Editorial

Stunned myocardium—an unfinished puzzle

Franz R. Eberli*

Cardiovascular Center, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland

Received 7 June 2004; accepted 9 June 2004

See article by Colantonio et al. [15] (pages 217–225) in this issue.

Myocardial stunning describes the phenomenon of reversible contractile dysfunction of viable myocardium after restoration of normal or nearly normal epicardial coronary flow following myocardial ischemia. In man, myocardial stunning has been observed following cardiac surgery (global ischemia and reperfusion), in myocardial infarction after timely reperfusion therapy (regional prolonged severe ischemia and reperfusion with stunned myocardium around areas of necrosis), in unstable angina and coronary spasm (regional transient ischemia without or with minimal necrosis), and in exercise-induced angina (demand ischemia with almost normal blood flow). Recurrent episodes of stunning might result in hibernating myocardium [1,2]. The designs of experiments used to elucidate the cellular and molecular mechanisms involved in myocardial stunning were as heterogeneous as the clinical settings involved in this phenomenon.

Oxygen-free radicals and intracellular calcium overload have been identified as two pathogenetic mechanisms inducing intracellular damage that results in contractile dysfunction [1–3]. These pathogenetic processes adversely affect several molecular targets that can potentially contribute to myocardial stunning (Table 1 and Refs. [1–3]), and will result in a change of the proteomics of the myocyte.

Contractile function is the result of a complex and fine-tuned interplay of the myofilament proteins. Disturbances of each of the parts composing this intricate system may lead to contractile dysfunction and contribute to myocardial stunning [3,4]. Accordingly, changes in the thick filament, mainly composed of myosin heavy chains and the associated light chains, and in the thin filament, composed of polymeric actin, troponin (Tn), tropomyosin, and the structural proteins, e.g. α-actinin, have been reported in myocardial stunning. In recent years, research has focused on proteolytic TnI degradation as a possible molecular mechanism of stunning. Strong indirect evidence has revealed that calcium-activated proteases such as calpain I, which cleaves both TnI and TnT in vitro, are activated by the intracellular calcium overload and result in specific troponin I degradation by proteolysis [4,5]. The main degradation product is a fragment, TnI $_{1-193}$, from the C-terminus. In addition, calcium activates transglutaminases, which form covalent complexes of the troponin degradation products. The TnI degradation product and its interference with the myofilaments were thought to exert the contractile dysfunction of myocardial stunning in the isolated perfused rat heart [4,5]. Several observations suggested that the proteolytic troponin I degradation product TnI $_{1-193}$ could replicate the phenotype of stunning. First, incorporation of the troponin complex from stunned myocardium into rabbit skeletal muscle fibers resulted in a decreased calcium sensitivity and force generation as compared to controls [6]. Second, in a transgenic mouse model over-expressing the cleaved TnI $_{1-193}$, the phenotype of stunned myocardium, i.e. diminished calcium-activated tension but intact response to β-adrenergic stimulation, was reproduced [7]. According to this evidence, it was proposed that damage to the myofilament proteins caused contractile dysfunction, and, likewise, the repair or restoration of normal proteins over time was responsible for the transient nature of stunning. This attractive explanation has been questioned based on recent experiments. In biochemical assays, the degradation product TnI $_{1-193}$ actually showed an increased calcium sensitivity compared to normal TnI [8,9]. In isolated rat hearts subjected to ischemia and reperfusion that resulted in myocardial stunning, the degradation of TnI could be prevented by keeping normal left ventricular filling pressures [10,11]. Furthermore, in stunned myocardium induced by regional ischemia in the swine [12,13] and the dog [14], degradation of TnI was absent. Therefore, TnI degradation was able to

* Tel.: +41-1-255-33-97, +41-1-255-22-16; fax: +411-255-44-01.
E-mail address: franz.eberli@usz.ch (F.R. Eberli).

Table 1
Possible mechanisms involved in myocardial stunning

- Triggers: oxygen-free radicals and calcium overload
- Abnormalities of proteins of the contractile apparatus (e.g. troponin I, troponin T, myosin-regulatory light chain 2)
- Abnormalities of calcium-handling proteins (e.g. phospholamban, SR-Ca^{2+}-ATPase)
- Impaired energy production and impaired energy use by myofibrils
- Damage to extracellular matrix

cause contractile dysfunction, but appeared not to be the primary mechanism of myocardial stunning.

In this issue of *Cardiovascular Research*, Colantonio et al. [15] add further evidence to the notion that TnI degradation may not be essential for myocardial stunning. In an in vivo animal model of myocardial infarction, the authors examined whether in viable, but stunned, peri-infarct tissue, degradation of TnI or other troponins (TnT or TnC) contributed to myocardial stunning. No degradation of TnI and of TnC was detected. However, they found that TnT was degraded. In a few animals, covalent complexes were detected. Covalent complexes are purportedly composed of TnI fragments bound to TnT, suggesting that minimal TnI degradation had taken place in some animals; but neither the extent of TnT degradation nor the presence or absence of covalent complexes were associated with myocardial stunning. The authors conclude that, similar to TnI degradation, TnT degradation plays no integral role in the pathogenesis of stunned myocardium.

An ancillary aim of the paper was to test whether preconditioning affected troponin degradation. As in many previous experiments [1,2], preconditioning did not affect myocardial stunning during the 3-h recovery period. Nevertheless, preconditioning did decrease the rate of TnT degradation. The authors speculate that this could result in better long-term functional recovery.

Where do these findings leave us? Can we discard TnI degradation or abnormalities of the myofilaments as a cause of myocardial stunning? As a primary cause of myocardial stunning in large animals, we probably can. However, troponin degradation and other changes in the myofilaments occur during ischemia and reperfusion. These abnormalities of the myofilaments induce contractile dysfunction. It has been pointed out that such changes may contribute to global LV dysfunction in other disease states [5,8]. Given the wide variety of experimental and clinical situations accompanied by myocardial stunning, it is also conceivable that abnormalities in the contractile proteins contribute to some forms of stunning and not to others.

With respect to molecular mechanisms responsible for myocardial stunning, it is generally assumed that reactive oxygen species and calcium trigger stunning. Experimentally scavenging free oxygen radicals and preventing calcium overload during reperfusion attenuate myocardial stunning. Questions remain about the source of reactive oxygen species in ischemia–reperfusion and how they

contribute to contractile dysfunction [3,16]. However, the central role of reactive oxygen species as a signaling mechanism for a large array of molecular networks and metabolic pathways is recognized [16], and it is well conceivable that they are important in protection mechanisms such as preconditioning and in damaging the cell as in myocardial stunning. The role of abnormalities of calcium handling in myocardial stunning has recently been re-examined [13,14]. Kim et al. [13] delineated nicely the difference of calcium handling in stunned myocardium of the swine and the rat. They found decreased calcium transients and slowed relaxation in the swine, whereas in rats the calcium transient was unchanged and relaxation was accelerated despite contractile dysfunction. Although the examined expression of calcium handling proteins was not altered in the stunned myocardium, the phosphorylation of phospholamban was reduced [13,14]. Therefore, abnormalities in calcium handling may well play an important role in myocardial stunning in large animals [3]. Further exploration of the signaling pathways of reactive oxygen species and calcium and, in addition, renewed examination of abnormalities in calcium handling might advance our understanding of the mechanisms involved in myocardial stunning.

What about some of the previously discarded causes of myocardial stunning [17]? Impaired energy production by mitochondria or impaired energy use by myofibrils as causes of myocardial stunning have been discarded, since decreased adenosine triphosphate (ATP) levels and the extent of contractile dysfunction were dissociated. However, it has been subsequently demonstrated that not ATP content but rather ATP turn-over and, more importantly, the free energy released from ATP hydrolysis determine the energetics of the cell [18]. The normal function of enzymes depends on the free energy available to them. SR-Ca^{2+}-ATPase requires the highest free energy (52 kJ/mol) for normal function and will be the first ion pump to be impaired by inadequate energy production. Given the dependence of calcium-handling proteins on normal energetics [19] and the importance of calcium in myocardial stunning, it is plausible that abnormalities of energy metabolism contribute to myocardial stunning after all. Furthermore, in a series of experiments Taegtmeyer et al. [20] have shown that efficient myocardial contractile function is dependent on a series of moiety-conserved cycles, of which the citric acid cycle seems to be the most important. Ischemia and reperfusion result in a transformation of efficient metabolic cycles to less efficient linear pathways [20]. In addition, during ischemia and reperfusion, residual oxidative phosphorylation is dominated by free fatty acid oxidation, and glucose becomes uncoupled from oxidation; thus, mitochondrial ATP production is impaired [19]. Replenishment of the citric acid cycle by anaplerotic reactions during reperfusion has been shown to improve post-ischemic contractile dysfunction [20]. In that regard, it is interesting to note that pyruvate, which can directly enter the citric acid

cycle and can replenish the moiety-deprived citric acid cycle via anaplerotic reactions, attenuated myocardial stunning in the swine [21]. Therefore, by indirect effects on ion homeostasis or direct effects on contractile function, energetics could influence myocardial stunning to some extent.

In summary, several complex molecular systems, i.e. reactive oxygen species, calcium-handling proteins, metabolic pathways including oxidative phosphorylation, and proteins of the contractile apparatus, are directly or indirectly involved in myocardial stunning. There are likely additional, yet unknown mechanisms associated with myocardial stunning. New methods such as proteomic and genomic studies might help elucidate how these multiple systems are linked and which ones are the most important pieces of the puzzle.

Acknowledgements

References

[1] Kloner RA, Jennings RB. Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: Part 1. Circulation 2001;104(24):2981–9.

[2] Kloner RA, Jennings RB, Rasmussen MM, et al. Consequences of brief ischemia: stunning, preconditioning, and their clinical implications: Part 2. Circulation 2001;104(25):3158–67.

[3] Kim SJ, Depre C, Vatner SF. Novel mechanisms mediating stunned myocardium. Heart Fail Rev 2003;8(2):143–53.

[4] Van Eyk JE, Murphy AM. The role of troponin abnormalities as a cause for stunned myocardium. Coron Artery Dis 2001;12(5):343–7.

[5] Canty JM, Lee TC. Troponin I proteolysis and myocardial stunning: now you see it—now you don't. J Mol Cell Cardiol 2002;34(4):375–7.

[6] McDonald KS, Moss RL, Miller WP. Incorporation of the troponin regulatory complex of post-ischemic stunned porcine myocardium reduces myofilament calcium sensitivity in rabbit psoas skeletal muscle fibers. J Mol Cell Cardiol 1998;30(2):285–96.

[7] Murphy AM, Kogler H, Georgakopoulos D, et al. Transgenic mouse model of stunned myocardium. Science 2000;287(5452):488–91.

[8] Foster DB, Noguchi T, VanBuren P, Murphy AM, Van Eyk JE. C-terminal truncation of cardiac troponin I causes divergent effects on ATPase and force: implications for the pathophysiology of myocardial stunning. Circ Res 2003;93(10):917–24 [Electronic publication 2003 Oct 9].

[9] Rarick HM, Tu XH, Solaro RJ, Martin AF. The C terminus of cardiac troponin I is essential for full inhibitory activity and Ca^{2+} sensitivity of rat myofibrils. J Biol Chem 1997;272(43):26887–92.

[10] Feng J, Schaus BJ, Fallavollita JA, Lee TC , Canty JM Jr.. Preload induces troponin I degradation independently of myocardial ischemia. Circulation 2001;103(16):2035–7.

[11] Prasan AM, McCarron HC, Hambly BD, Fermanis GG, Sullivan DR, Jeremy RW. Effect of treatment on ventricular function and troponin I proteolysis in reperfused myocardium. J Mol Cell Cardiol 2002;34(4):401–11.

[12] Luss H, Boknik P, Heusch G, et al. Expression of calcium regulatory proteins in short-term hibernation and stunning in the in situ porcine heart. Cardiovasc Res 1998;37(3):606–17.

[13] Kim SJ, Kudej RK, Yatani A, et al. A novel mechanism for myocardial stunning involving impaired Ca(2+) handling. Circ Res 2001;89(9):831–7.

[14] Luss H, Meissner A, Rolf N, et al. Biochemical mechanism(s) of stunning in conscious dogs. Am J Physiol, Heart Circ Physiol 2000;279(1):H176–84.

[15] Colantonio DA, Van Eyk JE, Przyklenk K. Stunned peri-infarct canine myocardium is characterized by degradation of T Tropinin, not Troponin I. Cardiovasc Res 2004;63:217–25.

[16] Becker LB. New concepts in reactive oxygen species and cardiovascular reperfusion physiology. Cardiovasc Res 2004;61(3):461–70.

[17] Bolli R, Marban E. Molecular and cellular mechanisms of myocardial stunning. Physiol Rev 1999;79(2):609–34.

[18] Cave AC, Ingwall JS, Friedrich J, et al. ATP synthesis during low-flow ischemia: influence of increased glycolytic substrate. Circulation 2000;101(17):2090–6.

[19] Opie LH, Sack MN. Metabolic plasticity and the promotion of cardiac protection in ischemia and ischemic preconditioning. J Mol Cell Cardiol 2002;34(9):1077–89.

[20] Taegtmeyer H, Goodwin GW, Doenst T, Frazier OH. Substrate metabolism as a determinant for postischemic functional recovery of the heart. Am J Cardiol 1997;80(3A):3A–10A.

[21] Kristo G, Yoshimura Y, Niu J, et al. The intermediary metabolite pyruvate attenuates stunning and reduces infarct size in in vivo porcine myocardium. Am J Physiol, Heart Circ Physiol 2004;286(2):H517–24.