Effect of p38 MAP kinase on cellular events during ischemia and reperfusion: possible therapy

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Myocardial adaptation to ischemia occurs through the activation of several tyrosine kinases (3). Phosphorylation of tyrosine kinases has been shown to be linked with the activation of both phospholipase C and phospholipase D, leading to the activation of multiple kinases including PKC and mitogen-activated protein (MAP) kinases. The MAP kinases play an essential role in mediating intracellular signal transduction. The intracellular signaling mechanisms that lead to adaptation require one or more members of MAP kinase cascades. Among the three distinct MAP kinase families, stress-activated protein kinases (SAPKs), also known as c-Jun NH2-terminal kinases (JNKs) and p38 MAP kinase, are known to be regulated by extracellular stresses. JNKs and p38 MAP kinase appear to be involved in distinct cellular functions, because they possess different cellular targets and are located on different signaling pathways.

In a recent study (12), adaptation to myocardial ischemia triggered a tyrosine kinase-regulated signaling pathway leading to the translocation and activation of p38 MAP kinase and MAP kinase-activated protein (MAPKAP) kinase 2. The expression of JNK1, c-Jun, and p38 MAP kinase was also shown to increase during ischemia-reperfusion (14).

In this issue of American Journal of Physiology-Heart and Circulatory Physiology, the recent study by Okada et al. (13) examined the effect of the p38 MAP kinase inhibitor SB-203580 on cardiomyocyte (CMC) apoptosis and necrosis (currently referred to as oncosis) to implicate a primary increase in membrane permeability and resultant cellular swelling as opposed to necrosis that can be induced secondary to apoptosis (11) during simulated ischemia and reperfusion. The study describes several important and interesting findings. The authors found that p38 MAP kinase was activated after 2 h of simulated ischemia and especially during the early reoxygenation. This was associated with phosphorylation and translocation of heat shock protein 27 (HSP27) from the soluble to the insoluble fraction and reorganization of F-actin cytoskeleton. Cytochrome c was released from mitochondria, caspase-3 was activated, and DNA fragmentation was increased during simulated ischemia and reoxygenation. Robust lactate dehydrogenase (LDH) release was induced only under the hyposmotic condition during reoxygenation. SB-203580 or the F-actin disrupting agent cytochalasin D abrogated F-actin reorganization, cytochrome c release, caspase-3 activation, and DNA fragmentation during simulated ischemia and reoxygenation under the isosmotic condition. Conversely, SB-203580 and cytochalasin D enhanced LDH release under the hyposmotic condition. Under isosmotic condition, when reoxygenation was conducted, the osmotic stress prevented LDH release associated with an increase in ATP. Moreover, SB-203580 inhibited the extent of apoptosis after osmotic stress. This indicates that temporary blockade of physical stress during reoxygenation prevented both apoptotic and oncosis CMC death. On the basis of the above observations, Okada et al. (13) concluded that p38 MAP kinase activation during simulated ischemia and reoxygenation aggravates CMC apoptosis but conversely protects oncosis induced by osmotic stress presumably through F-actin cytoskeletal reinforcement. Thus inhibition of p38 MAP kinase during simulated ischemia and reoxygenation provides an antiapoptotic effect as well as a prooncotic effect. We know that temporary blockade of physical stress during reoxygenation converts CMCs from the prooncotic to the antioncotic phenotype. However, SB-203580 is not a specific inhibitor of p38 MAP kinase and inhibits both α- and β-isoforms of p38 MAP kinase. In addition, the dose of SB-203580 used in the study of Okada et al. (13) is relatively higher than that used in other studies. Thus interpretation of the results should be carried out with caution. Despite these study limitations, Okada et al. (13) for the first time shed a light on the novel aspect of p38 MAP kinase inhibition therapy that had been paid little attention or even ignored in the field of cardioprotection utilizing p38 MAP kinase inhibitors.

CMCs are unique in that their plasma membranes are subjected to physical stress during each cycle of contraction and relaxation in the intact heart. Therefore, it is likely that the effect of p38 MAP kinase inhibition differs between CMCs with and without physical stress if p38 MAP kinase is involved in maintaining the cytoskeletal and membrane integrity during ischemia and reperfusion. Okada et al. (13) have indeed raised this possibility. They found that although p38 MAP kinase inhibition was cytoprotective against apoptosis during reoxygenation in the absence of physical stress, the same inhibition provoked a robust increase in oncosis in the presence of physical stress. Thus physical stress is a critical determinant of the fate of CMCs with p38 MAP kinase inhibition at the time of reperfusion. Fragility of the sarcolemma appears to underlie the detrimental effect of p38 MAP kinase inhibition on CMCs during reperfusion. The same group of investigators (8, 9, 16) has recently reported that ischemic loss of sarcomemmal dystrophin, which plays a crucial role in stabilizing sarcolemma, predisposed the sarcolemmal fragility at the time of reperfusion. Temporary blockade of contractility on reperfusion prevented oncosic CMC death only when the heart was protected by ischemic preconditioning or by p38 MAP kinase inhibition with SB-203580 (8, 16). These previous studies indicate that a benefit of contractile arrest at the time of reperfusion appears to depend on protection of the mitochondria from ischemia-reperfusion damage and the ability of the mitochondria to produce sufficient ATP for repairing sarcolemmal fragility. Unless CMCs were capable of fixing the sarcolemmal fragility during contractile arrest, reintroduction of contractile force would have resulted in membrane disruption and oncosis. Osmotic stress has been a standard technique to impose physical stress on the sarcolemma in mechanically separated CMCs that do not become disrupted even...
when they hypercontract during reoxygenation (15). Okada et al. (13), using isolated CMCs subjected to osmotic stress during simulated ischemia and reperfusion, demonstrated that p38 MAP kinase inhibition was protective against both apoptotic and oncotic death of CMCs when physical stress was temporarily blocked during reperfusion.

Although the exact mechanism of p38 MAP kinase activation-mediated reinforcement of fragile sarcolemmal membrane is unclear at present, accumulating evidence suggests that phosphorylation of HSP27 plays a role in p38 MAP kinase-mediated F-actin organization and cytoprotection (1, 6, 7, 10). Because phosphorylation of HSP27 dissipates molecular chaperon activity and renders cells susceptible to oxidative stress and apoptosis, the mechanism of cytoprotection conferred by phosphorylation of HSP27 should be different from that mediated by unphosphorylated form of HSP27. The unphosphorylated form of HSP27 acts as F-actin cap-binding proteins and inhibits actin polymerization, whereas phosphorylation of HSP27 promotes actin polymerization and increases filament stability (10). This reorganization of F-actin appears to be involved in resistance against actin fragmentation and cell death induced by oxidative stress (7).

Thus Okada et al. (13) delineated the protective role of either form of HSP27 during ischemia and reperfusion, dependent on cellular redox state and the magnitude of physical stress. However, increasing the net amount of HSP27 seems to confer the most appropriate protection against both apoptosis and oncosis during ischemia and reperfusion because CMCs are subjected to both oxidative and physical stress at various degrees during these periods. Abundant presence of HSP27 could counteract either stress even when the ratio of unphosphorylated and phosphorylated forms of HSP27 is changed during ischemia and reperfusion in response to the activation status of p38 MAP kinase. Consistent with this notion is the fact that transgenic overexpression of wild-type HSP27 or nonphosphorylatable HSP27 provided equal protection against ischemia-reperfusion injury in the mouse heart (5).

Finally, the present and the previous studies (8, 13, 16) from H. Otani’s group provided an important concept in myocardial protection, emphasizing that physical stress at the time of reperfusion should be eliminated from “the protected heart” to avoid accidental cell death, i.e., oncosis. Accidental cell death occurs at the time of reperfusion through disruption of the sarcolemma in potentially viable CMCs that possess many functional mitochondria. Currently, mitochondria are the primary target of protection against ischemia-reperfusion injury, and an enormous effort has been exerted on this purpose. Nevertheless, protection of mitochondria during ischemia and reperfusion may not always be associated with the reduction of infarction. In addition to p38 MAP kinase inhibitors, there are many cardioprotective drugs that are potentially protective for mitochondria against ischemia-reperfusion damage. However, the efficacy of these drugs in preventing ischemia-reperfusion injury is not consistent. For example, despite unquestionable involvement of reactive oxygen species in mitochondrial damage during ischemia and reperfusion, none of the free radical scavengers has been proven to limit infarction (4). One of the therapeutic potentials of these drugs is alleviation of myocardial stunning (2). Provided that free radical scavengers mitigate stunning, improvement of contractile dysfunction may para-doxically aggravate oncosis of CMCs that otherwise survive after reperfusion. This assumption should be seriously taken into account as a possible explanation for the existing controversy on antioxidant as well as p38 MAP kinase inhibition therapies aiming at reducing myocardial infarction. Although many issues remain to be addressed to translate in vitro findings into the clinical arena, the elaborate study conducted by Okada et al. (13) opened a new avenue in the research of cardioprotection.

REFERENCES