Cytoskeletal Proteins

– Hidden Targets of Cytoprotective Signaling? –

Tetsuji Miura, MD, PhD

Sarcolemmal rupture, a hallmark of cardiomyocyte necrosis after ischemia–reperfusion, is induced by an increase in sarcolemmal fragility during ischemia, as well as by mechanical stress on the sarcolemma imposed by cell edema and the restoration of contraction upon reperfusion.¹ How ischemia induces fragility of the sarcolemma remains unclear, but a putative mechanism is loss of cytoskeletal proteins. Long-sustained ischemia reduces sarcolemmal levels of dystrophin, β -dystroglycan, γ -sarcoglycan, vinculin, spectrin and ankyrin, and dystrophin is the most sensitive of these structural proteins to ischemia.^{2–4} Dystrophin connects F-actin in the subsarcolemmal cytoskeleton or the sarcomeres to δ -dystroglycan spanning the cell membrane, and is a member of the dystrophin glycoprotein complex (DGC).⁵ Ischemia induces translocation of dystrophin from the sarcolemma to the intracellular pools, and this ischemia-induced loss of dystrophin in the sarcolemma has been shown to correlate with formation of sarcolemmal blebs.² On the other hand, in isolated cardiomyocytes the loss of spectrin from the sarcolemma has correlated with fragility of the cell to hypo-osmotic stress.² However, these findings do not necessarily indicate that loss of dystrophin and/or spectrin is sufficient for priming cardiomyocytes for sarcolemmal rupture upon reperfusion.

Article p 2393

Dystrophin and other members of the DGC are crucial for physiological regulation of membrane permeability. In myocardium in which dystrophin or sarcoglycans is genetically deleted, permeability of the sarcolemma assessed by influx of cell-impermeable tracers (eg, Evans Blue [EB] and albumin) is significantly increased.^{5,6} Intracellular Ca^{2+} levels are elevated in these cardiomyocytes possibly by upregulation of the Ca^{2+} -permeable, growth-factor-regulated channel (TRPV2).⁷ Ca^{2+} influx via this channel potentially aggravates cell injury by activation of a Ca^{2+} -activated protease, calpain, leading to a vicious cycle of loss of structural membrane protein, increased sarcolemmal instability and Ca^{2+} overload. This vicious cycle has been proposed as a mechanism of progressive cell loss and heart failure in animals and patients with mutations in the DGC.^{5,6}

Otani et al have conducted a series of studies to address the causative relationship between dystrophin loss from the sarcolemma during ischemia and reperfusion-induced cardiomyocyte necrosis.^{4,8–10} They first showed in the rat myocar-

dium that approximately 60% of membrane dystrophin was lost after 30 min of ischemia and that further loss of dystrophin occurred after reperfusion.⁴ Inhibition of ventricular contraction by 2,3-butanedione monoxime (BDM) during reperfusion did not affect the loss of sarcolemmal dystrophin, but did inhibit the increase in sarcolemmal permeability to EB after reperfusion, indicating that mechanical stress is crucial for the pathological increase in sarcolemmal permeability upon reperfusion. They found that ischemic preconditioning (IPC) restores sarcolemmal dystrophin and suppresses the increase in sarcolemmal permeability after reperfusion.^{8,9} These effects of IPC were enhanced by using BDM to temporarily arrest the heart beat during the early reperfusion period. Interestingly, IPC-induced restoration of sarcolemmal dystrophin after reperfusion was dependent on mitochondrial ATP synthesis.⁹ These results support the notion that both the sarcolemmal level of dystrophin and mechanical stress during reperfusion are factors determining sarcolemmal permeability and thus susceptibility to reperfusion injury.

In this issue of the Journal, Moriguchi et al¹⁰ report that ischemic post-conditioning (IPost) alone did not restore sarcolemmal dystrophin during reperfusion but preserved sarcolemmal impermeability by a PI3K-Akt-dependent mechanism. Interestingly, as with IPC, IPost enhanced BDM-induced dystrophin restoration. Their results suggest that IPost affords sarcolemmal protection by a dystrophin-independent and PI3K-Akt-dependent mechanism and that IPost signaling has a target in the process of dystrophin restoration. The mechanism by which dystrophin is restored in the sarcolemma by IPC or by BDM plus IPost remains unclear, but phosphorylation of a C-terminal region of dystrophin, which is close to a cysteine-rich domain binding to β -dystroglycan in the membrane,^{5,11} appears to be a possibility.

Circumstantial evidence supports the hypothesis that loss of sarcolemmal dystrophin during ischemia contributes to reperfusion-induced cardiomyocyte necrosis. However, because this hypothesis has been examined by indirect (ie, BDM, IPC, IPost) manipulation of the sarcolemmal dystrophin level, the causal relationship between dystrophin loss in the sarcolemma and ischemia–reperfusion injury is not as unequivocal as the relationship between genetic defects of DGC proteins and cardiomyopathy.^{5,6} Rodriguez et al reported that ischemia-induced depletion of structural proteins was associated with significant alterations in gene transcription and translation in cardiomyocytes.³ IPC and IPost activate multiple signal

The opinions expressed in this article are not necessarily those of the editors or of the Japanese Circulation Society.

Received September 14, 2010; accepted September 14, 2010; released online October 16, 2010

Second Department of Internal Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan

Mailing address: Tetsuji Miura, MD, PhD, Second Department of Internal Medicine, Sapporo Medical University School of Medicine, South-1 West-16, Chuo-ku, Sapporo 060-8543, Japan. E-mail: miura@sapmed.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-10-0935

All rights are reserved to the Japanese Circulation Society. For permissions, please e-mail: cj@j-circ.or.jp

pathways before and upon reperfusion, which target multiple extra-sarcolemmal molecules, including mitochondrial permeability transition pores (mPTPs).^{12,13} Recently, Millay et al¹⁴ found that myocyte necrosis in dystrophin-deficient mice can be attenuated by deletion of the cyclophilin D gene or by pharmacological inhibition of mPTPs without any effects on enhanced sarcolemmal permeability. Both IPC and IPost have been shown to inhibit mPTP opening after ischemia–reperfusion in the myocardium,¹² and thus their protective actions, at least in part, might be independent of their effects on the sarcolemma.

Dystrophin may not be the only target sarcolemmal protein for IPC or IPost to be able to stabilize the membrane, and the relative importance of membrane stabilization compared with other mechanisms (such as mPTP inhibition) in the protection afforded by IPC and by IPost is still unclear. Nevertheless, accumulating evidence indicates that alterations in DCG play significant roles not only in the pathogenesis of ischemia–reperfusion injury,^{4,8–10} but also in that of heart failure^{5,6} and ventricular remodeling.¹⁵ Important targets of cytoprotective signaling appear to be hidden somewhere in the cytoskeletal proteins, and further investigation is necessary to obtain a clear picture.

References

1. Reimer KA, Jennings RB. Myocardial ischemia, hypoxia, and infarction. In: Fozzard HA, Haber E, Jennings RB, Katz AM, Morgan HE, editors. The heart and cardiovascular system. New York: Raven Press, 1986; 1133–1201.
2. Armstrong SC, Latham CA, Shivell LC, Ganote CE. Ischemic loss of sarcolemmal dystrophin and spectrin: Correlation with myocardial injury. *J Mol Cell Cardiol* 2001; **33**: 1165–1179.
3. Rodriguez M, Cai WJ, Kostin S, Lucchesi BR, Schaper J. Ischemia depletes dystrophin and inhibits protein synthesis in the canine heart: Mechanisms of myocardial ischemic injury. *J Mol Cell Cardiol* 2005; **38**: 723–733.
4. Kyo S, Otani H, Sumida T, Okada T, Osako M, Imamura H, et al. Loss of intracellular dystrophin: A potential mechanism for myocardial reperfusion injury. *Circ J* 2003; **67**: 725–727.
5. Wallace GQ, McNally EM. Mechanisms of muscle degeneration, regeneration and repair in the muscular dystrophies. *Annu Rev Physiol* 2008; **71**: 37–57.
6. Toyooka T, Kawada T, Nakata J, Xie H, Urabe M, Masui F, et al. Translocation and cleavage of myocardial dystrophin as a common pathway to advanced heart failure: A scheme for the progression of cardiac dysfunction. *Proc Natl Acad Sci USA* 2004; **101**: 7381–7385.
7. Iwata Y, Katanosaka Y, Arai Y, Komamura K, Miyatake K, Shigekawa M. A novel mechanism of myocyte degeneration involving the Ca²⁺-permeable growth factor-regulated channel. *J Cell Biol* 2003; **161**: 957–967.
8. Kido M, Otani H, Kyo S, Sumida T, Fujiwara H, Okada T, et al. Ischemic preconditioning-mediated restoration of membrane dystrophin during reperfusion correlates with protection against contraction-induced myocardial injury. *Am J Physiol Heart Circ Physiol* 2004; **287**: H81–H90.
9. Kyo S, Otani H, Hamano A, Matsuhisa S, Akita Y, Fujiwara H, et al. Dystrophin is a possible end-target of ischemic preconditioning against cardiomyocyte necrosis during the early phase of reperfusion. *Cardiovasc Res* 2006; **70**: 354–363.
10. Moriguchi A, Otani H, Yoshioka K, Shimazu T, Fujita M, Okazaki T, et al. Inhibition of contractile activity during postconditioning enhances cardioprotection by restoring sarcolemmal dystrophin through phosphatidylinositol 3-kinase. *Circ J* 2010; **74**: 2393–2402.
11. Michalak M, Fu SY, Milner RE, Bussaan JL, Hance JE. Phosphorylation of the carboxyl-terminal region of dystrophin. *Biochem Cell Biol* 1996; **74**: 431–437.
12. Hausenloy DJ, Ong SB, Yellon DM. The mitochondrial permeability transition pore as a target for preconditioning and postconditioning. *Basic Res Cardiol* 2009; **104**: 189–202.
13. Miura T, Tanno M, Sato T. Mitochondrial kinase signaling pathways in myocardial protection from necrosis after ischemia/reperfusion. *Cardiovasc Res* 2010; **88**: 7–15.
14. Millay DP, Sargent MA, Osinska H, Baines CP, Barton ER, Vagniaux G, et al. Genetic and pharmacologic inhibition of mitochondrial-dependent necrosis attenuates muscular dystrophy. *Nat Med* 2008; **14**: 442–447.
15. Han F, Lu YM, Hasegawa H, Kanai H, Hachimura E, Shirasaki Y, et al. Inhibition of dystrophin breakdown and endothelial nitric-oxide synthase uncoupling accounts for cytoprotection by 3-[2-[4-(3-chloro-2-methylphenyl)-1-piperazinyl]ethyl]-5,6-dimethoxy-1-(4-imidazolylmethyl)-1H-indazole dihydrochloride 3.5 hydrate (DY-9760e) in left ventricular hypertrophied Mice. *J Pharmacol Exp Ther* 2010; **332**: 421–428.