Microvascular damage during myocardial ischemia-reperfusion: pathophysiology, clinical implications and potential therapeutic approach evaluated by myocardial contrast echocardiography

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During myocardial ischemia produced by coronary occlusion, coronary microvessels and cardiac myocytes undergo progressive functional and structural changes. The prompt reopening of the epicardial vessel is the main therapeutic strategy to limit the vascular and cellular damage. However, the full benefit of reperfusion can be limited by progressive microvascular obstruction and cell death occurring after the reestablishment of flow. During ischemia-reperfusion, preservation of the integrity of the coronary microvasculature is a fundamental prerequisite to ensuring myocardial viability. Therefore, therapeutic approaches should be developed to prevent and treat microvascular impairment resulting from ischemia-reperfusion. Also, given the importance of the assessment and treatment of post-reperfusion disorders of coronary microvasculature, a diagnostic tool able to evaluate the structural and functional status of the microcirculation *in vivo* is needed. Myocardial contrast echocardiography has been demonstrated to be extremely useful in this setting.

In this review, the anatomic and functional characteristics of the coronary microcirculation are described during normal conditions, as well as in the presence of ischemia-reperfusion injury. The role of myocardial contrast echocardiography in the assessment of microvascular dysfunction and specific potential therapeutic approaches to the treatment of microvascular damage during ischemia and after reperfusion are also discussed.

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As a result of ischemia induced by acute occlusion of an epicardial coronary artery, intramyocardial microvessels and myocytes undergo progressive functional and structural changes. Vascular and cellular damage follows a temporal sequence that begins with functional abnormalities of the microcirculation, then functional and anatomic damage of myocytes develops, lastly the microvascular network is structurally deranged[1]. Reopening of an occluded vessel with consequent coronary reperfusion is capable of limiting the extent of irreversible myocardial and microvascular damage. Nevertheless, restoration of blood flow may itself be responsible for additional damage, a condition referred to as reperfusion injury[2]. The extent of preserved microvascular integrity following an ischemia-reperfusion cycle is very likely determined by both the duration and severity of ischemia as well as the relative damage produced by reperfusion.

During ischemia-reperfusion, preservation of the integrity of the coronary microvasculature is a prerequisite to ensuring myocardial salvage and recovery of function[3]. Therefore, it is necessary that approaches be developed to study the pathophysiology, diagnosis, prevention and treatment of post-ischemic damage at the microvascular level. Myocardial contrast echocardiography has been demonstrated to be extremely useful in this setting. Gas microbubbles flow freely within the microcirculation, with a rheologic behavior similar to that of red blood cells[4], and can be readily detected by ultrasound[5].

Based on experimental and clinical studies, several agents have shown the potential to limit the extension of myocardial necro-

sis and facilitate the recovery of post-ischemic contractile dysfunction, if administered before or soon after reperfusion.

In this review, the anatomic and functional characteristics of the coronary microcirculation are described during normal conditions, as well as in the presence of ischemia-reperfusion injury. Specific potential diagnostic and therapeutic approaches to the microvascular damage accompany ing ischemia and reperfusion are presented.

Anatomy and physiology of coronary microcirculation

The myocardial microvascular network is comprised of capillaries, pre-capillary arterioles and post-capillary venules[6]. The pre-capillary arteriole is < 100 μm in diameter with a wall formed by intima, media and adventitia. Small arterioles merge into capillary vessels which have a lumen diameter of 8-10 μm, a wall of endothelial cells and the absence of pores. Myocardial tissue is characterized by a high and uniform density of capillary vessels[7] (approximately 3000 per mm^3 of tissue) with a capillary-myocardial fiber ratio of about 1:1 (Fig. 1). Post-capillary venules have a diameter of 10-35 μm and differ from arterioles in having only one or two layers of smooth muscle cells in the media and no internal elastic lamina.

Under normal conditions, the physiology of the coronary microcirculation is designed to assure adequate fluid and nutrient exchanges through the capillary wall. To achieve this, hydrostatic pressure must be maintained constant at a level of approximately 30 mmHg. Arterioles control this pressure gradient through vasodilation or vasoconstriction in response to changes in the perfusion pressure. This process of autoregulation ensures adequate perfusion to achieve normal myocardial function.

Functional and structural abnormalities of coronary microcirculation due to ischemia-reperfusion

As a result of ischemia-reperfusion, the coronary microcirculation may be functionally impaired and the microvascular network structurally damaged. Experimental studies using models of short-term ischemia (10-20 min) have demonstrated that functional disturbances of the microcirculation precede any evidence of morphologic damage of either myocytes or microvessels[1,8]. These functional disturbances are characterized by a decrease in the number of perfused capillaries, by

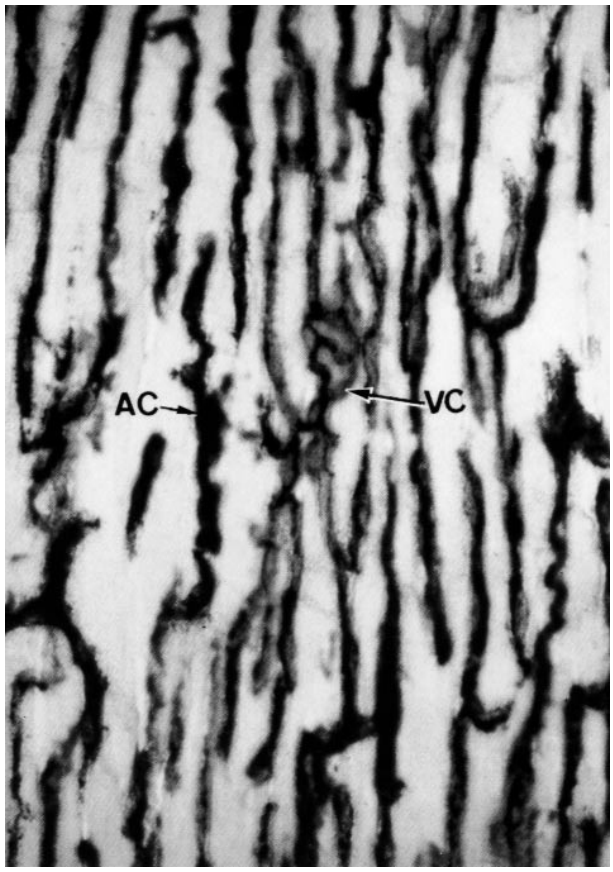

Figure 1. *Microphotography of the left ventricle of the rat heart, stained by the histochemical method[7]. Arteriolar capillaries (AC) stain blue, venular capillaries (VC) stain red. The capillaries run nearly along the vertical axis. Scale bar = 25 μm. Courtesy of Sanjay Batra, PhD.*

a reduction in the absolute volume of blood flow, by increased permeability, and especially by impairment of vasodilatory reserve[9-11].

Several mechanisms have been postulated to explain the reduction of microvasculature reserve seen after short duration ischemia: 1) loss of endothelium-mediated vasomotion[10]; 2) alteration of the sympathetic nervous system[12]; 3) increased vascular resistance of arterioles and capillaries due to interstitial edema[13] or neutrophil plugging[14]; 4) spasm of coronary resistance vessels[15]. This impairment occurs only at the microvascular level, as confirmed by the observation that large coronary arteries maintain their vascular reactivity after ischemia-reperfusion[10].

Anatomic damage of the coronary microvasculature develops as a result of more prolonged ischemia (Table I). Kloner et al.[1] demonstrated that, after 40 min of ischemia followed by reperfusion, the microvascular structure is still intact while there is already evidence of irreversible myocyte damage. However, after 90 min of ischemia, microvessels of the subendocardial layers are structurally damaged and prevented intramyocardial reperfusion (Fig. 2). They termed this condition the no-reflow phenomenon [16].

Table I. Myocardial cell grade and concomitant microvascular damage.

Duration of ischemia	Myocardial cell grade	Concomitant microvascular abnormality
20 min	Grade 1 Nuclear chromatin clumping alone or with occasional vacuoles; wide I bands	Normal
40 min	Grade 2 Criteria for grade 1 plus intermyofibrillar edema and more vacuoles; swollen mitochondria	Endothelium usually normal; occasionally swollen (< 2%) occasional red blood cell stasis
60 min	Grade 3 Criteria for grade 2 plus numerous vacuoles and sarcolemmal membrane lifted off myofibrils	Endothelium often normal; or decreased pinocytotic vesicles, swelling, blebs, gaps; red blood cell stasis
90-180 min	Grade 4 Severe swelling and architectural disruption	Endothelial blebs, gaps; foci of hemorrhage

From Kloner et al.[1], modified.

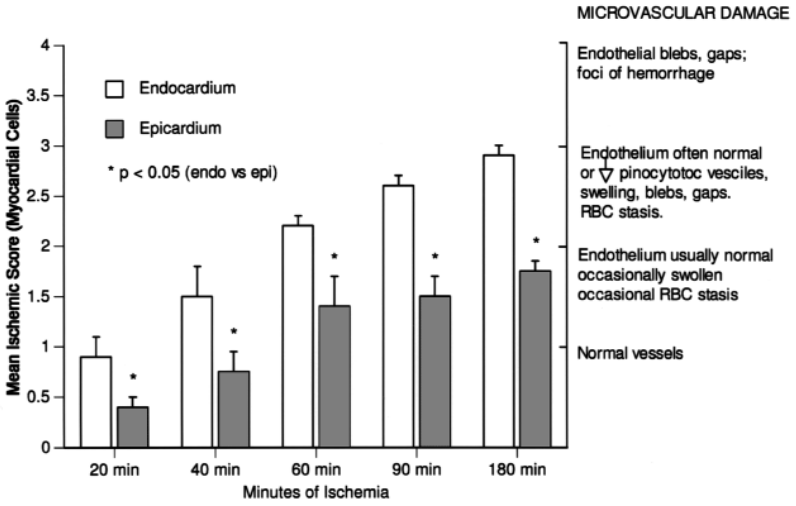

Figure 2. *Mean ischemic score for myocardial cells and the concomitant microvascular damage and duration of the occlusion for both subendocardial and subepicardial myocardium. The subendocardium always showed a more severe damage at any given time-interval than the subepicardium. RBC = red blood cell. From Kloner et al.[1], modified.*

In the course of acute myocardial infarction, early reperfusion is the most effective way to decrease necrosis and to preserve left ventricular function[17-19]. However, reperfusion itself may have deleterious effects on post-ischemic myocardium[20]. The death of viable cells due to reperfusion has been designated as *reperfusion injury* . In the microvasculature, myocardial reperfusion can lead to dynamic and evolving damage, characterized by a progressive reduction of flow to the ischemic area.

Ambrosio et al.[21] provided evidence of the existence of reperfusion injury. They analyzed the reflow data of two groups of dogs subjected to 90 min occlusion, the first reperfused for 2 min and the second for 3.5 hours. In animals in which blood flow was restored for 2 min, 9.5% of the risk region was characterized by true absence of reflow and coagulation necrosis. In dogs reperfused for 3.5 hours, the area of impaired perfusion was nearly 3 times larger (25.9% of the risk region, $p < 0.05$) and manifested contraction band necrosis, a marker of tissue damage induced by reperfusion. Reperfusion injury developed in regions not supplied by collateral flow during ischemia and was associated with neutrophil accumulation and progressive capillary plugging. Regional blood flow data obtained by microspheres[21] as well as by rubidium-82 positron emission tomography[22] demonstrated a delayed, progressive fall in flow to areas that had initially received adequate reperfusion.

The microvascular damage produced by prolonged ischemia-reperfusion may have several mechanisms. Moreover, it is difficult to distinguish the damage due to ischemia from that due to reperfusion. For example, chemical mediators produced during ischemia, such as oxygen free radicals and endothelin exert maximal damage during reperfusion at both endothelial and myocyte level[23]. Other proposed mechanisms are increased deposition of platelets after a period of ischemia[24]; marked increase in the leukocyte deposition, with histologic evidence of plugging of the microvascular bed by leukocytes[25] (Fig. 3); fibrin plugs and development of microthrombi[26]; microvascular compression due to myocyte edema[13] or contracture[27] or microvascular obstruction due to endothelial cell swelling[28]. Regardless of the mechanism, the final result is impedance to flow.

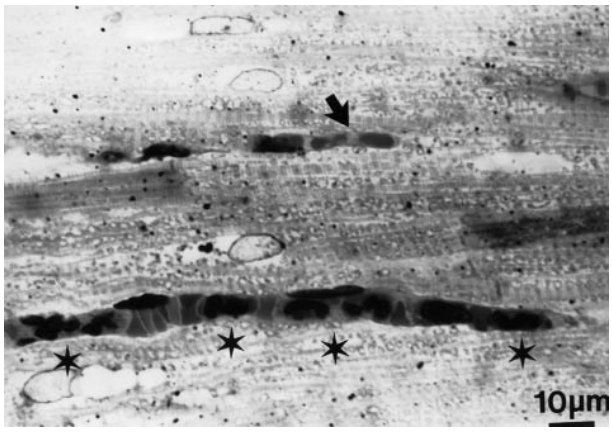

Figure 3. *Light micrographs of histological section in canine mid-endocardium after 3 hours of ischemia followed by reperfusion showing a normal capillary* (arrow) *and a capillary obstructed by several neutrophilic granulocytes and red blood cells* (stars).

Assessment of post-ischemic microvascular damage in humans

Although primarily examined in experimental preparations, ischemia-reperfusion injury of the coronary microvasculature has been studied in humans mainly by means of myocardial contrast echocardiography. In patients with acute anterior myocardial infarction in whom the infarct-related artery was reopened within 6 hours of the onset of symptoms by intracoronary thrombolysis or angioplasty, Ito et al.[29] performed myocardial contrast echo by direct intracoronary injection of sonicated Ioxaglate. They demonstrated that angiographically documented recanalization of epicardial vessels does not necessarily produce evidence of intramyocardial reperfusion by echo. A residual perfusion defect at contrast echo was evident in approximately 20% of patients after successful reopening of the infarct-related artery. Functional contractile recovery in patients who manifested effective intramyocardial reflow was superior than in patients with evidence of no-reflow. More recently, Porter et al.[30] detected no-reflow areas in patients after acute myocardial infarction using intravenous injection of a new ultrasound contrast agent.

The clinical relevance of the no-reflow phenomenon has clearly been demonstrated by Ito et al.[31] in a series of 126 successfully reperfused patients with first anterior infarction. From the intracoronary myocardial contrast pattern, 47 patients (37%) were identified as non-uniformly reperfused, the remaining 79 patients had complete reflow. Outcome data showed that in patients with no reflow left ventricular remodeling was more severe, pericardial effusion and early congestive heart failure were more frequent, and congestive heart failure was more prolonged.

Microvascular damage and myocardial dysfunction

In order to understand the functional and prognostic consequences of myocardial reperfusion, the interactions between flow and function in the post-ischemic reperfused myocardium need to be investigated more thoroughly. After acute myocardial infarction, dysfunctional but viable myocardium has a normal ultrastructure and retains its contractile reserve[32]. This reserve can be detected by two-dimensional echocardiography after inotropic stimulation with agents such as dobutamine[33,34].

Although investigating different pathophysiologic aspects, both contrast echocardiography and dobutamine echocardiography can provide information on the presence of post-infarct viable myocardium. The former detects microvascular integrity, while the latter defines the contractile reserve of dysfunctioning myocardium. In patients with recent myocardial infarction, we compared the relative potential of myocardial contrast echo and low dose dobutamine to define myocardial viability within the post-infarct dysfunctional risk area[3,35]. Regional left ventricular function improved both during dobutamine infusion and at follow-up only in patients with evidence of preserved microvascular integrity by contrast echo. On the other hand, in the presence of anatomic damage of microcirculation, neither contractile reserve nor functional recovery were observed (Fig. 4). These results are concordant with the data of Ito et al.[31] and Sabia et al.[36] which demonstrated that regional and global myocardial function at follow-up is superior in the presence of preserved microvascular integrity.

In our study, the sensitivity and specificity for identify ing post-infarct segments with functional recovery at follow-up were 100 and 46% for contrast echo and 71 and 88% for dobutamine echo[3]. Similar re-

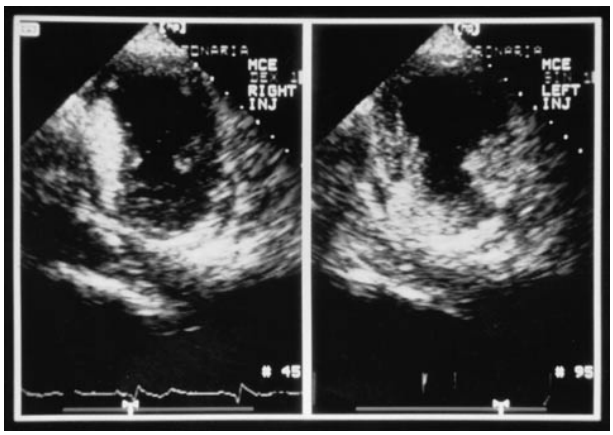

Figure 4. *This figure shows a myocardial contrast echo (MCE) study of a patient with acute myocardial infarction of the anterior wall in whom the infarct-related artery was patent. Selective contrast injection of sonicated Ioxaglate into the right coronary artery* (left panel) *depicts the perfusion bed of the right coronary artery. However, when the contrast agent was injected into the left infarct-related coronary artery* (right panel), *the anterior wall showed a clear perfusion defect indicative of no-reflow, a sign of irreversible damage of the microvascular network. The intramyocardial perfusion pattern did not correlate with the angiographic patency of the infarct-related vessel, thus myocardial contrast echocardiography provided additional valuable information on myocardial perfusion after infarct.*

sults have been reported by Bolognese et al.[37] in a group of 30 patients with acute myocardial infarction revascularized by angioplasty. Contrast echo showed a sensitivity in predicting functional recovery similar to that of dobutamine echo (96 vs 89%, respectively). However, the specificity of contrast echo was lower than that of dobutamine echo (18 vs 91%, $p < 0.001$). In fact, in a subset of dysfunctional segments with evidence of microvascular integrity at contrast, regional function failed to improve during dobutamine and at follow-up. The explanation for this discordance between anatomic microvascular integrity, contractile reserve and recovery of function at follow-up requires further investigation. The most likely mechanism is related to the presence of incomplete myocardial necrosis either only subendocardial or transmural, but patchy. In these conditions, the preserved microcirculation provides blood flow to myocytes which remain viable, although unable to manifest contractile reserve or functional recovery at follow-up[38]. Residual stenosis of the infarct-related vessel may also be responsible for the absence of myocardial contraction during dobutamine or at follow-up, because of inadequate blood supply to the preserved microcirculation.

Effects of therapeutic interventions on post-ischemic microvascular dysfunction

A variety of drugs have been tested, mostly in experimental models, in an attempt to reduce the microvascular damage produced by ischemia-reperfusion. The main agents, their mechanisms of action in the treatment of ischemia-reperfusion injury and their evaluation by myocardial contrast echocardiography are reported.

Vasodilators. Intracoronary adenosine, administered in dogs after 90 min of ischemia followed by reperfusion, produced a 2-fold increase in myocardial blood flow during reperfusion and resulted in a 75% reduction in infarct size and a significant improvement in ventricular function[39]. There are at least three mechanisms that can explain these beneficial effects. The potent endothelial-independent vasodilator effect of adenosine may partially reverse any vasoconstriction of the coronary bed, therefore enhancing oxygen delivery to the ischemic myocardium[40]. Preservation of the structural and functional integrity of the endothelium in adenosine-treated animals would both permit the physiologic release of endothelial-derived relaxing factors and reduce the degree of capillary obstruction by endothelial cell protrusions. Adenosine may also protect reversibly damaged myocardium by interfering with interaction of neutrophils with the endothelial cells, thus reducing their adherence and cytotoxicity[41]. Furthermore, adenosine has been shown to decrease oxygen free radical production by neutrophils[42]. Recently, vesnarinone, a quinolinone derivative, which may lead to increases in adenosine levels in the heart[43], has also been demonstrated to significantly limit infarct size compared with controls (6.8 – 2.2 vs 44.7 – 3.9%).

Several potent vasodilators have been tested in humans to assess whether they could attenuate the no-reflow phenomenon. Intracoronary papaverine has produced a significant improvement of the flow grade in post-angioplasty patients with myocardial infarction and no-reflow defined angiographically as TIMI flow grade 1 or 2 without obstruction in the epicardial artery[44]. Nicorandil is a new agent which has hybrid properties as a nitrate type vasodilator and a potassium channel opener[45]. Both actions have been shown to be of benefit against ischemia-reperfusion injury in animals[46-48]. Myocardial blood flow by contrast echo and regional left ventricular function have been assessed in a group of post-infarct patients after coronary reperfusion and nicorandil administration[49]. Reflow and regional wall motion significantly improved in 1 month in patients receiving nicorandil.

Captopril may scavenge free radicals, blunt the catecholamine response, elicit coronary vasodilation, and increase prostacycline and bradykinin levels[50]. Recently, ACE-inhibition at the time of reperfusion has been demonstrated to enhance coronary blood flow and prevent impairment of endothelium-dependent arteriolar responses in a swine model[51]. However, in the same model, neither captopril or enalaprilat were able to enhance

regional ventricular function acutely, despite improved microvascular endothelial function and augmented post-ischemic coronary blood flow[51].

Since arteriolar spasm has been proposed as a possible mechanism of post-ischemic injury, attention has been focused on the potent vasoconstrictor, endothelin[52]. It has been observed that a simultaneous increase in the synthesis and release of endothelin, along with an enhanced vascular reactivity to exogenous endothelin, occurs during acute myocardial infarction[53]. This has reinforced interest in the possible pathophysiological role of endogenous endothelin in the development of ischemia-reperfusion injury[54,55]. Several studies have investigated this issue by using various agents that block endothelin-1 synthesis or receptors[56,57]. Even though conflicting results exist regarding the cardioprotective effects of these anti-endothelin agents, most of the experimental evidence shows that the administration of endothelin receptor antagonists during ischemia-reperfusion limits infarct size and improves the recovery of flow as well as the endothelium-dependent vasodilation[55]. Furthermore, we have recently shown that an endothelin A selective antagonist was able to reduce no-reflow and infarct size in dogs, when administered at the time of reperfusion[58].

Calcium channel blockers. Because of their potent effect on coronary microvessels, calcium channel antagonists have been proposed for the therapy of microvascular dysfunction due to ischemia-reperfusion. Tillmanns et al.[59] demonstrated the efficacy of the treatment with nifedipine and verapamil in reducing microcirculatory disturbances in a model of reversible post-ischemic dysfunction induced in rats. Several mechanisms have been proposed to explain the beneficial effects of calcium channel antagonists: 1) dilation of larger arterioles in addition to the terminal vessels affected by ischemia, 2) limitation of myocardial flow decrease, 3) attenuation of the reduction in blood cell content with more homogeneous capillary perfusion, 4) reduction in the number of leukocytes adhering to capillaries and post-capillary venules, and 5) block of intracellular calcium overload[59].

In an experimental model of reversible coronary occlusion, the extension of no-reflow and myocardial necrosis was significantly reduced by the administration of gallopamil during ischemia[60]. When administered during ischemia, calcium antagonists reduced oxygen demand due to a reduction in heart rate, arterial pressure and double product; during reperfusion, they were also able to indirectly reduce the damage due to oxygen free radicals.

In a subgroup of patients who underwent coronary angioplasty (2% of the total population), Piana et al.[61] observed a substantial reduction in coronary flow at angiography (TIMI flow grade < 3 in the absence of dissection, thrombosis or embolization of distal vessels). Most of these patients (89%) with angiographic no-reflow phenomenon showed a substantial improvement in coronary blood flow after intracoronary administration of verapamil, with augmented perfusion of distal vessels and blood flow velocity. With return to normal flow, angina and ST segment elevation, which were present in 78% of these patients with no-reflow, were also reduced. To explain this no-reflow phenomenon after mechanical reopening of a coronary artery, the authors postulated the existence of microcirculatory spasm due to the release of potent vasoconstrictor substances, such as serotonin, from cellular elements contained in the thrombus. In this particular clinical setting, the endoarterial administration of verapamil was demonstrated to be particularly effective in attenuating the no-reflow phenomenon, probably due to vasodilation with release of microvascular spasm.

In 19 patients with a first uncomplicated anterior myocardial infarction, Taniyama et al.[62] tested the potential of verapamil and nicorandil to limit infarct area and vascular damage, and attenuate reperfusion injury. Chronic oral therapy followed acute administration. Regional myocardial contractile function and contrast enhancement were evaluated using echocardiography at 1 and 28 days after acute myocardial infarction. The extension of the no-reflow area, and thus of microvascular damage, was reduced after acute administration of verapamil and nicorandil. In addition, the amount of perfused myocardium at contrast echo, an expression of microvascular integrity, was increased. In the first 28 days after infarction, regional contractile function was improved compared to the control group, but the amount of improvement was greater in patients treated with verapamil. Thus, the acute intracoronary administration of verapamil was able to improve vascular function in the ischemic area and to positively influence left ventricular function.

Oxygen radical scavengers. Adequate tissue oxygenation through enhanced oxygen delivery[63] or reduced oxygen demand[64] has been shown to reduce post-ischemic injury. Nevertheless, the generation of toxic oxygen free radicals at the time of, and shortly after, reperfusion is the most widely accepted mechanism for reperfusion injury[23]. Numerous recent studies show that treatment with free radical scavengers can improve structural and functional recovery after myocardial ischemia. Benefit was found by superoxide dismutase alone[65] or by superoxide dismutase combined with either catalase[66] or mannitol[67]. However, not all studies are concordant on this issue. In fact, Gallagher et al.[68] have found that superoxide dismutase provided no protection against reperfusion injury in the conscious dog. Microvascular injury is a sensitive index of the effects of

oxygen radical scavengers, because neutrophils accumulate within the microvasculature during ischemia-reperfusion and because microvascular endothelium with its relatively high xanthine oxidase content is a significant source of reactive oxygen metabolites[23]. Thus far, therapy with oxygen radical scavengers has been successful in decreasing the functional coronary microvascular injury due to ischemia-reperfusion both by reducing protein leaking[69] and improving microvascular flow reserve[70].

Conclusions

The therapy of acute myocardial infarction is based on the effective and immediate reestablishment of myocardial perfusion. The reopening of the epicardial infarct-related artery does not imply the achievement of adequate myocardial reperfusion; in fact microcirculatory flow may be absent resulting in the no-reflow phenomenon. This phenomenon is not merely a pathophysiological curiosity , but an important clinical entity which needs to be promptly recognized. At the current time, contrast echocardiography performed by intra-arterial injection of contrast agents has been the primary diagnostic modality applied to identify the no-reflow phenomenon. New developments involving both contrast agents[71] and ultrasound technology[72] which now permit myocardial opacification by the intravenous approach, will make clinical applications of myocardial contrast echocardiography more feasible and useful (Fig. 5). A variety of therapeutic agents have now been demonstrated to produce salutary effects upon ischemia-reperfusion injury.

In light of the functional and clinical implications of post-ischemic microvascular damage, it appears logical that the optimal management of evolving myocardial infarction requires a two-pronged approach. Therapy should consist of the pharmacological or mechanical reopening of the infarct-related artery, but also of the protection of microvessels and myocytes prior or during reperfusion, in order to ensure an adequate intramyocardial reperfusion and myocyte functional recovery.

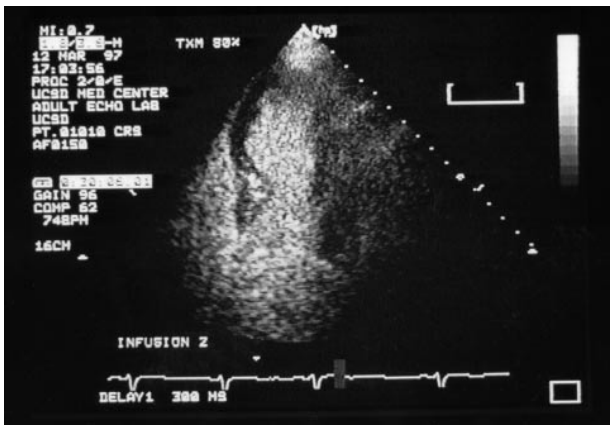

Figure 5. *In this example, a patient with acute anterior myocardial infarction has been studied by contrast echocardiography. The contrast agent was intravenously injected, 48 hours after successful reperfusion of the infarct-related artery by percutaneous coronary angioplasty. The contrast filled the left ventricular cavity and perfused the basal interventricular septum and the lateral wall. A clear perfusion defect was observed in the mid-apical part of the septum, indicating extensive post-infarct damage.*

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