The coronary circulation in acute myocardial ischaemia/reperfusion injury - a target for cardioprotection

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Abstract

The coronary circulation is both culprit and victim of acute myocardial infarction. The rupture of an epicardial atherosclerotic plaque with superimposed thrombosis causes coronary occlusion, and this occlusion must be removed to induce reperfusion. However, ischaemia and reperfusion cause damage not only in cardiomyocytes but also in the coronary circulation, including microembolisation of debris and release of soluble factors from the culprit lesion, impairment of endothelial integrity with subsequently increased permeability and oedema formation, platelet activation and leukocyte adherence, erythrocyte stasis, a shift from vasodilation to vasoconstriction, and ultimately structural damage to the capillaries with eventual no-reflow, microvascular obstruction and intramyocardial haemorrhage. Therefore, the coronary circulation is a valid target for cardioprotection, beyond protection of the cardiomyocyte. Virtually all of the above deleterious endpoints have been demonstrated to be favourably influenced by one or the other mechanical or pharmacological cardioprotective intervention. However, no-reflow is still a serious complication of reperfused myocardial infarction and carries, independently from infarct size, an unfavourable prognosis. Microvascular obstruction and intramyocardial haemorrhage can be diagnosed by modern imaging technologies, but still await an effective therapy. The current review provides an overview of strategies to protect the coronary circulation from acute myocardial ischaemia/reperfusion injury. This article is part of a Cardiovascular Research Spotlight Issue entitled ‘Cardioprotection Beyond the Cardiomyocyte’, and emerged as part of the discussions of the European Union (EU)-CARDIOPROTECTION Cooperation in Science and Technology (COST) Action, CA16225.

1. Introduction

Reperfusion is the only way to salvage ischaemic myocardium from infarction, but reperfusion *per se* also inflicts additional injury, such that the resulting myocardial infarct (MI) size is determined by both ischaemia- and reperfusion-induced injury.[1-3] There is still an unmet medical need for adjunct cardioprotection on top of timely reperfusion.[4, 5] In type II myocardial infarction and in the absence of epicardial coronary artery occlusion, the distinction of ischaemia and reperfusion is less obvious, but there is still infarction and cardioprotection is needed.[6] Numerous animal experiments have provided robust evidence that MI size can be reduced by mechanical or pharmacological interventions before (preconditioning), during (perconditioning) or after (postconditioning) myocardial ischaemia. However, the translation of cardioprotection to clinical practice has been largely disappointing so far, for many reasons, including lack of rigor and reproducibility in experimental studies, as well as conceptual and technical faults in clinical trial design.[7-10] One important conceptual reason for failure of translation may relate to the focus of cardioprotection studies on the cardiomyocyte, and the neglect of other tissues in the heart, notably the coronary circulation.[11]

2. The coronary circulation in acute myocardial ischaemia/reperfusion injury

The coronary circulation is both culprit and victim of acute myocardial ischaemia/reperfusion injury (IRI), and as such a prime target for cardioprotection. Acute ST-segment elevation myocardial infarction (STEMI) is induced by rupture of an epicardial coronary atherosclerotic plaque with superimposed thrombosis, which occludes the epicardial coronary artery completely and renders the dependent perfusion territory ischaemic; residual blood flow to the perfusion territory then depends entirely on the coronary collateral circulation which varies interindividually and largely depends on its prior adaptation to pre-existing epicardial coronary atherosclerotic narrowing. More recent studies have emphasised the increasing importance of atherosclerotic plaque erosion rather than rupture, particularly in statin-treated patients and particularly for the induction of non-STEMI.[12] The epicardial coronary artery with its culprit lesion is also the target of interventional therapy by dilatation/stenting with or without

thrombectomy. Such percutaneous coronary intervention (PCI) may not only restore epicardial coronary blood flow but at the same tissue dislodge atherothrombotic debris from the culprit lesion and embolise it into the coronary microcirculation.[13]

The coronary circulation distal to the epicardial atherosclerotic culprit lesion is not virgin, but characterised by endothelial dysfunction through the typical risk factors (aging, hypertension, hyperlipidaemia; diabetes etc.) which characterise atherosclerosis in general.[11] More specifically, the coronary circulation distal to epicardial stenoses remodels, with atrophy of the vascular wall in larger coronary arteries and hypertrophy of the vascular wall in smaller arteries and arterioles,[14, 15] and its autoregulatory vasomotor responses are attenuated.[15] The coronary microcirculation as such is not only exposed to atherothrombotic debris which is dislodged from the epicardial culprit lesion and causes microembolisation, microinfarcts and a subsequent inflammatory response,[16-18] but also the release of vasoconstrictor, pro-thrombotic and pro-inflammatory soluble substances from the culprit lesion, notably serotonin, thromboxane A_2 and TNFα.[19, 20] In consequence of coronary microembolisation and in response to these soluble substances, coronary vasodilator reserve is severely impaired.[18, 21]

3. Effects of acute myocardial ischaemia/reperfusion injury on the coronary vasculature

Endothelium, pericytes, and glycocalyx

Coronary endothelial cells are relatively resistant to ischaemia and survive hypoxia *in vitro* for several days.[22] However, *in vivo*, the interruption of antegrade pulsatile flow and shear stress induces swelling and blebbing of endothelial cells.[23] The actual disruption of the endothelium and subsequent extravasation of cells after reperfusion are probably facilitated by destabilisation of the cellular junctions. Reperfused endothelium experiences altered Ca^{2+} homeostasis, increased cytosolic calcium activates the endothelial contractile elements and their contraction promotes the formation of intercellular gaps which increase permeability to large molecules.[24] Activated endothelial cells and platelets result in the expression of adhesion molecules and subsequent adhesion of platelets and platelet-leukocyte aggregates to the

coronary microvasculature.[25] Also, the release of cytokines impairs the stability of cell junctions and increases vascular permeability via activation of Src[26] and dissociation of the VEGFR2/vascular endothelial (VE)-cadherin complex (Figure 1).[27] NLRP3 inflammasome activation in endothelial cells may initiate caspase 1-mediated cell death.[28] Endothelium-initiated inflammation together with pro-inflammatory effects of debris from cardiomyocyte necrosis result in recruitment of inflammatory cells and release of pro-inflammatory factors, including vascular endothelial growth factor (VEGF),[29] matrix metalloproteases, thrombin, myeloperoxidase,[30] and platelet activating factor.[31] These factors, in turn, increase vascular permeability and result in myocardial oedema by different mechanisms, including activation of eNOS in caveolae by VEGF.[32, 33] Angiopoietin-1 and angiopoietin-like peptide 4 have protective effects via stablisation of endothelial cell junctions.[29, 34]

Pericytes induce vasoconstriction of the cerebral microvasculature, thereby contributing to entrapment of red and white blood cells in areas of no-reflow in the post-ischaemic brain.[35] Although pericytes are present in high numbers in the coronary microvasculature,[36] their role in the heart remains unclear. In the acutely reperfused rat heart, capillary obstruction was associated with the presence of pericytes, with reduced capillary diameter, suggesting that cardiac pericytes may also constrict coronary capillaries and reduce microvascular blood flow after acute myocardial infarction (AMI). The pericyte relaxant adenosine increased capillary diameter, decreased capillary obstruction, and increased perfusion volume.[37] Cardiac pericytes may therefore represent a novel therapeutic target for protecting the coronary microvasculature following AMI.

The glycocalyx is a matrix structure which covers endothelial cells and pericytes. The coronary glycocalyx is sensitive to acute myocardial IRI,[38] and its shedding contributes to the development of oedema,[39] and leukocyte[40] and platelet[41] adherence. TNFα is involved in glycocalyx degradation,[42] and nitric oxide (NO) is protective.[43] Thus, the glycocalyx may be a novel target for coronary vascular cardioprotection.

Oedema

Intracellular water accounts for more than 75% of myocardial water content, and reperfusion induces cardiomyocyte swelling immediately upon coronary reflow.[44] Osmotic swelling contributes to sarcolemmal rupture and cell death, and hyperosmotic reperfusion can reduce myocardial oedema and MI size.[45, 46] In surviving cardiomyocytes, intracellular oedema is reversed by restoration of activation of ion pumps, notably sarcolemmal Na^+/K^+-ATPase.[47] During ischaemia, the accumulation of metabolites increases interstitial osmolality, and the exposure to normo-osmotic blood at reperfusion induces immediate interstitial oedema. Interstitial oedema then diminishes as catabolite washout eliminates the osmotic gradient between the intravascular and the interstitial compartments,[44] but there is a second wave of oedema caused by increased vascular permeability. Serial cardiovascular magnetic resonance (CMR) imaging studies have revealed such bimodal pattern of myocardial oedema after reperfusion in pigs and humans.[48, 49]

Platelets

Platelets contribute to many processes relevant to acute IRI, including vascular integrity, lymphangiogenesis and tissue regeneration.[50] After AMI, platelets play a biphasic role, initially recruiting neutrophils and amplifying the inflammatory response, and later releasing factors that actively support the resolution of inflammation.[50] Upon activation, platelets release a variety of nucleotides, neurotransmitters, and over 300 proteins from secretory α-granules, dense granules and lysosomal granules.[51] Activated platelets also release microvesicles and exosomes which contain miRNA and lipids. The released substances are involved in platelet aggregation and coagulation. Some, such as sphingosine-1-phosphate (S1P),[52, 53 54, 55] and platelet-activating factor,[56, 57] can exert direct cardioprotective effects on cardiomyocytes, but their protective effect depends on the actual concentrations and circumstances. Other factors can affect the coronary microvasculature, including serotonin, growth factors, cytokines and chemokines. Intriguingly, both anti- and pro-angiogenic factors (e.g.: VEGF and SDF1α) can be released from platelet α-granules under different circumstances.[58]

Endothelial cells produce prostacyclins, NO and adenosine that inhibit platelet aggregation and adhesion. When activated, however, they express adhesion molecules and release von Willebrand factor, which activates platelets, causing them to form a plug. Conversely, activated platelets release vasoconstrictive compounds such as ADP, serotonin and thromboxane A2.[59]

Studies in isolated, perfused hearts have shown that platelets can be cardioprotective. The barrier function of coronary microvessels in the isolated perfused rat heart is improved after perfusion of platelet-rich plasma.[60] Myocardial injury measured by cardiac enzymes and function in rat hearts subject to IRI was decreased by perfusion with either washed rat platelets or with the supernatant of activated rat platelets.[61] The precise mechanism is unclear but may involve the release of S1P, adenosine, serotonin or thromboxane A2.[61] Perfusion of guinea pig hearts with constituents released by platelets helped to maintain the integrity of the coronary endothelium after IRI.[62] The specific action of platelets in a given situation appears to depend on their state of activation.[56, 57, 63] In rat hearts subjected to acute myocardial IRI, perfusion with platelets from AMI patients increased coronary resistance and myocardial injury when compared to perfusion with platelets from healthy volunteers.[64] Such injury was prevented by the $P2Y_{12}$ receptor antagonist cangrelor and the glycoprotein IIb/IIIa receptor blocker abciximab, suggesting that early inhibition of platelet activation may be cardioprotective.[64]

Given the complex, multi-factorial role of platelets, *in vivo* studies provide more clinically relevant information than *in vitro* studies which are more reductionist and mechanistic in nature.[65] Pigs were administered the platelet integrin $\alpha_{IIb}\beta_3$ receptor antagonist lamifiban prior to reperfusion after 55 min myocardial ischaemia. Lamifiban inhibited platelet aggregation and had a potent antithrombotic effect at the culprit lesion as expected, but did not reduce microvascular platelet accumulation or MI size.[66] Similarly, in a mouse *in vivo* model of 30 min left coronary artery ligation followed by 24 h reperfusion, MI size was not affected by inhibition of platelet adhesion or aggregation, but reduced by inhibition of platelet activation along with improved perfusion, suggesting a possible effect on the

microvasculature.[67] Ultimately, even if activated platelets do release substances with protective effects on the endothelium, treatment of AMI patients will always include platelet inhibition, given the importance of their primary pro-thrombotic activity.[64] To complicate matters even further, experimental data suggest that $P2Y_{12}$ receptor inhibition using ticagrelor or cangrelor at the onset of reperfusion can itself reduce MI size,[68] but whether this cardioprotective effect is mediated on the coronary vasculature or the cardiomyocyte is not clear.

4. Microvascular obstruction as a target for cardioprotection

Microvascular obstruction (MVO) following AMI is primarily a reperfusion phenomenon, which manifests clinically as coronary no-reflow in the infarct-related artery following primary PCI, and has been defined as the "inability to reperfuse a previously ischaemic region".[69] The pathophysiology underlying MVO is complex and multifactorial, and has been attributed to: endothelial swelling and blebbing obstructing capillary blood flow, cardiomyocyte swelling compressing capillaries, platelet activation and aggregation, capillary obstruction due to red and white blood cell stasis, and coronary microembolisation (reviewed in [11]). Severe MVO can result in capillary destruction and extravasation of red blood cells into the myocardium - termed intramyocardial haemorrhage (IMH), a condition which portends to worse prognosis following AMI. MVO following reperfusion of sustained myocardial ischaemia is always associated with infarction.[70] The MVO and no-reflow areas are always contained within the infarcted tissue and not seen in the risk area which has remained viable.[71] Also, there is infarction without MVO/no-reflow. These observations would put MVO as a consequence of myocardial infarction rather than its cause. However, MI size is robustly identified and quantified no earlier than after several hours of reperfusion, for technical reasons.[70] Therefore, any early and transient MVO which may have contributed to infarct extension may have gone unnoticed. In response to cardioprotective interventions, effects on MI size and on MVO can be dissociated. In pigs, local and remote ischaemic conditioning procedures reduce MI size but not areas of no-reflow.[72] Conversely, delayed hypothermia during reperfusion only reduces no-reflow but

not MI size.[73] Mechanistically, the same factors which cause cardiomyocyte death (necrosis, apoptosis, etc.) can also cause death of endothelial and vascular smooth muscle cells, i.e. hypoxia *per se* with re-oxygenation and consequent enhanced formation of reactive oxygen species (ROS). Intracellular and interstitial oedema, intravascular platelet and erythrocyte aggregates and early inflammatory responses contribute to MVO and cardiomyocyte death, but their contribution to MVO and cardiomyocyte death may differ. At this point, the causality between MVO and cardiomyocyte cell death remains unresolved, and the two phenomena must be considered as separate but intimately related, possibly because of their identical underlying mechanisms. MVO and coronary no-reflow occur frequently even after prompt epicardial recanalisation of the infarct-related artery,[74] and strongly impact on patient prognosis.[75] Several therapies for preventing MVO, which have been successfully tested in experimental models of AMI, have failed in the translation to AMI patients.[10, 11]

Invasive and non-invasive methods for assessment of coronary no-reflow and MVO

The thrombolysis in myocardial infarction (TIMI) score grades blood flow in epicardial vessels.[76] However, MVO may occur in nearly 50% of patients with TIMI flow 3. Angiographic methods characterising dye penetration within the myocardium, the myocardial blush grade (MBG) and TIMI myocardial perfusion grade, have been developed to shift attention to coronary microcirculatory flow.[77, 78] The gold standard for assessing coronary microvascular function is coronary blood flow by thermodilution or flow velocity by Doppler which in combination with quantitative coronary angiography of epicardial coronary arteries also provides volumetric coronary blood flow.[79] MVO is characterised by systolic retrograde and diminished anterograde flow, and by rapid deceleration of diastolic flow. Such impaired coronary flow velocity pattern following primary PCI is associated with future cardiovascular events.[80] The index of microvascular resistance assessed by thermodilution provides a more reproducible assessment of the coronary microcirculation and predicts acute microvascular injury, left ventricular functional recovery and clinical outcomes after STEMI.[81, 82]

Incomplete ST-segment resolution (STR) has been related to MVO and worse clinical outcome after primary PCI.[83] A consensus is still lacking over which ECG leads should be analysed, the optimal timing of electrocardiogram analysis, and whether standard ECG or continuous ECG monitoring is preferable.[84] Myocardial contrast echocardiography (MCE) utilises ultrasound to visualise contrast microbubbles with a rheology similar to that of erythrocytes, and lack of contrast opacification due to MVO predicts poor functional recovery after STEMI.[85] MCE, however, is limited by moderate spatial resolution and operator dependency. CMR allows multi-slice imaging with high tissue contrast and high spatial resolution, enabling accurate quantification and localisation of MVO and MI size. CMR-defined MVO correlates with angiographic and invasive indices of MVO,[86] and is associated with worse outcome.[87] MVO is diagnosed as: (i) lack of gadolinium uptake on first pass perfusion (<1 min of contrast administration), (ii) lack of early gadolinium enhancement (<2-3 min of contrast administration) (ii) lack of late gadolinium enhancement (LGE) (10–15 min after contrast administration).[88] Although first pass perfusion and early contrast gadolinium enhancement detect the presence of MVO with greater sensitivity than LGE, the presence of MVO on LGE is a stronger predictor of clinical outcomes following STEMI.[88]

5. Intramyocardial haemorrhage as a target for cardioprotection

IMH can develop after reperfusion of an infarct-related coronary artery. In dog hearts with 50 to 60 min coronary occlusion and reperfusion IMH develops in the central core of the infarct; ultrastructurally, the endothelium is interrupted at several locations.[89, 90] In patients, IMH was first observed at autopsy after lytic therapy of AMI.[91] IMH is not germane to thrombolysis but frequently observed also after mechanical reperfusion and associated with unfavourable clinical outcome.[92] This relation with adverse clinical outcome is even stronger than that of MI size or MVO.[93] IMH is associated with larger MI size, longer treatment delay and the use of glycoprotein IIb/IIIa inhibitors.[94] IMH is not only a bystander phenomenon; extravasation of erythrocytes, leukocytes and finally iron deposition further increase myocardial damage via a sustained inflammatory reaction.[95, 96] Without reperfusion, IMH will not occur as shown both in

experimental models,[97] and at autopsy of patients with non-reperfused AMI.[98] In an *ex-vivo* reperfusion rat model, the endothelial barrier function for microspheres of 0.1 µm diameter was lost in hearts exposed to initial 30 min ischaemia followed by 60 min reperfusion whereas the barrier function remained intact after 30 min ischaemia without reperfusion, along with better preservation of endothelial cellular junctions and less endothelial cell damage.[99] Given this sequence of events, a therapeutic window apparently exists to prevent microvascular damage and subsequent IMH upon reperfusion.

The first large series of CMR-scanning acutely after STEMI demonstrated specific changes in the infarct core in up to 50% of patients treated with primary PCI.[87] Using LGE, many patients displayed infarct areas completely devoid of contrast.[87] Subsequently, contrast-free sequences were introduced to specifically detect IMH.[100, 101] The degradation of erythrocytes and release of oxyhaemoglobin, de-oxyhaemoglobin and methaemoglobin change the CMR tissue characteristics, as reflected by a relative decrease in relaxation time and thus relative signal attenuation within the infarct zone. Iron deposition in the form of ferritin and hemosiderin also induces signal attenuation (Figure 2). T2* shows the lowest increase upon oedema and the highest relative decrease upon haemorrhage and thus theoretically is the most accurate sequence to detect IMH.[95]Whether or not CMR-defined MVO and IMH are separate entities is still debated. In a combined patient and pig study, there was a very large overlap between LGE detected MVO and T2-detected IMH. These areas were confined to the infarct core and displayed massive haemorrhage and complete microvascular destruction. Actual MVO was only observed in the infarct border zone.[102]

6. Coronary collateral angiogenesis

Brief episodes of ischaemia and reperfusion induced by ischaemic preconditioning (IPC) enable the preservation of endothelial function of coronary arterioles following acute myocardial IRI.[103] Coronary endothelial function is sensitive to acute myocardial IRI, in that the vasodilatory action of thrombin under normal conditions is reversed to a vasoconstrictive effect following IRI,[104] and this original observation by Ku has been confirmed by many

groups.[105, 106] A well-developed coronary collateral circulation protects against lethal acute myocardial IRI by maintaining perfusion to the area at risk. Apparently, similar underlying mechanisms are shared by both IPC of cardiomyocytes and coronary collateral growth. Activation of hypoxia-inducible factor (HIF) ap dissecting whether the cardioprotective effects of ischaemic ears critical for IPC,[107] and HIF-dependent genes are required for coronary collateral growth in a model of episodic myocardial ischaemia.[108, 109] Mitochondrial function also appears to be critical for both IPC,[110] and for coronary collateral growth.[111] Collateral angiogenesis cannot be recruited acutely for cardioprotection, but is important for the healing and remodelling following acute myocardial infarction.[112, 113]

7. Targeting the coronary vasculature for cardioprotection

Interventions to protect the coronary vasculature following acute IRI sustained during AMI have been targeted to endothelial dysfunction, loss of endothelial integrity, microembolisation, impaired vasomotor function, cardiomyocyte and endothelial swelling compressing capillaries, and capillary rupture with IMH (Figure 3).

The heart can be protected from cell death induced by different endogenous cardioprotective strategies, collectively termed 'ischaemic conditioning' (reviewed in [114] and comprising the application of one or more brief cycles of non-lethal ischaemia and reperfusion to the heart itself, either prior to the lethal ischaemic episode (IPC),[115] or at the onset of reperfusion (ischaemic postconditioning [IPost]).[116] Such cardioprotective stimulus can also be applied to an organ or tissue away from the heart (remote ischaemic conditioning [RIC]),[117-121] either prior to (remote ischaemic preconditioning [RIPC]),[122] or during the lethal ischaemic episode (remote ischaemic perconditioning [RIPerC]),[123] or at the onset of reperfusion (remote ischaemic postconditioning [RIPost]).[124] The majority of experimental and clinical studies have focused on the cardioprotective effects of ischaemic conditioning on cardiomyocytes and neglected the coronary vasculature. However, dissecting whether the cardioprotective effects of ischaemic conditioning protects the coronary vasculature independently of cardiomyocytes

is challenging, given the intimate and potentially causal relationship between damage to the coronary vasculature and cardiomyocyte death following AMI.[70]

Protecting the coronary vasculature with IPC

IPC, in addition to reducing MI size, can protect the coronary vasculature, as evidenced by less endothelial damage,[125] increased flow-mediated dilator response to vasodilators such as adenosine and nitric oxide or a reactive hyperaemia stimulus,[103, 126-129] less neutrophil adherence,[126] and improved endothelial integrity.[130] Mechanisms implicated in IPC include adenosine,[131, 132] K_{ATP} channel opening,[131, 133] signalling ROS,[134] bradykinin B1 receptor activation,[135] prostaglandin E2,[136], NO,[137] attenuated formation of detrimental ROS,[138] reduced endothelin-1,[139] enhanced eNOS function,[140] and preservation of endothelial tight junctions.[130] However, some studies failed to show beneficial effects with IPC on coronary no-reflow [72, 141] or coronary vasomotor response.[142] The interaction of coronary microembolisation with ischaemic conditioning is complex.[13] Prior coronary microembolisation does not induce IPC,[13] and conversely IPC does not protect from coronary microembolisation.[143] Coronary microembolisation induces however delayed protection from infarction through upregulation of TNFα.[144]

In patients with pre-infarction angina (a clinical example of IPC)[145, 146] reperfusion,[147] coronary microvascular reflow and flow reserve were improved following AMI, suggesting coronary vascular protection with endogenous IPC by pre-infarct angina.[148] Whether or not pre-infarction angina is a form of IPC is still under debate, and whether or not pre-infarction angina is protective under all circumstances is questionable, given the phenomenon of hyperconditioning.[149] In any event, the need to apply the protective stimulus prior to the lethal ischaemic insult has prevented the clinical application of IPC in AMI patients in whom the onset of acute myocardial ischaemia cannot be anticipated.

Protecting the coronary vasculature with IPost

IPost can be applied at the onset of reperfusion, making its use in STEMI patients at the time of primary PCI possible. In the first description of MI-limitation by IPost,[116] less myocardial oedema, reduced neutrophil adherence and decreased endothelial P-selectin expression, and improved vasodilator response to acetylcholine were observed. In pigs, smaller MI size, less MVO, improved endothelial function, and preserved coronary blood flow were observed after 2 hours of reperfusion with IPost.[150] A more recent study reported less oedema and MVO, but no reduction in MI size with IPost and RIC in a closed-chest pig infarction model.[151] Other studies failed to show any beneficial effects of IPost on MVO[72, 152, 153]; one of these studies also found no reduction in MI size with IPost,[152] but the others did demonstrate a smaller MI size with IPost.[72, 153] The dissociation between the beneficial effects of IPost on MVO and MI size are difficult to interpret at this time. Concomitant IPost and coronary microembolisation, as probably occurs during further manipulation of the culprit lesion just after established reperfusion, has been shown to not impair protection by IPost.[154]

In the clinical setting, the beneficial effects of IPost on MVO appeared to mirror its MI-limiting effect.[155] Reduction of MI size went along with limitation of MVO by 50% with IPost (both by CMR).[155] In primary PCI-treated STEMI patients less coronary no-reflow with IPost was reflected by improved TIMI grade, STR, MBG, and corrected TIMI frame count.[156] Also, IPost reduced MI size, and improved coronary blood flow and endothelium-dependent vasodilator function following STEMI.[157] However, other clinical studies have failed to demonstrate an effect of IPost on MVO, but these studies also showed no effect of IPost on MI size.[152, 158] Some studies have even reported detrimental effects of IPost with larger MI size, but in these studies there was no detrimental effect on coronary microvascular function.[159, 160]

Protecting the coronary vasculature with limb RIC

IPost requires further manipulation of the culprit coronary lesion, thereby limiting its clinical application. In contrast, RIC can be induced non-invasively by one or more cycles of brief non-lethal ischaemia and reperfusion to the limb.[161] In human volunteers, serial inflations and

deflations of a pneumatic cuff on the upper arm improved post-ischaemic endothelial function (as measured by increased blood flow response to acetylcholine) in the contralateral arm.[161] Using the same model, limb RIC induced an early and a delayed vasculoprotective effect 24-48 hours following the stimulus in healthy volunteers and in patients with atherosclerosis which was blocked by the K_{ATP} channel blocker glibenclamide,[162] required a neural pathway which was blocked by pharmacological ganglionic blockade,[163] and was effective even when limb RIC was performed during the acute forearm IRI. An endothelial-protective effect from limb RIC was also present with daily limb RIC for 7 days,[164] and still present 8 days following the protective stimulus,[165] suggesting that a chronic daily limb RIC stimulus may be able to extend the window of vascular protection. Long-term nitroglycerine and limb RIC each separately reduced MI size in rats and attenuated the endothelial dysfunction from forearm ischemia/reperfusion in healthy volunteers, but in combination abrogated any protection both in the heart and in the peripheral vasculature.[166]

Coronary vascular resistance was reduced and coronary blood flow improved with limb RIC in pigs at baseline and following acute myocardial IRI, and this effect was blocked by K_{ATP} channel blockade with glibenclamide but not by femoral nerve transection.[167] In healthy human volunteers, limb RIC increased coronary flow velocity (by Doppler), suggesting a hyperaemic response with RIC.[168] In patients undergoing PCI for stable CAD, limb RIC reduced periprocedural myocardial injury and rapidly increased distal coronary occlusive pressure, reflecting improved coronary collateral blood flow.[169] Also in patients undergoing PCI for stable CAD, RIC improved coronary vasomotor responses to acetylcholine, reflecting better endothelial function.[170, 171] However, several clinical studies have reported reductions in MI size with limb RIC in STEMI patients treated by primary PCI, but have not found any beneficial effects on coronary no-reflow or MVO,[158, 172] suggesting that the cardioprotective effects of limb RIC in STEMI patients may be targeted towards ischaemic cardiomyocytes rather than the coronary vasculature.

Pharmacological strategies for protecting the coronary vasculature

Many pharmacological agents have been tested for their protective effects on the coronary vasculature, and only an overview is provided here. A number of drugs are currently given in the cardiac characterisation laboratory to treat coronary no-reflow in STEMI patients following PCI, and these include nitrates, calcium channel blockers and adenosine. Although these drugs can induce coronary vasodilation and in some case reduce MVO, these interventions do not appear to improve clinical outcomes following primary PCI.[173-175] Most pharmacological agents used to induce coronary vascular protection also have protective effects on the cardiomyocyte, i.e. adenosine, NO donors, calcium antagonists and $P2Y_{12}$ inhibitors, making it difficult to separate vascular from cardiomyocyte protection. Some novel approaches have been tried to reduce coronary no-reflow and prevent MVO in experimental studies.[9]

Administration of angiopoietin-like peptide 4 at reperfusion to target the endothelial gap-junction VE-cadherin complex and preserve coronary endothelial integrity following acute myocardial IRI reduced MI size, decreased myocardial oedema, and prevented MVO and IMH.[29] Opening of the mitochondrial permeability transition pore (MPTP) during reperfusion is a critical determinant of cell death from acute IRI, and its inhibition at reperfusion using cyclosporine-A (CSA) reduced MI size in small animal AMI models,[176, 177] although in large animals the effect of CSA has been mixed.[178-180] CSA reduced MI size in an initial clinical study of primary PCI-treated STEMI patients,[181] but failed to improve clinical outcomes in 2 subsequent large clinical studies.[182, 183] In one pig study, CSA reduced both MI size and MVO;[153] however, whether this was due to a direct vasculoprotective effect of CSA or occurred secondary to myocardial salvage is not clear. Nitroglycerine can induce a preconditioning-like protection of the coronary vasculature, the peripheral vasculature and the myocardium,[146, 166] and its mechanisms are still not fully elucidated, may depend on dose and duration of administration and may include hitherto unrecognised effects on the MPTP.[184]

Therapeutic hypothermia limits MI size in experimental IRI studies when initiated during ischaemia, whereas clinical studies using invasive interventions to achieve hypothermia have had limited success primarily due to logistical issues. Hypothermia in rabbit hearts reduced coronary no-reflow following acute IRI, when delayed into reperfusion, even

when there was no MI limiting effect,[73] raising the possibility for an extended window for vascular protection following AMI. Mild hypothermia using a non-invasive ThermoSuit System initiated during ischaemia reduced MI size and prevented coronary no-reflow in rabbit and rat models of acute myocardial IRI;[185] whether or not such protection would be effective if applied at the onset of reperfusion needs to be tested.

8. Effect of co-morbidities and co-medications on coronary vascular protection

Co-morbidities and co-medications can confound cardioprotection elicited by ischaemic conditioning strategies.[186] In pigs with acute IRI, IPost improved endothelial function and reduced MVO in healthy animals, but failed to do so in the presence of hypercholesterolemia.[150] The abrogation of IPost-induced cardioprotection was attributed to detrimental effects of hypercholesterolemia on NOS levels. In another study, IPC provided significant microvascular protection in the skeletal muscle from prolonged IRI in normal, but not in diabetic rats.[187] In young men, flow-mediated dilation (FMD) decreased significantly after IRI without but not with prior IPC; such protection by IPC was attenuated in elderly patients.[188] In smokers, the IPC-induced increase in forearm blood flow response to acetylcholine seen in healthy volunteers was blunted while the responses to sodium nitroprusside before and after the IPC stimulus were similar.[189] In contrast to age and smoking, neither hypertension,[190] nor reduced left ventricular ejection fraction[191] affected the protective response of RIC on FMD,[190] or coronary flow reserve (by transthoracic Doppler).[191]

Of note, in most studies on co-morbidities animals are untreated. Acute rosuvastatin prevented the development of IRI-induced conduit artery endothelial dysfunction.[192] In contrast, chronic rosuvastatin did not prevent the development of IRI-induced endothelial dysfunction.[193] The anti-diabetic sulfonylurea glibenclamide abolished RIC- and IPost- induced protection on forearm endothelial function in humans during acute IRI.[162, 194] On the other hand, re-establishment of normoglycemia by islet cell transplantation restored the cardioprotection, as reflected by reduced infarct size, from IPost which had been lost in diabetes.[195] The RIC-induced prevention of FMD impairment following IRI was abrogated by

cyclooxygenase (COX) 2 inhibition.[196] Non-selective COX inhibition with aspirin 325mg and ibuprofen or specific COX-2 inhibition with celecoxib inhibited the protective effects of rosuvastatin in the setting of IRI. In contrast, low dose aspirin (81mg daily) – as given for the prevention on coronary artery disease - did not have such inhibitory effects.[197] Often, low dose aspirin is combined with $P2Y_{12}$-inhibition: clopidogrel given 24 hours prior to an episode of IRI limited the adverse effects of ischaemia on endothelial function.[198] While acute treatment with NO donors might protect endothelial function, such protection might be lost with the development of nitrate tolerance, and nitrate tolerance may also interfere with the vascular protection by RIC.[166] In contrast, inhibition of phosphodiesterase 5 with sildenafil provided sustained protection of the endothelium from adverse IRI effects on vascular function.[199]

In summary, while there appears to be an effect of co-morbidities and co-treatments in peripheral vascular beds, almost nothing is known on their interactions on cardioprotective interventions in the coronary circulation.

9. Future perspectives

MVO and no-reflow are serious consequences of reperfused AMI which carry an adverse prognosis. As such these phenomena require attention. Currently, the causal relationship between cardiomyocyte and coronary microvascular injury is not clear. Likewise it is not clear to what extent protective interventions target the cardiomyocyte, the coronary circulation or both. Clearly, however, there is a need for protection of the coronary circulation beyond infarct size reduction. At this point, there is no intervention or substance which would specifically protect the coronary circulation from ischaemia/reperfusion injury. However, the development of specific or additive protective strategies for the coronary circulation is an unmet medical need. Protection is needed from enhanced permeability, enhanced platelet and leukocyte adherence and transmigration, impaired vasomotion, capillary obstruction by erythrocytes, platelets and leukocytes and ultimately capillary destruction and haemorrhage. Thus, all structural elements of the coronary vascular wall from glycocalyx to endothelium to smooth muscle and adventitia need protection. At this point, the most promising protective

substance/molecule to achieve such multi-faceted protection appears to be angiopoietin-like peptide 4.[29]

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Figure 1: Potential mechanisms underlying capillary damage following AMI
During thrombotic coronary occlusion and interruption of flow, the endothelium shows morphological and functional changes, including swelling and blebbing and loss of endothelial junctions via release of angiopoietins and VEGF. Instantaneous opening of the coronary vessel by placement of a coronary stent induces additional damage leading to endothelial gaps, extravasation of erythrocytes and intramyocardial haemorrhage. Figure reproduced from[200].

Figure 2: Intramyocardial haemorrhage following AMI on cardiac MRI.
(a) On T2-weighted images relaxation times and thus signal strength increase due to myocardial oedema formation after AMI (white arrow heads). In case of intramyocardial haemorrhage (IMH), haemoglobin degradation products lead to a relative decrease in relaxation time, and thus a relative signal attenuation within the MI zone (black arrow heads). **(b)** On T2* images a relatively lower increase is observed with myocardial oedema (white arrow heads), and a relative higher decrease is observed upon IMH (black arrow heads), providing a stronger signal separation as compared to T2**. (c)** On late gadolinium enhancement (LGE) images the hypointense core indicates that no gadolinium entered the infarct core (yellow arrow heads). Overall infarct area is indicated by the hyperintense signal of the gadolinium that is retained within the tissue (white line). Note the large overlap between microvascular obstruction (MVO) as assessed by LGE and IMH as assessed by T2 and T2*. Figure reproduced from [200].

Figure 3: Effects of acute myocardial ischaemia/reperfusion injury on the coronary vasculature, and therapeutic vascular targets for cardioprotection
This scheme depicts the diverse consequences of acute myocardial ischaemia/reperfusion injury on the coronary vasculature following acute myocardial infarction, and highlights the vascular targets of endogenous cardioprotective strategies (IPC, ischaemic preconditioning, IPost, ischaemic postconditioning and RIC, remote ischaemic conditioning) and Pharmacological agents (Pharm). Figure modified from [11].

Figure 1

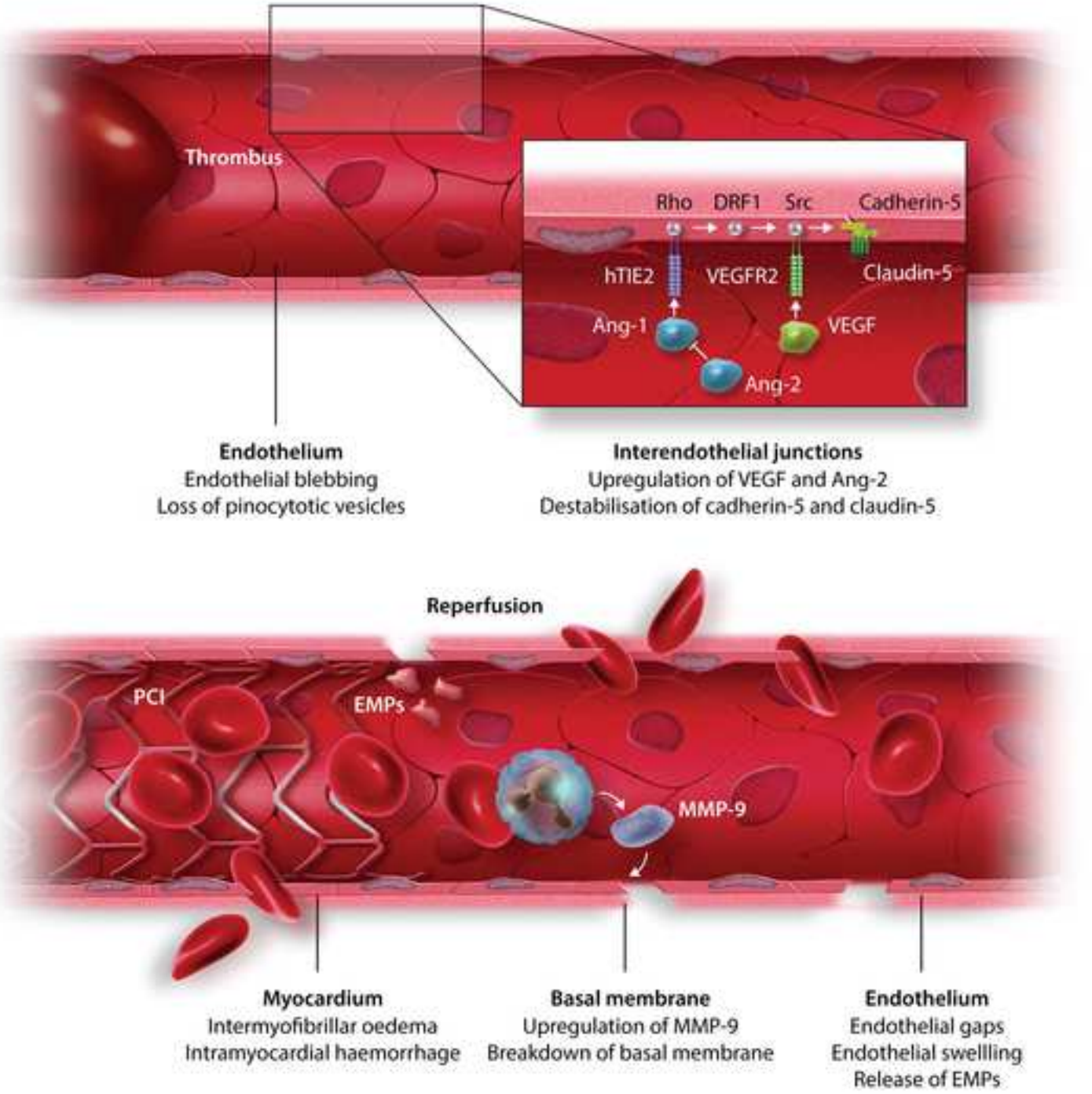

Figure 2

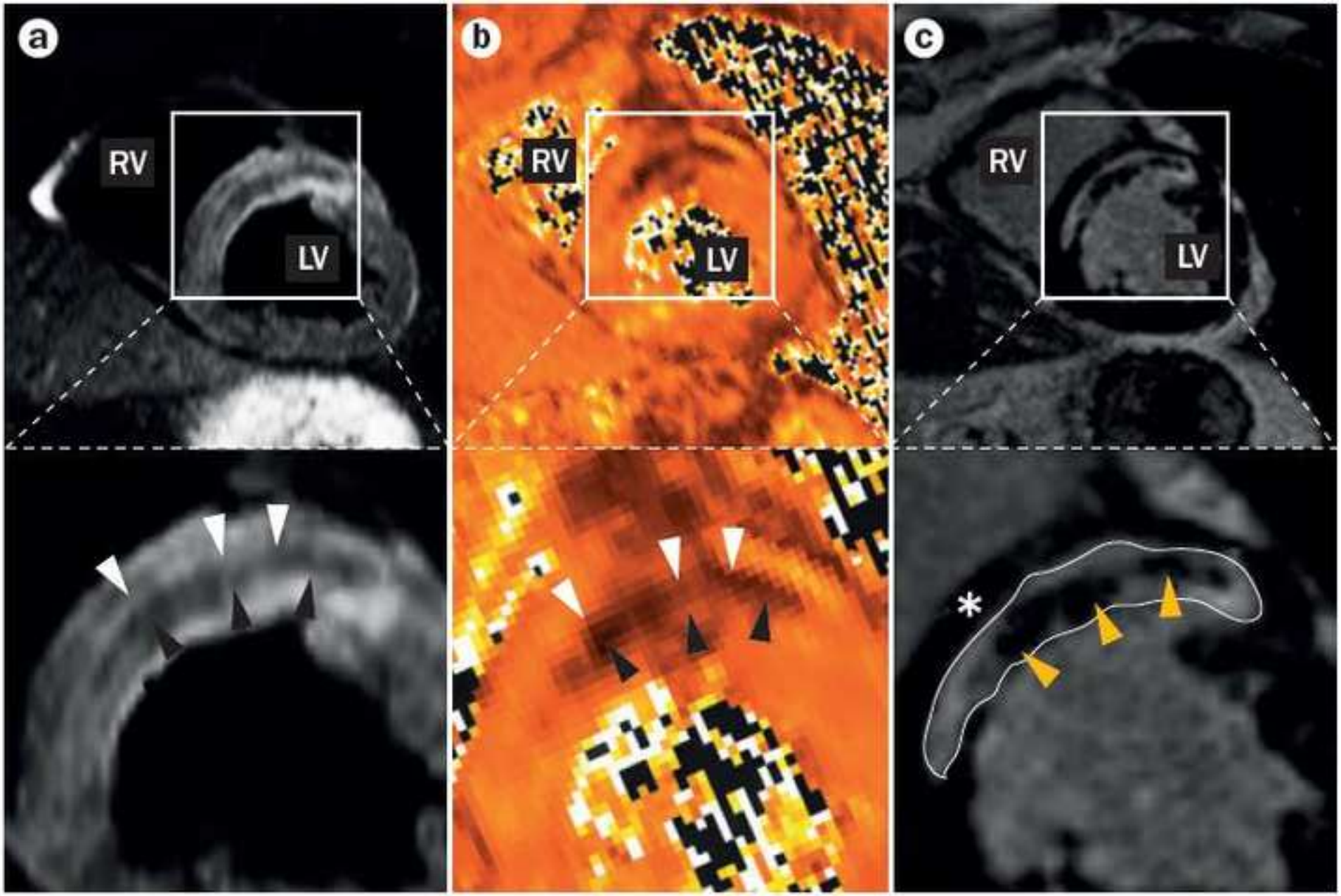

Figure 3