International standardization of diagnostic criteria for vasospastic angina. Eur Heart J 2017;38:2565–8. https://doi.org/10.1093/eurheartj/ehv351; PMID: 26245334.

82. Suda A, Takahashi J, Hao K, et al. Coronary functional abnormalities in patients with angina and nonobstructive coronary artery disease. J Am Coll Cardiol 2019;74:2350–60. https://doi.org/10.1016/j.jacc.2019.08.1056; PMID: 31699275.

83. Odaka Y, Takahashi J, Tsuburaya R, et al. Plasma concentration of serotonin is a novel biomarker for coronary microvascular dysfunction in patients with suspected angina and unobstructive coronary arteries. Eur Heart J 2017;38:489–96. https://doi.org/10.1093/eurheartj/ehw448; PMID: 27694191.

84. Hao K, Takahashi J, Kikuchi Y, et al. Prognostic impacts of comorbid significant coronary stenosis and coronary artery spasm in patients with stable coronary artery disease. J Am Heart Assoc 2021;10:e017831. https://doi.org/10.1161/JAHA.120.017831; PMID: 33455423.

85. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J 2020;41:407–77. https://doi.org/10.1093/eurheartj/ehz425; PMID: 31504439.

86. Kunadian V, Chieffo A, Camici PG, et al. An EAPCI expert consensus document on ischaemia with non-obstructive coronary arteries in collaboration with European Society of Cardiology working group on coronary pathophysiology and microcirculation endorsed by coronary vasomotor disorders international study group. Eur Heart J 2020;41:3504–20. https://doi.org/10.1093/eurheartj/ehaa503; PMID: 32626906.

87. Nishimiya K, Matsumoto Y, Shindo T, et al. Association of adventitial vasa vasorum and inflammation with coronary hyperconstriction after drug-eluting stent implantation in pigs in vivo. Circ J 2015;79:1787–98. https://doi.org/10.1253/

circj.CJ-15-0149; PMID: 26027445.

88. Ohyama K, Matsumoto Y, Nishimiya K, et al. Increased coronary perivascular adipose tissue volume in patients with vasospastic angina. *Circ J* 2016;80:1653–6. https://doi.org/10.1253/circj.CJ-16-0213; PMID: 27194468.

89. Ohyama K, Matsumoto Y, Shimokawa H. Impact of epicardial adipose tissue volume quantified by non-contrast electrocardiogram-gated computed tomography on ergonovine-induced epicardial coronary artery spasm. *Int J Cardiol* 2017;229:40. https://doi.org/10.1016/j.ijcard.2016.10.030; PMID: 27751596.

90. Ohyama K, Matsumoto Y, Amamizu H, et al. Association of coronary perivascular adipose tissue inflammation and drug-eluting stent-induced coronary hyperconstricting responses in pigs: 18F-fluorodeoxyglucose positron emission tomography imaging study. *Arterioscler Thromb Vasc Biol* 2017;37:1757–64. https://doi.org/10.1161/ATVBAHA.117.309843; PMID: 28751570.

91. Ohyama K, Matsumoto Y, Shimokawa H. Coronary artery spasm and perivascular adipose tissue inflammation: insights from translational imaging research. *Eur Cardiol* 2019;14:6–9. https://doi.org/10.15420/ecr.2019.3.2; PMID: 31131030.

92. Uzuka H, Matsumoto Y, Nishimiya K, et al. Renal denervation suppresses coronary hyperconstricting responses after drug-eluting stent implantation in pigs *in vivo* through the kidney-brain-heart axis. *Arterioscler Thromb Vasc Biol* 2017;37:1869–80. https://doi.org/10.1161/ATVBAHA.117.309777; PMID: 28818859.

93. Amamizu H, Matsumoto Y, Morosawa S, et al. Cardiac lymphatic dysfunction causes drug-eluting stent-induced coronary hyperconstricting responses in pigs in vivo. *Arterioscler Thromb Vasc Biol* 2019;39:741–53. https://doi.org/10.1161/ATVBAHA.119.312396; PMID: 30816801.

94. Watanabe T, Matsumoto Y, Nishimiya K, et al. Low-intensity pulsed ultrasound therapy suppresses coronary adventitial inflammatory changes and hyperconstricting responses after coronary stent implantation in pigs in vivo. *PLoS One* 2021;16:e0257175. https://doi.org/10.1371/journal.pone.0257175; PMID: 34516572.

95. Shiroto T, Yasuda S, Tsuburaya R, et al. Role of Rho-kinase in the pathogenesis of coronary hyperconstricting responses induced by drug-eluting stents in pigs in vivo. *J Am Coll Cardiol* 2009;54:2321–9. https://doi.org/10.1016/j.jacc.2009.07.045; PMID: 19958969.

96. Tsuburaya R, Yasuda S, Shiroto T, et al. Long-term treatment with nifedipine suppresses coronary hyperconstricting responses and inflammatory changes induced by paclitaxel-eluting stent in pigs *in vivo*: possible involvement of Rho-kinase pathway. *Eur Heart J* 2012;33:791–9. https://doi.org/10.1093/eurheartj/ehr145; PMID: 21624903.

97. Nishimiya K, Matsumoto Y, Uzuka H, et al. Beneficial effects of a novel bioabsorbable polymer coating on enhanced coronary vasoconstricting responses after drug-eluting stent implantation in pigs in vivo. *J Am Coll Cardiol Intv* 2016;9:281–91. https://doi.org/10.1016/j.jcin.2015.09.041; PMID: 26847120.

98. Nishimiya K, Matsumoto Y, Uzuka H, et al. Accuracy of optical frequency domain imaging for evaluation of coronary adventitial vasa vasorum formation after stent implantation in pigs and humans – a validation study -. *Circ J* 2015;79:1323–31. https://doi.org/10.1253/circj.CJ-15-0078; PMID: 25843557.

99. Nishimiya K, Matsumoto Y, Takahashi J, et al. Enhanced adventitial vasa vasorum formation in patients with vasospastic angina: assessment with OFDI. *J Am Coll Cardiol* 2016;67:598–600. https://doi.org/10.1016/j.jacc.2015.11.031; PMID: 26846957.

100. Ohyama K, Matsumoto Y, Takanami K, et al. Coronary adventitial and perivascular adipose tissue inflammation in patients with vasospastic angina. *J Am Coll Cardiol* 2018;71:414–25. https://doi.org/10.1016/j.jacc.2017.11.046; PMID: 29389358.

101. Nishimiya K, Suda A, Fukui K, et al. Prognostic links between OCT-delineated coronary morphologies and coronary functional abnormalities in patients with INOCA. *J Am Coll Cardiol Intv* 2021;14:606–18. https://doi.org/10.1016/j.jcin.2020.12.025; PMID: 33736768.

102. Ridker PM, Everett BM, Pradhan A, et al. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* 2019;380:752–62. https://doi.org/10.1056/NEJMoa1809798; PMID: 30415610.

103. Sandler A, Gray R, Perry MC, et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med* 2006;355:2542–50. https://doi.org/10.1056/NEJMoa061884; PMID: 17167137.

104. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* 2005;25:2054–61. https://doi.org/10.1161/01.ATV.0000178991.71605.18; PMID: 16037567.

105. Hanawa K, Ito K, Aizawa K, et al. Low-intensity pulsed ultrasound induces angiogenesis and ameliorates left ventricular dysfunction in a porcine model of chronic myocardial ischemia. *PLoS One* 2014;9:e104863. https://doi.org/10.1371/journal.pone.0104863; PMID: 25111309.

106. Shindo T, Ito K, Ogata T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates left ventricular dysfunction in a mouse model of acute myocardial infarction. *Arterioscler Thromb Vasc Biol* 2016;36:1220–9. https://doi.org/10.1161/ATVBAHA.115.306477; PMID: 27079882.

107. Eguchi K, Shindo T, Ito K, et al. Whole-brain low-intensity pulsed ultrasound therapy markedly improves cognitive dysfunctions in mouse models of dementia – crucial roles of endothelial nitric oxide synthase. *Brain Stimul* 2018;11:959–73. https://doi.org/10.1016/j.brs.2018.05.012; PMID: 29857968.

108. Ogata T, Ito K, Shindo T, et al. Low-intensity pulsed ultrasound enhances angiogenesis and ameliorates contractile dysfunction of pressure-overloaded heart in mice. *PLoS One* 2017;12:e0185555. https://doi.org/10.1371/journal.pone.0185555; PMID: 28957396.

109. Monma Y, Shindo T, Eguchi K, et al. Low-intensity pulsed ultrasound ameliorates cardiac diastolic dysfunction in mice: a possible novel therapy for heart failure with preserved left ventricular ejection fraction. *Cardiovasc Res* 2021;117:1325–38. https://doi.org/10.1093/cvr/cvaa221; PMID: 32683442.

110. Ichijo S, Shindo T, Eguchi K, et al. Low-intensity pulsed ultrasound therapy promotes recovery from stroke by enhancing angio-neurogenesis in mice *in vivo*. *Sci Rep* 2021;11:4958. https://doi.org/10.1038/s41598-021-84473-6; PMID: 33654156.

111. Nakata T, Shindo T, Ito K, et al. Beneficial effects of low-intensity pulsed ultrasound therapy on right ventricular dysfunction in animal models. *J Am Coll Cardiol Basic Trans Science* 2023;8:283–97. https://doi.org/10.1016/j.jacbts.2022.08.010.

112. Shimokawa H, Shindo T, Ishiki A, et al. A pilot study of whole-brain low-intensity pulsed ultrasound therapy for early stage of Alzheimer's disease (LIPUS-AD): a randomized, double-blind, placebo-controlled trial. *Tohoku J Exp Med* 2022;258:167–75. https://doi.org/10.1620/tjem.2022.J078; PMID: 36104179.

113. Oikonomou EK, Marwan M, Desai MY, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. *Lancet* 2018;392:929–39. https://doi.org/10.1016/S0140-6736(18)31114-0; PMID: 30170852.

114. Shindo T, Shimokawa H. Therapeutic angiogenesis with sound waves. *Ann Vasc Dis* 2020;13:116–25. https://doi.org/10.3400/avd.ra.20-00010; PMID: 32595786.