Proteasome inhibition during myocardial infarction

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The ubiquitin-proteasome system (UPS) plays a central role in protein degradation and regulates a variety of critical cellular processes. During recent years, the cardiac UPS has become increasingly recognized as a key regulator of cardiac function under both physiological and pathological conditions. Numerous studies have demonstrated that altered UPS function is involved in the pathogenesis of cardiac disease including myocardial ischaemia or infarction. The expression and activity of the E3 ubiquitin ligases, which confer substrate specificity in the UPS pathway, affect the apoptosis and severity of disease in myocardial ischaemia and reperfusion. Although impaired proteasome function is commonly associated with myocardial ischaemic injury, recent evidence also supports a cardioprotective role for proteasome inhibitors in myocardial ischaemia. We will review these studies and data, discuss controversies regarding the UPS alterations and use of proteasome inhibitors in myocardial ischaemia, and attempt to identify strategies that may enhance their clinical application.

Keywords
Ubiquitin-proteasome system • Proteasome inhibitor • Myocardial infarction • Myocardial ischaemia/reperfusion injury • Cardioprotection • G-protein-coupled receptor kinase

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1. Introduction

The ubiquitin-proteasome system (UPS) represents the major non-lysosomal pathway for degradation of ubiquitinated proteins. In addition to the removal of misfolded or damaged proteins, the UPS also plays a crucial role in the regulation of many critical cellular processes such as the cell cycle, transcriptional control, apoptosis, and responses to stress. Recent studies indicate that alterations in the UPS contribute to the pathogenesis and progression of a variety of cardiac diseases. This review focuses on recent findings regarding the roles of the UPS in pathophysiology of myocardial ischaemia and discusses the potential therapeutic use of proteasome inhibitors in myocardial ischaemia or infarction.

2. The cardiac 26S proteasome

The 26S proteasome is a multi-subunit complex composed of a 20S proteolytic core and two 19S regulatory caps. Evidence suggests that the 20S core functions independently of ATP, whereas the 26S proteasome is an ATP-dependent system that is responsible for efficient degradation of ubiquitinated proteins. The assembly of the proteasome is a complex process that involves the association of multiple and different subunits. Cell-specific proteasomes have been recognized by their distinct proteasome subunit composition in different cell types. The cardiac proteasome has unique properties with specific molecular composition and post-translational modifications, and there are specific associating partners with regulatory activity that may increase the diversity of proteasome function in the heart. The substrate specificity of the UPS mainly lies in the E3 ubiquitin ligases, which identify targeted proteins for ubiquitination and subsequent degradation. Already, several cardiac E3 ligases including atrogin-1/MAFbx (muscle atrophy F-box), MuRF (muscle RING finger), CHIP (carboxyl terminus of Hsp70-interacting protein), and MDM2 (murine double minute 2) have been reported to regulate specific cellular processes involved in cardiac disease. Overall cardiac proteasome activity has also been found to decrease with age, as demonstrated by increased oxidized and ubiquitinated proteins as well as decreased 20S proteasome content and loss of specific activities. The loss of proteasome function may impair the ability of myocytes...
to elicit an appropriate response to stress and thus enhance the susceptibility of the ageing heart to cardiovascular disease.

3. Role of the UPS in myocardial ischaemia

Myocardial infarction results from irreversible myocardial necrosis caused by prolonged ischaemia and hypoxia. Patients with acute myocardial infarction are at increased risk for heart failure, arrhythmias, and death. It is important, however, to realize that these infarcts are frequently heterogeneous with islands of ischaemic tissue alongside frankly infarcted and otherwise normal-appearing cellular groups. Moreover, the ischaemic zones may include cells which will survive and others moving on to overt apoptosis and death. Although early restoration of blood flow during myocardial ischaemia is essential for salvaging the myocardium, reperfusion itself can paradoxically exacerbate myocardial damage (reperfusion injury). The mechanisms of reperfusion injury involve the generation of reactive oxygen species, intracellular calcium overload, microvascular and endothelial dysfunction, altered myocardial metabolism, and concurrent activation of neutrophils, platelets, and complement. Recent investigations of the proteasome pathway in animal models of myocardial ischaemia suggest that impaired proteasome function is associated with the pathophysiology of myocardial ischaemia and reperfusion. Evidence for proteasome dysfunction is usually derived from in vitro measurement of the accumulation of increased ubiquitinated proteins and/or by demonstrating altered proteasome activity. These abnormalities in proteasome function and changes in the UPS components have been observed to variable degrees in myocardial ischaemia (Table 1).

3.1 The ubiquitin ligases in myocardial ischaemia

The importance of the E3 ligases including CHIP, MDM2, and MuRF in myocardial ischaemia is underlined by several recent studies using genetic engineering. CHIP-deficient mice are more prone to ischaemia/reperfusion injury with increased frequency of reperfusion arrhythmias and increased infarct size compared with wild-type control. These data support a cardioprotective function for CHIP. The mechanism of cardioprotection lies in the ability of CHIP to bind to damaged proteins in association with the molecular chaperone Hsp70 or Hsp90, and coordinate their refolding or mediate their degradation through ubiquitination. A similar protective effect by MDM2 against cardiac ischaemia/reperfusion injury has also been documented. Reduced MDM2 expression in a genetic mouse model rendered the isolated hearts more sensitive to ischaemia/reperfusion injury, whereas overexpression of MDM2 in cultured neonatal rat cardiomyocytes led to protection against hypoxia/reoxygenation-induced apoptosis. These data indicate that MDM2 may be required for preserving myocardial function and survival in response to ischaemia/reperfusion injury. MDM2 is a critical regulator of the pro-apoptotic transcription factor p53 and is responsible for ubiquitination and proteasome degradation of p53, therefore the cardioprotective effects of MDM2 are at least in part due to the inhibition of p53-induced apoptosis. Also, mice lacking MuRF3 display susceptibility to cardiac rupture after acute myocardial infarction, suggesting that MuRF3 is essential for maintenance of ventricular integrity and function after myocardial infarction. MuRF3 targets FHL2 (four-and-a-half LIM domain) and γ-filamin proteins for proteasome-mediated degradation, and the loss of MuRF3 results in accumulation of these proteins leading to abnormal sarcomere structure. Recent data suggest that the E3 ligases may have additional distinct effects on stressed cardiac tissues. In a study using rat H9c2 cardiomyocytes, Foo et al. demonstrated that oxidative stress-induced apoptosis was associated with decreased ARC (apoptosis repressor with caspase recruitment domain) and concurrent up-regulation of MDM2. MDM2 promotes ARC protein turnover via ubiquitination and proteasome-dependent degradation. Loss of the anti-apoptotic protein ARC has been reported to increase myocardial infarct size following ischaemia/reperfusion injury. The seemingly contradictory results in contrast to the previous study may be related to the fact that ARC expression is also regulated by p53. p53 has recently been shown to induce apoptosis in cardiomyocytes exposed to oxidative stress.

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<td>Rat model of chronic myocardial infarction</td>
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<td>Rat H9c2 cardiomyocytes exposed to oxidative stress</td>
<td>Increased E3 ligase (MDM2)</td>
<td>Foo et al.</td>
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I/R, ischaemia/reperfusion; MuRF-1, muscle ring finger 1; MAFbx, atrogin-1/muscle atrophy F-box; MDM2, murine double minute 2.
oxidative stress by transcriptionally suppressing ARC. Therefore, the cardioprotective effects by overexpression of MDM2 may result from the degradation of p53 and subsequent inhibition of p53-induced ARC repression that surpasses MDM2-mediated degradation of ARC. Conversely, when MDM2 is knocked down, the p53-mediated pro-death signals may exceed the protection by ARC. Increased expression of the atrophy-related E3 ligases, MuRF-1 and MAFbx, has also been observed in chronic infarcted rat heart. This up-regulation was attenuated by exercise training and a negative correlation was shown between MuRF-1/MAFbx expression and myocardial function.

3.2 The proteasome system in myocardial ischaemia

Proteasome dysfunction plays an important role in myocardial ischaemia. The first evidence for decreased proteasome function in myocardial ischaemia was derived from examination of the cardiac proteasome in an in vivo rat model of myocardial ischaemia/reperfusion. A loss of the 20S trypsin-like activity was observed in association with oxidative modification (4-hydroxy-nonenal) of several proteasome subunits as well as accumulation of oxidized and ubiquitinated proteins. This finding was confirmed by an ex vivo study of ischaemia/reperfusion, which showed decreased activities of both 20S and 26S in parallel with increased levels of myocardial ubiquitinated proteins. A similar decrease in the 26S proteasome activities along with accumulated ubiquitinated proteins was also documented in an in vivo canine model of myocardial ischaemia/reperfusion. The mechanisms underlying ischaemia-induced proteasome inhibition are not clear. It is suggested that some proteasome subunits may be suppressed or inactivated by oxidative stress. Modification of the 20S proteasome subunits by the lipid peroxidation product 4-hydroxy-nonenal results in selective inactivation of the cardiac 20S activity. Similar inactivation occurs in isolated proteasomes exposed to other oxidants. Moreover, the 26S proteasome subunit S6 ATPase has been identified to be very sensitive to oxidative inactivation. Reductive pre-treatment of isolated rat hearts with a vitamin E analogue preserved post-ischaemic proteasome function, whereas pre-treatment with the proteasome inhibitor lactacystin led to greater accumulation of oxidized proteins in the post-ischaemic heart. These data suggest an association between proteasome activity and the degree of oxidation. It is also important to note that protein ubiquitination and unfolding are ATP-dependent; therefore, ischaemia-mediated decreases in ATP levels may contribute to diminished 26S proteasome activity by dissociating the 26S complex.

Myocardial ischaemia/reperfusion injury is associated with increased necrosis and apoptosis. Since the UPS degrades numerous proteins including pro-death proteins and regulates multiple signalling pathways, proteasome dysfunction during ischaemia would be expected to have a significant impact on myocardial function. A novel protein kinase C (PKC) isozyme, PKCδ, has recently been shown to play a key role in mediating the cardiac ischaemia/reperfusion injury. During reperfusion, PKCδ is activated and translocated to the mitochondria where it negatively modulates mitochondrial function and induces apoptosis. PKCδ is ubiquitinated and degraded by the proteasome upon phorbol ester activation, and inhibition of the proteasome results in mitochondrial accumulation of PKCδ with associated mitochondrial dysfunction and induction of apoptosis. Evidence for the role of proteasome-regulated PKCδ in myocardial ischaemia injury has been found in an ex vivo rat model of myocardial infarction. Thus, decreased degradation of pro-apoptotic PKCδ due to ischaemic inactivation of cardiac proteasome may contribute to myocardial ischaemic injury by impairing mitochondrial function and inducing myocardial apoptosis.

Although proteasome impairment has been shown to contribute to myocardial ischaemic injury, proteasome activity is not consistently decreased in diseased myocardium. A recent study has demonstrated a selective rather than global proteasome inhibition in a rat model of cardiac ischaemia/reperfusion injury, suggesting that some proteasome functions remain active. In addition, increased proteasome activity and up-regulation of the 11S, 19S, and 20S proteasome subunits have been observed in a mouse model of chronic myocardial infarction. Similar activation of the cardiac proteasome, including increased levels of UPS components and proteasome activities, has also been shown in pressure overload-induced cardiac hypertrophy. Moreover, pharmacological blockade of the UPS seems to confer cardioprotection against ischaemic injury under certain conditions. The inconsistencies may arise from variations in the type and duration of ischaemia, the type of proteasome assay, the specificity and dosage/frequency of proteasome inhibitors, and the experimental model (cultured cardiomyocytes, ex vivo or in vivo heart model) used in the studies. A firm distinction must be made between studies using cultured cardiomyocytes in medium, ex vivo (Langendorf with buffered perfusate) and in vivo heart models wherein formed elements of the blood are also present to contribute cytokines and other interactions. Such differences may not explain some of these discrepancies, but their impact will certainly be recognized with adequate comparative studies.

4. Proteasome inhibitors and their effects in myocardial ischaemia

Several different types of proteasome inhibitors have recently been developed for potential clinical use in oncology and autoimmune diseases. Targeting the proteasome in cancer therapy was validated by using the dipeptide boronate, bortezomib (Velcade), the first FDA-approved proteasome inhibitor for the treatment of multiple myeloma. Common side effects associated with bortezomib repetitive chemotherapy regimens include neurological symptoms, orthostatic hypotension, gastrointestinal disturbances, and thrombocytopenia. Unexpected cardiac complications such as arrhythmias and heart failure have also been reported during sustained therapy, suggesting that chronic inhibition (3 weeks or more exposure) of the cardiac proteasome may be detrimental. The effects of proteasome inhibition during myocardial ischaemia on cardiac function have been controversial, as both beneficial and deleterious effects have been reported (Table 2). Nonetheless,
Table 2 Use of proteasome inhibitors in myocardial ischaemia

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<th>Proteasome inhibitor</th>
<th>Dosage and time of administration</th>
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<th>Experimental model</th>
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<td>Bortezomib</td>
<td>0.0875 mg/kg, pre- or post-LAD ligation</td>
<td>Dog</td>
<td>In vivo myocardial infarction</td>
<td>Ischaemic loss of GRK2 and ventricular tachyarrhythmias are prevented</td>
<td>Yu et al.65</td>
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<td>Epoxomicin</td>
<td>2.5 μg/kg, pre-ischaemia</td>
<td>Dog</td>
<td>In vivo myocardial I/R injury</td>
<td>No change in infarct size</td>
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<td>Epoxomicin</td>
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<td>Mouse</td>
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<td>Cardiac remodeling and contractile function are improved</td>
<td>Hedhli et al.35</td>
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<tr>
<td>LCL961</td>
<td>2.5 μmol/L, pre-anoxia</td>
<td>Rat</td>
<td>In vitro anoxia/reoxygenation in cultured neonatal cardiomyocytes</td>
<td>Cardiomyocyte necrosis/apoptosis is prevented</td>
<td>Dosenko et al.70</td>
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<td>Lactacystin</td>
<td>2 μmol/L, pre-ischaemia</td>
<td>Rat</td>
<td>Ex vivo myocardial I/R injury in isolated perfused heart</td>
<td>No effect on post-ischaemic haemodynamic recovery, protein carbonylation is increased</td>
<td>Divald et al.25</td>
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<td>MG132</td>
<td>1 μmol/L, pre-oxidative stress</td>
<td>Rat</td>
<td>In vitro oxidative stress in cultured neonatal cardiomyocytes</td>
<td>LDH release from cardiomyocytes is decreased</td>
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<td>MG132</td>
<td>10 μmol/L, pre-hypoxia</td>
<td>Rat</td>
<td>Ex vivo hypoxia/reoxygenation in isolated papillary muscles</td>
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<td>MG132</td>
<td>6–25 μmol/L, pre-ischaemia</td>
<td>Rat</td>
<td>Ex vivo myocardial I/R injury in isolated perfused heart</td>
<td>Post-ischaemic recovery of haemodynamic function is decreased</td>
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<td>PR-39</td>
<td>60 μg/kg/d × 7d, post-LAD ligation</td>
<td>Mouse</td>
<td>In vivo myocardial infarction</td>
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<td>Li et al.74</td>
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<tr>
<td>PR-39</td>
<td>1 μg/kg/d × 7d, post-LAD ligation</td>
<td>Mouse</td>
<td>In vivo myocardial infarction</td>
<td>Myocardial infarct size is reduced</td>
<td>Gao et al.30</td>
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<td>PR-39</td>
<td>14 mg/kg, pre-LAD occlusion</td>
<td>Mouse</td>
<td>In vivo myocardial I/R injury</td>
<td>Leucocyte recruitment is inhibited, myocardial infarct is reduced</td>
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<td>PR-39</td>
<td>10 mmol/kg, at reperfusion</td>
<td>Rat</td>
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<td>Bao et al.51</td>
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<td>PS-519</td>
<td>1 mg/kg, pre-LAD occlusion</td>
<td>Mouse</td>
<td>In vivo myocardial I/R injury</td>
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<td>Stansfield et al.48</td>
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<tr>
<td>PS-519</td>
<td>1 mg/kg, at reperfusion</td>
<td>Mouse</td>
<td>In vivo myocardial I/R injury</td>
<td>Myocardial infarct size is attenuated, ejection fraction is preserved</td>
<td>Moss et al.45</td>
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<tr>
<td>PS-519</td>
<td>1 mg/kg, pre-LAD occlusion</td>
<td>Pig</td>
<td>In vivo myocardial I/R injury</td>
<td>NF-κB activation is inhibited, myocardial infarct size is reduced, regional myocardial function is preserved</td>
<td>Pye et al.47</td>
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<tr>
<td>PS-519</td>
<td>0.01–1.0 mg/kg, during I/R</td>
<td>Rat</td>
<td>Ex vivo myocardial I/R injury in isolated heart perfused with PMN</td>
<td>Cardiac contractile function is preserved, leucocyte accumulation is reduced</td>
<td>Campbell et al.46</td>
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Table 2: Use of proteasome inhibitors in myocardial ischaemia

Recent data from both in vitro and in vivo studies support the concept that short-term (acute) proteasome inhibition under certain conditions may serve as a novel therapeutic strategy for myocardial ischaemia and reperfusion, although the therapeutic potential of proteasome inhibition in human cardiac diseases is yet to be tested. Several mechanisms involved in cardioprotection by proteasome inhibition have been proposed including inhibition of the nuclear factor κ-B (NF-κB) inflammatory pathway activation, prevention of ventricular tachyarrhythmias associated with inactivation of G-protein-coupled receptor kinase 2 (GRK2), and inhibition of cardiomyocyte apoptosis (Figure 1).

4.1 Cardioprotection through inhibition of proteasome degradation of inhibitory κB and subsequent activation of NF-κB

A major mechanism underlying proteasome inhibition-mediated cardioprotection is inactivation of NF-κB, a nuclear transcription factor that regulates pro-inflammatory cytokine expression. Activation of NF-κB has been documented in myocardial ischaemia/reperfusion41,42 and specific inhibition of NF-κB is cardioprotective.43–45 Since NF-κB activity depends on proteasome ubiquitination and subsequent degradation of the inhibitory
κB (IkB), inhibition of the proteasome pathway should exert similar protection in ischaemic heart.

PS-519, a synthetic analogue of lactacystin, is a highly selective and potent proteasome inhibitor. Proteasome inhibition by PS-519 in rats,\textsuperscript{46} porcine,\textsuperscript{47} and murine models of myocardial ischaemia/reperfusion injury is cardioprotective. In the isolated perfused rat heart model (20 min ischaemia and 45 min reperfusion) in the presence of polymorphonuclear leucocytes, PS-519 improved cardiac contractile function and coronary flow that was associated with significantly reduced polymorphonuclear leucocyte accumulation in the ischaemic myocardium.\textsuperscript{46} In the porcine in vivo left anterior descending (LAD) coronary occlusion model (1 h occlusion and 3 h reperfusion), PS-519 administered prior to occlusion inhibited NF-κB activation, decreased the size of myocardial infarction, reduced reperfusion injury, and preserved regional myocardial function.\textsuperscript{47} In the in vivo mouse model (30 min LAD occlusion and 24 h reperfusion), PS-519 delivered prior to occlusion reduced infarct size, decreased ischaemic injury, and improved myocardial function.\textsuperscript{48} These were associated with decreased p65 and TNF-α expression and preserved IkBα expression, indicating that PS-519 inhibited NF-κB inflammatory pathway activation. Consistent with the effects of this proteasome inhibition of IkB degradation, blockade of IkB phosphorylation through IkB kinase β (Inkβ) inhibition before ischaemia resulted in similar cardioprotection in the same mouse infarct model.\textsuperscript{44} At the onset of reperfusion, both IkB inhibition and PS-519 attenuated infarct size and preserved myocardial function. However, only IkB inhibition provided cardioprotection through specific suppression of NF-κB signalling, suggesting that the protective effects of proteasome inhibition after ischaemia are mediated through other proteasome-regulated pathways.\textsuperscript{49} In addition, the delayed cardioprotection by IkB inhibition was present even when the IkB inhibitor was delivered 2 h after reperfusion.\textsuperscript{35}

PR-39, a naturally occurring antibacterial peptide originally isolated from porcine intestine, is a non-competitive and reversible inhibitor of the 20S proteasome. The potent anti-neutrophil action of PR-39 makes it a likely cardioprotectant against ischaemia/reperfusion injury. Indeed, in a mouse model of myocardial ischaemia/reperfusion injury (30 min LAD occlusion and 24 h reperfusion), injection of PR-39 prior to occlusion inhibited leucocyte recruitment into inflamed myocardium and attenuated myocardial reperfusion injury.\textsuperscript{49} It has also been shown that PR-39 inhibits proteasome-mediated IkBα degradation in vitro and decreases infarct size in a mouse model of acute myocardial infarction (LAD ligation).\textsuperscript{50} The protective mechanism of PR-39 through inhibition of IkBα degradation was confirmed in a rat LAD occlusion model (30 min ischaemia and 24 h reperfusion).\textsuperscript{51} PR-39 at the time of reperfusion effectively decreased infarct size and improved post-ischaemic cardiac function, which was mediated by inhibition of IkBα degradation and subsequent inhibition of NF-κB-dependent adhesion molecule expression.

Epoxomicin, a specific inhibitor of the 25 protein responsible for the chymotryptic activity of the 20S proteasome, has also been tested in myocardial infarction. In a canine model of myocardial ischaemia/reperfusion (90 min ischaemia and 6 h reperfusion), epoxomicin administered before ischaemia did not alter infarct size.\textsuperscript{19} However, proteasome inhibition by epoxomicin after volume overload induced by chronic myocardial infarction in the mouse (LAD ligation) improved cardiac remodelling and contractile function.\textsuperscript{35} This is likely related to the inhibition of NF-κB-mediated increase in gene expression and hypertrophic growth of cardiomyocytes, as demonstrated in aorta-banded mice.\textsuperscript{52} After the onset of pressure overload, epoxomicin decreased NF-κB activity and reversed cardiac remodelling by stabilizing cardiac function and suppressing hypertrophy progression.

4.2 Cardioprotection through inhibition of proteasome degradation of GRK2 and hypersensitivity to β-adrenergic response

GRK2, along with β-arrestin, is a primary homologous desensitizer of G-protein receptor signalling.\textsuperscript{53} Receptor desensitization occurs as a consequence of G-protein uncoupling from the receptor in response to binding of β-arrestins to agonist-occupied receptor following phosphorylation of the receptor by GRKs. Changes in GRK2 expression and activity and their involvement in cardiovascular pathophysiology have been documented in recent years.\textsuperscript{54} Although the majority of data have focused on the reduced β-adrenergic responsiveness associated with increased GRK2 levels in cardiac hypertrophy and heart failure,\textsuperscript{55} there are a number of studies showing down-regulation of GRK2 in ischaemic heart\textsuperscript{57} and brain.\textsuperscript{58,59} A differential expression of GRK2 has also been described in cardiac hypertrophy with or without heart failure after myocardial infarction in the rat.\textsuperscript{60} GRK2 was

Figure 1 Proposed mechanisms for proteasome inhibition-mediated cardioprotection during acute myocardial ischaemia. (i) Inhibition of proteasome degradation of IkB blocks the NF-κB inflammatory pathway activation. (ii) Inhibition of proteasome degradation of GRK2 protects against increased sensitivity to β-adrenergic stimulation and increased susceptibility to ventricular tachyarrhythmias. (iii) Inhibition of proteasome degradation of ARC protects against cardiomyocyte apoptosis. Proteasome inhibition may also induce HSP production that exerts anti-apoptotic effects. ARC, apoptosis repressor with caspase recruitment domain; GRK2, G-protein-receptor kinase 2; HSP, heat shock protein; IkB, inhibitory κB; NF-κB, nuclear factor-κB.
down-regulated in animals with cardiac hypertrophy in the absence of heart failure, whereas GRK2 expression and activity were elevated in animals with heart failure thereby supporting the role of GRK2 in the development of heart failure. GRK2 expression is tightly regulated by its degradation via the proteasome pathway. Upon β-adrenergic receptor stimulation, GRK2 undergoes polyubiquitination and is rapidly degraded by the proteasome. The mechanisms triggering GRK2 degradation are proposed to involve the β-arrestin recruitment of c-Src and MAPK resulting in GRK2 phosphorylation and subsequent degradation. The E3 ligase MDM2 has recently been shown to target GRK2 for ubiquitination in a similar β-arrestin-dependent manner, therefore may play a crucial role in GRK2 degradation.

We have reported a loss of GRK2 expression and activity in the arrhythmia-prone ischaemic cardiac tissue and increased sensitivity to β-adrenergic stimulation 6–24 h after LAD ligation in the dog. It is known that ventricular fibrillation and myocardial ischaemia are often inseparable, and the first manifestation of myocardial ischaemia or infarction may be sudden cardiac death. In our studies, GRK2 was decreased by bortezomib 1 h before or after the onset of myocardial ischaemia effectively blocked the GRK2 reduction in ischaemic cardiac tissue and suppressed malignant ventricular tachyarrhythmias and sudden death during the first 24 h after myocardial ischaemia. The delayed cardioprotection by bortezomib supports the concept that proteasome inhibition within a clinical window following myocardial infarction may be of use in suppressing fatal tachyarrhythmias and sudden death. The proteasome-mediated down-regulation of GRK2 has also been observed in rat organotypic hippocampal cultures subjected to oxygen and glucose deprivation, which is consistent with a marked GRK2 reduction in the ischaemic brain in an in vivo model of hypoxia/ischaemia. Thus, we have demonstrated a mechanism whereby proteasome degradation of GRK2 during acute myocardial ischaemia leads to hypersensitivity to β-adrenergic response and a resultant predisposition to ventricular tachyarrhythmias. In contrast, GRK2 preservation by proteasome inhibition confers protection against the onset of tachyarrhythmias and consequent sudden death.

4.3 Other mechanisms involved in proteasome inhibition-mediated cardioprotection

The proteasome system is known to regulate the proteolysis of proteins involved in a variety of cellular processes including apoptosis, differentiation, inflammation, and proliferation. Proteasome inhibition by clasto-lactacystin β-lactone in cultured neonatal rat cardiomyocytes has been shown to prevent cardiomyocyte necrosis and apoptosis after exposure to anoxia/reoxygenation. Degradation of the endogenous apoptosis inhibitor ARC was mediated by the UPS, which was markedly decreased in the mouse heart following ischaemia/reperfusion, suggesting that proteasome inhibition at the onset of reperfusion may block ARC degradation and protect ischaemic myocardium against reperfusion-induced apoptosis. In addition, proteasome inhibition has also been found to induce heat shock proteins in cardiomyocytes and exert cardioprotection after oxidative stress. MG132, a less specific proteasome inhibitor, protected neonatal rat cardiomyocytes from oxidative injury and enhanced the contractile recovery of isolated rat papillary muscles following hypoxia/reoxygenation, both being likely related to induction of heat shock proteins that have direct anti-apoptotic activities. Interestingly, PR-39 has been shown to induce angiogenesis both in vitro and in vivo by inhibiting proteasome degradation of the hypoxia-inducible factor-1α protein, which regulates expression of many angiogenesis-related genes. In the mouse model of acute myocardial ischaemia, PR-39 administration after LAD ligation produced a significant increase in vascularity along the infarct border zone, suggesting that proteasome inhibition may be effective in inducing therapeutic angiogenesis.

4.4 Limitations on use of proteasome inhibitors during myocardial ischaemia

In contrast to the beneficial effects of proteasome inhibitors in myocardial ischaemia, other studies suggest that inhibition of the cardiac proteasome may be associated with post-ischaemic cardiac dysfunction. Pre-treatment of isolated rat hearts with the proteasome inhibitor lactacystin resulted in increased levels of oxidized proteins, but had no significant impact on recovery of post-ischaemic function (30 min ischaemia and 60 min reperfusion). Pre-ischaemic treatment of the same isolated rat hearts with MG132 resulted in dose-dependent decreases in recovery of post-ischaemic function and increased accumulation of ubiquitinated proteins. As discussed earlier, the conflicting results may arise from variations in the duration of ischaemia, the specificity of the proteasome inhibitors, the degree of proteasome inhibition, and the specific models used in the studies. Bortezomib, epoxomicin, and PS-319 are highly selective and potent inhibitors of the proteasome, whereas MG132 and lactacystin are less specific in their inhibition of the 26S proteasome. In addition, in vivo models of ischaemia/reperfusion are generally more complex and subject to more regulatory variables than the isolated perfused heart preparation. Recent studies have shown that proteasome inhibition results in activation of autophagy (enzyme-mediated self digestion), whereas suppression of autophagy promotes accumulation of ubiquitinated proteins, suggesting that there is an inter-regulation between the two pathways. Autophagy is a summation of highly conserved degradative processes in eukaryotic cells. Many studies have demonstrated that autophagy is up-regulated during myocardial ischaemia/reperfusion. However, the functional consequences of enhanced autophagy and the inter-relationship between the UPS and autophagy pathways in the heart remain unclear. It appears that proteasome inhibition may exert cardioprotective effects when administered during acute myocardial ischaemia when proteasome function is minimally affected. Chronic or additional inhibition of the cardiac proteasome when proteasome function is already significantly impaired following
myocardial infarction may ultimately lead to the accumulation of misfolded proteins and cellular apoptosis, thereby exacerbating cardiac dysfunction.

5. Therapeutic strategies and future directions

Considering the variety of cellular processes regulated by the UPS, therapeutic approaches that specifically target the cardiac proteasome are highly desired to minimize toxicity. Recent studies on the dynamics and complexity of cardiac proteasome suggest the existence of distinct subpopulations of the cardiac proteasome with different subunit composition, post-translational modification, and associating partners.4–6 Therefore, a certain subpopulation may be targeted for specific functional regulation. This is confirmed by a recent report that provided evidence for distinctive cardiac proteasome subtypes.47 The results showed that different proteasome subtypes displayed different levels of proteolytic activities, and importantly, different proteasome inhibitors had differential inhibitory effects on the various cardiac proteasome subtypes. In addition, different cardiac proteasome subtypes were inhibited by the same dose of proteasome inhibitor to a different extent. These data suggest that investigation of alterations in cardiac proteasome subtypes is required in order to better understand the roles of proteasome dysregulation in cardiac diseases including myocardial ischaemia. Consequently, therapeutic designs that target the subtypes/subpopulations of cardiac proteasome will provide better efficacy and safety.

Figure 2  Potential therapeutic targets of the UPS. Ubiquitination is mediated by the sequential action of a multi-enzymatic system consisting of E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin ligase). The ubiquitinated protein is then directed to the proteasome for degradation. Inhibitors of the E3 ligases which confer substrate specificity prevent protein ubiquitination and subsequent degradation by the proteasome. Proteasome inhibitors bind to the proteolytic subunits of the proteasome and block the degradation of the ubiquitinated proteins. Selective pharmacological interventions of the E3 ligases or subpopulations of the cardiac proteasome may offer higher efficacy with less toxicity and warrant further studies on their therapeutic value in cardiac disease. Adapted from Ostrowska.79

6. Conclusions

The UPS is responsible for the degradation of most intracellular proteins and plays a key role in regulating many biological processes. As a generality, UPS dysfunction has been found to be associated with myocardial ischaemic injury. However, the specific molecular components, function, and regulation of the cardiac proteasome still remain largely unknown. Short-term use of proteasome inhibitors in acute myocardial ischaemia has been shown to be cardioprotective in some cases when cardiac proteasome is functionally active. Novel experimental and therapeutic approaches that target the cardiac-specific UPS may offer potential new therapies and warrant further clinical studies. The development of selective inhibitors for the E3 ligases and subpopulations of the cardiac proteasome may provide the means for diminishing the associated side effects of UPS inhibitors without compromising the utility of this approach.

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