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Review

Extracellular and intracellular proteases in cardiac dysfunction due to ischemia–reperfusion injury

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ABSTRACT

Various procedures such as angioplasty, thrombolytic therapy, coronary bypass surgery, and cardiac transplantation are invariably associated with ischemia–reperfusion (I/R) injury. Impaired recovery of cardiac function due to I/R injury is considered to be a consequence of the occurrence of both oxidative stress and intracellular Ca^{2+}-overload in the myocardium. These changes in the ischemic myocardium appear to activate both extracellular and intracellular proteases which are responsible for the cleavage of extracellular matrix and subcellular structures involved in the maintenance of cardiac function. It is thus intended to discuss the actions of I/R injury on several proteases, with a focus on calpain, matrix metalloproteinases, and cathepsins as well as their role in inducing alterations both inside and outside the cardiomyocytes. In addition, modifications of subcellular organelles such as myofibrils, sarcoplasmic reticulum and sarcolemma as well as extracellular matrix, and the potential regulatory effects of endogenous inhibitors on protease activities are identified. Both extracellular and intracellular proteolytic activities appear to be imperative in determining the true extent of I/R injury and their inhibition seems to be of critical importance for improving the recovery of cardiac function. Thus, both extracellular and intracellular proteases may serve as potential targets for the development of cardioprotective interventions for reducing damage to the heart and retarding the development of contractile dysfunction caused by I/R injury.

1. Introduction

The pathophysiology of cardiac dysfunction in heart disease is a complex process that involves the interplay and alterations of various extracellular and intracellular proteins and molecules. One of the key families of enzymes involved is the proteases which specialize in cleaving protein peptide bonds [1]. Various studies in the area of heart disease have shown their involvement in cardiac remodeling, a phenomenon that occurs partly as a result of ischemia–reperfusion (I/R) injury [1]. This injury arises from cessation of nutrients perfusing the heart due to lack of blood flow and is associated with initial damage that becomes significantly amplified upon reperfusion. Bursts of reactive oxygen species (ROS) occurring upon reperfusion lead to oxidative stress and the development of intracellular Ca^{2+}-overload thus impairing a complete recovery of cardiac function [2–4]. The cessation and subsequent reperfusion of blood flow occurs in a number of clinical procedures including angioplasty, thrombolytic therapy, coronary bypass surgery, and cardiac transplantation [2]. The detrimental action of I/R injury occurs during the requisite reperfusion period, where oxidative stress induced by ROS and increases in intracellular Ca^{2+} can catalyze the activation and modification of numerous proteins in the cell, plausibly altering their functions [2]. Thus, the disequilibrium of Ca^{2+} homeostasis and generation of ROS have the potential to directly and/or indirectly activate different proteases in the heart to promote the development of cardiac dysfunction following I/R injury.

Proteases are essential for the homeostatic maintenance of the cell, allowing for the degradation of misfolded or malfunctioning proteins, and routine turnover of the extracellular matrix (ECM) and other subcellular organelles [5–7]. Proteases are active at a basal level in cardiomyocytes. However, their actions are controlled via regulation of their transcription, translation, chaperone molecules, and endogenous inhibitors. On the other hand, under pathological conditions, including I/R injury, these regulatory and control mechanisms are altered leading to marked increases in protease activities [1,6–11]. These changes can occur both intracellularly and extracellularly, depending on the type of protease and location of the activation, as well as on interactions with its target. The proteases present in the myocardium that have demonstrated involvement in I/R injury include calpain, matrix metalloproteinases (MMPs), and cathepsins. The details regarding the structure, localization and methods of activation of calpains, MMPs, and cathepsins can be found in extensive reviews [1,2,4,5,12–22]. An overall scheme involving different proteases and their targets in the I/R-induced cardiac dysfunction, invariably

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seen upon subjecting the heart to various cardiovascular procedures, is depicted in Fig. 1.

2. Extracellular and intracellular proteases

Proteolysis of both intracellular and extracellular proteins is important as the homeostasis of the intracellular milieu in conjunction with the stability of the surrounding cardiac interstitium is crucial in normal heart function. The digression from normal cardiac architecture by proteolytic degradation leads to abnormal cardiac function and cellular damage [23]. Traditionally perceived as being a relatively static cellular scaffold, it has become clear that the cardiac extracellular environment is as important in the function of the heart as the intracellular milieu of the cardiomyocyte. The ECM environment is made up of signaling molecules, proteases, cytokines and growth factors that appear to be compartmentalized throughout the interstitium [13,24–30]. Regardless of the bioactive molecules present throughout the cardiac interstitial space, the architecture of matrix proteins themselves is distinctively arranged to optimize communication between cardiomyocytes and the overall pumping action of the heart [31,32]. During I/R injury, ECM is degraded via proteolysis to enable infiltration of fibroblasts to the damaged area for commencing the process of wound repair [33]; however, such a change in ECM, as well as in cardiomyocytes, may also result in cardiac dysfunction. Extracellular integrity is as important to maintain as cellular integrity, and thus alteration of the ECM by proteases is of critical importance to consider when targeting proteolytic activity.

The majority of extracellular damage is caused by the MMP family which degrades a number of ECM structural proteins including collagen, fibronectin, elastin, and proteoglycans [34]. Vanhoutte et al. [35] have analyzed the temporal alterations in the ECM with respect to MMP activity and have suggested that damage of the ECM is dependent on the duration of ischemic insult. An imbalance between MMPs and their endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs), has been noted indicating that the potential inhibitory effect of TIMPs is overpowered by the accentuation of MMP activity [36–38]. MMPs have also recently been attributed to causing proteolysis of various sarcomeric structural proteins [39–42]. Changes in the intracellular milieu, such as an increase in the concentration of Ca^{2+}, activate calpain causing it to degrade numerous subcellular organelle proteins where this increase in calpain activity is partially caused by the degradation of its endogenous inhibitor, calpastatin [9,15–17,43–45]. It is also important to note that ECM damage can occur via cathepsins by the proteolytic cleavage of collagen and other interstitial structural proteins [46–53]. In addition, lysosomal changes occur within the ischemic cardiomyocyte and have been observed during prolonged I/R and alterations in cathepsins occur during peak ischemic injury [54–57]. Although the activities of the above mentioned proteases are modified by changes in pH, it is not clear whether the activities of endogenous inhibitors of these proteases are altered by pH as well. Overall, it is reasonable to suggest that the extent of alterations in the heart, both to the ECM and cardiomyocytes, by I/R injury is influenced by the vigor of proteolytic activities in the myocardium.

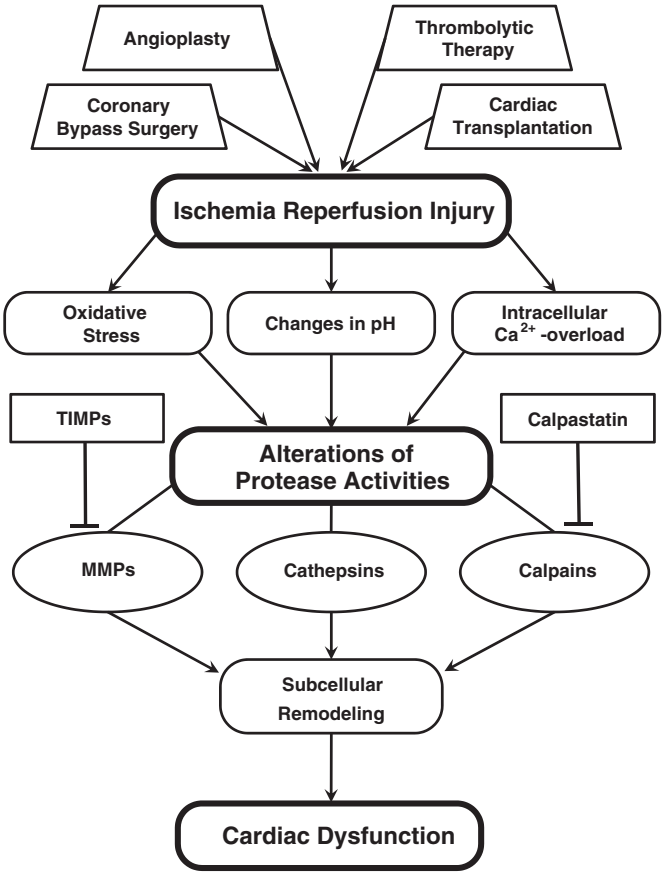

Fig. 1. Schematic representation of the mechanisms of I/R induced changes in cardiac dysfunction involving different proteases and their associations with some cardiovascular interventions. TIMPs: tissue inhibitors of matrix metalloproteinases, MMPs: matrix metalloproteinases.

It should be noted that the decrease in pH during ischemia occurs as a result of anaerobic by-products, including lactate and hydrogen ions despite the presence of the Na^+/H^+ exchanger [58–60]. Low pH has been demonstrated to affect both ECM remodeling and intracellular protease activities as protease classes are sensitive to various pH levels [59–61]. Acidic proteases, particularly those belonging to the family of cysteine proteases and aspartyl cathepsins, have been reported to increase their proteolytic activities under acidic conditions which further exacerbate ECM remodeling [59]. Cathepsins are effective at acidic pH values where low extracellular pH (pHe) has been demonstrated to increase the release of cathepsin B [59]. In cells cultured in an acidic environment, there was increased secretion of active cathepsin B [62]. With respect to the MMP family, although their proteolytic activities are reduced at low pH, conversion from their zymogen form to an active form requires proteolytic cleavage from other types of proteases [63]. In fact, a member of the gelatinase MMP family has shown to be activated by acid treatment [64]. Even at an acidic pH of 6.8, MMPs have been observed to retain up to 80% of their proteolytic activities [65]. A significant amount of acidosis has been noted to increase caspase-3 activity, which propagates the apoptotic cascade in cardiomyocytes [66]. However, it has also been observed that prolonging acidosis inside the cell can cause a cardioprotective effect, particularly concerning the attenuation of calpain proteolytic damage as it is not active until intracellular pH returns to normal [60,67]. Since pH is known to decrease in the ischemic myocardium and to show dramatic alterations upon reperfusion, it appears that the activities of different proteases are modified by changes in pH during the development of I/R injury. It is noteworthy that there is very little information regarding the influence of changes in pH on the behavior of endogenous inhibitors of proteases available in the literature.

3. Calpain in ischemia–reperfusion injury

Calpain is a Ca^{2+}-dependent cysteine protease involved in the degradation of various structural proteins and the cell death pathway. Its involvement in contributing to cardiovascular damage in I/R injury is demonstrated by its inhibition which results in a reduced infarct size [68,69]. However, it appears to only become activated post-reperfusion once cellular pHi has stabilized because when pHi is lowered, the decrease in calpain activity is comparable to when it is inhibited by a pharmacological calpain-specific inhibitor, MDL-28170 [60]. Calpain targets troponin I (TnI), a component of the actin–tropomyosin complex,