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The role of Caspase 1 in ischemia/reperfusion injury of the myocardium.

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Abstract (200 words)

Acute occlusion of a coronary artery can result in myocardial infarction - a leading cause of premature death. Prompt restoration of blood flow to the myocardium can prevent excessive death of cardiomyocytes and improve clinical outcome. Although the major mechanism of cell death after reperfusion is necrosis, it is now recognized that many other cell death pathways may be involved in ischemia-reperfusion (I/R) injury. Pyroptosis is one such cell death pathway that is caspase-1-dependent and induced in response to cellular insult. The activated caspase-1 protease cleaves and activates specific cellular targets including gasdermin D and the pro-inflammatory cytokines interleukin-1□ and interleukin-18. The N-terminal fragment of gasdermin D forms plasma membrane pores resulting in cytosolic leakage and cell rupture, releasing interleukin-1beta and interleukin-18. Evidence suggests that inflammation induced by I/R via the pyroptotic pathway contributes to cardiomyocyte death, excessive scar formation and poor ventricular remodeling. For this reason, there is growing interest in targeting components of the pyroptotic pathway as a means of reducing I/R injury.

Key words:

cardiac, ischemia, reperfusion, pyroptosis, caspase 1

Introduction

Although the pathogenesis of myocardial infarction is increasingly well understood, it remains a leading cause of premature death in the Western world. The widespread adoption of percutaneous coronary intervention has resulted in a significant reduction in the duration of coronary ischemia once the clinical diagnosis of coronary artery occlusion is made[1]. Early restoration of blood flow to the myocardium can prevent excessive oncotic cardiomyocyte death. Conversely, delaying treatment is associated with worse outcome and increased mortality[2]. It is now understood that in addition to the ischemic insult, the process of reperfusion paradoxically leads to further damage called myocardial ischemia-reperfusion (I/R) injury[3]. Myocardial I/R results in four recognized types of injury: reperfusion induced arrhythmias, myocardial stunning, microvascular obstruction and lethal myocardial reperfusion injury. Of these four injuries, lethal myocardial reperfusion injury, which is defined as reperfusion-induced death of cardiomyocytes that were viable at the end of a period of ischemia, may account for 50% or more of the total cell death[1].

Myocardial I/R injury is a complex multifactorial process involving a number of different processes that culminates in irreversible cardiomyocyte death. The process starts following occlusion of the coronary artery, most commonly due to atherothrombotic disease. This prevents blood supply to a segment of the myocardium, which becomes ischemic and is known as the “area at risk” - every cardiomyocyte in this area is at risk of death. The lack of circulating oxygen and nutrients in the area at risk causes the cardiomyocytes to switch to anaerobic metabolism in order to maintain cellular homeostasis. After a prolonged period of ischemia, continued anaerobic respiration results in a drop in intracellular pH and an overload of intracellular cations (particularly Na^+ and Ca^{2+}). During an extended period of ischemia,

the resultant swelling of the intracellular organelles and cell cytoplasm can result in irreversible breakdown of the sarcolemma in a process known as “oncotic” cell death[4]. Restoration of blood flow in the artery and reperfusion of the myocardium is necessary to prevent further cell death. However, the sudden arrival of oxygenated blood to an ischemic cell result in an increase in oxygen free radicals, and cytosolic and mitochondrial Ca^{2+} overload. With the reactivation of mitochondrial oxidative phosphorylation and generation of ATP, reperfusion permits the function of ion exchangers, washes out lactate from the myocardium and rapidly restores the neutral extracellular pH. Unfortunately, the neutral pH facilitates opening of the previously closed MPTP, resulting in mitochondrial membrane depolarization and uncoupling of oxidative phosphorylation; this combination leads to rapid ATP depletion and subsequent cell death. Delaying the restoration of pH or inhibiting MPTP opening at reperfusion has been shown experimentally to reduce infarct sizes by up to 40%[1]. The Reperfusion Injury Salvage Kinase (RISK) pathway refers to pro-survival protein kinases (including PI3K/Akt and MEK/ERK1/2), which confer powerful cardioprotection by preventing the opening of the MPTP, when activated specifically at the time of myocardial reperfusion[1,5,6]. Recent publications suggest that for cardioprotection to be clinically effective it will be necessary to use multi-target strategies, for example the combination of those activating pro-survival RISK pathway with those inhibiting cell death pathways[7,8].

Pyroptosis and the inflammasome

Although the major form of cell death after reperfusion is necrosis[4,9], it is now recognized that many other forms of cell death contribute to ischemia-reperfusion injury[10,11]. For example, some cells, primarily non-cardiomyocytes, die by apoptosis[7,12]. There is increasing evidence that the inflammatory response, while being necessary for post-infarction repair,

contributes to cardiomyocyte death, excessive scar formation and poor ventricular remodeling[13-15]. An inflammatory insult can cause the activation of a caspase-1-dependent programmed cell death pathway that culminates in plasma membrane pore formation, cytosolic leakage and cell rupture, in a process that has been termed pyroptosis[15-18]. Powerful pro-inflammatory cytokines such as IL-1□ and IL-18 can also be released leading to a pro-inflammatory cascade[14,19]. This has led to the proposal that targeting components of the pyroptotic pathway such as caspase-1 may be a means of reducing I/R injury.[15,20].

The inflammasome is an intracellular protein complex that is a central component of pyroptosis (**Figure 1**). It regulates caspase-1 activity and acts as a gate keeper to the sensing and subsequent initiation and amplification of the inflammatory response to injury[15]. Inflammasome activation relies on two independent steps, priming and triggering[21]. During priming, microbial molecules or endogenous factors stimulate inflammatory receptors such as the TNF-receptor or Toll-like receptors (TLR), important components of the innate immune system. These activate NF-κB and induce the expression of components such as NLRP3, ASC and pro-IL-1β.

In the setting of cardiovascular and cardiometabolic disease, priming of the NLRP3 inflammasome can be caused by diabetes, hyperlipidemia, inflammatory disease or atherosclerosis[22]. I/R can cause priming during reperfusion when necrotic cell death creates a milieu of fragmented cytoplasmic debris in the extracellular environment which act as "Danger Associated Molecular Patterns" (DAMPs). Examples of DAMPs include high mobility group box-1 (HMGB1)[23] heat shock proteins[24], adenosine, extracellular RNA[25], matrix fragments, and Interleukin (IL)-1α[26]. DAMPs can stimulate receptors such as TLRs, purine receptors and IL-1 receptor on surviving cardiac cells in the border zone of the infarct

area. Stimulation of TLRs activates downstream intracellular signaling pathways that result in NF-κB-mediated expression of the protein components that make up the inflammasome[27]. The accumulation of the individual components of the inflammasome within the cytoplasm marks the culmination of the priming phase.

Following priming, a secondary event or trigger is then required for the assembly of the inflammasome from its protein components into its macromolecular active form. One of the key components of the inflammasome which facilitates this transition is the NOD-like receptor protein 3 (NLRP3), a member of the class of cytosolic pattern recognition receptors[28,29]. NLRP3 acts as a sensor and responds to changes in intracellular $[K^+]$[30]. In the case of myocardial ischemia, mitochondrial dysfunction and lysosomal destabilization leads to increased K^+ efflux which is the main trigger that leads to activation of the NLRP3[30]. Reactive oxygen species from mitochondria or other intracellular sources can directly activate NLRP3[31-33]. Oxidized mtDNA escaping through the MPTP has also been proposed to be a trigger for NLRP3 inflammasome assembly[34].

Following stimulation of the NLRP3 receptor, a conformational change of the protein ensues which leads to recruitment of the adaptor protein ASC (apoptosis speck-like protein)[35,36], a protein containing the caspase-recruitment domain (CARD). This complex is now able to interact with pro-caspase 1, and leads to its cleavage to the active caspase form. The assembly of the NLRP3 inflammasome is now complete and caspase-1 begins the subsequent cleavage and activation of the pro-inflammatory cytokines IL-1□ and IL-18. An additional substrate of caspase-1 is the cytosolic protein, gasdermin D (GSDMD)[37,38]. Following its cleavage by caspase-1, the N-terminal fragments of GSDMD oligomerize within the plasma membrane to form pores. These pores result in loss of cell membrane integrity, leading to

deranged ion homeostasis, osmotic swelling and eventually cell lysis, which is the terminal event in pyroptotic cell death. The pores also increase membrane permeability to IL-1□ and IL-18 leading to their extracellular release and these cytokines then amplify the inflammatory response and mediate further injury[16,17,21,39,40]. IL-18 for instance, has been shown to affect cardiomyocyte contractile function and induce further cell death by apoptosis[41,42].

Several studies have implicated the NLRP3 inflammasome and its downstream effectors such as caspase-1 in the phenomenon of I/R injury (Table 1). Kawaguchi et al. demonstrated the presence of NLRP3 inflammasome components (ASC aggregates) in the hearts of patients who had died of myocardial infarction[40], which heralded the need for further pre-clinical investigation into the role of the inflammasome in MI. In their subsequent study of transient myocardial ischemia in mice, they showed that resident myocardial cells, primarily fibroblasts, form the NLRP3 inflammasome in response to I/R injury and release IL-1□[40]. Furthermore, they demonstrated a smaller infarct size in mice lacking caspase-1 or ASC, as compared with wild type mice, indicating the critical role of the inflammasome in the development of myocardial I/R injury[40]. Interestingly, data obtained following bone marrow transplantation from KO to WT mice (or *vice versa*) indicates that the inflammasome formation in both inflammatory cells and myocardial resident cells (such as cardiomyocytes and fibroblasts) contributes to infarct formation[40]. owever, inflammasome activation and Il-1□ production were only seen in fibroblasts and not cardiomyocytes, suggesting it is the fibroblasts which act as “sentinel” cells, detecting injury and recruiting leukocytes which in the situation of I/R leads to further cardiomyocyte damage[40].

Subsequent work by Mezzaroma et al. in a mouse model of non-reperfused MI showed increased protein expression of ASC and formation of the NLRP3 inflammasome in cardiac

fibroblasts as well as cardiomyocytes, endothelial cells, and leukocytes in the infarct and infarct border zones, but not in remote cardiac myocardium or sham-operated hearts[43]. They also showed increased caspase-1 enzyme activity in the heart, which was detectable as early as 6 hours after coronary ligation and continued to rise for several days, reaching a peak between day 3 and 7, and persisted for up to 14 days[43]. Gene silencing of NLRP3 with small interfering (si)RNA prevented the increase in caspase-1 activity 72 h after coronary artery ligation, and this attenuated cardiac remodeling at 7 days as reflected by less cardiac enlargement and dysfunction NLRP3. Furthermore, caspase-1 inhibition was shown to protect HL-1 cardiomyocytes against hypoxia and reoxygenation-induced cell death *in vitro*[43]. This supported the view of caspase-1 and potentially the pyroptotic pathway as having a causative role in mediating I/R injury and promoting adverse cardiac remodeling, thereby demonstrating the need to develop pharmacological therapies to target components of the pathway specifically.

Targeting caspase-1 in reperfusion injury

Experimental studies in mice with genetic deletion of caspase-1 have identified caspase-1 as a potential target for intervention in preventing excessive cell death after coronary ischemia (Table 1)[44-46]. For instance, caspase-1 knockout mice had reduced infarct size and attenuated left ventricular remodeling after I/R[44], whereas cardiomyocyte-restricted overexpression of caspase in mice increased infarct size[46].

One of the first studies utilizing a pharmacological caspase inhibitor to assess involvement of caspases in myocardial I/R injury was in a study by Yellon's group in which the pan-caspase inhibitor zVAD.fmk was used[47]. In this *ex vivo* experimental model, isolated, Langendorff-perfused rat hearts were subjected to 35 min of coronary occlusion and 120 min reperfusion.

The treatment group was perfused with zVAD.fmk during early reperfusion with a resultant significant reduction in infarct size. This identified the potential role for the caspase family of proteases as a therapeutic target. At the time, this was interpreted as demonstrating that inhibition of apoptosis was cardioprotective. However, the role of apoptosis in acute myocardial I/R injury is controversial[48,49]. While apoptotic nuclei can be detected by TUNEL staining for example, these are mostly due to vascular and interstitial cells dying by apoptosis[50]. Thus, it is possible to re-interpret studies such as the above using pan-caspase inhibitors[47] as having inhibited caspase-1 mediated death of cardiomyocytes.

Pomerantz et al were the first to use the more selective caspase-1 inhibitor, Ac-YVAD.cmk[51]. They utilized human atrial myocardium attached to force transducers in the presence of a buffer and subjected to bouts of hypoxia-reoxygenation. The force transducers allowed for the assessment of the myocardial contractile force. The treatment arm involved the addition of yVAD to the perfusate during and after the simulated ischemia/ reperfusion. After 30 min simulated ischemia the contractile force in the non-treatment was reduced by 35% of the non-ischemic control, however tissue treated with caspase-1 inhibitor only had a mean reduction to 75.8% of the control[51]. This significant attenuation of post I/R myocardial dysfunction was also associated with a significant increase in intracellular creatinine kinase levels, a positive marker of cell viability. Unfortunately, the authors did not investigate the mechanism, the rate of cell death using DNA fragmentation, or the cleavage of poly(ADP)-ribose polymerase, so they were unable to prove whether the cardio-protection due to the effect of caspase 1 inhibition was preventing cytokine-induced myocardial stunning. However, they did also demonstrate cardioprotection after neutralization of IL-18 with IL-18 binding protein, suggesting that this may have mediated the injury[51].

In a study by Toldo et al[52], alpha 1 antitrypsin (AAT) was used as an anti-inflammatory therapy in a mouse model of *in vivo* coronary artery ligation and reperfusion. The rationale for using this therapy was that AAT is a serine-protease inhibitor with anti-inflammatory properties, and plasma levels have been shown to be increased in patients with acute myocardial infarction, suggesting a possible protective role in response to ischemic injury. Furthermore, administration of human AAT had previously been shown to reduce tissue injury and organ dysfunction in a mouse model of renal I/R injury, and examination of renal tissues in this AAT treated group showed reduced caspase-1 activity. In Toldo's study, AAT not only reduced the size of a myocardial infarct after coronary artery ligation and reperfusion, but also inhibited the release of caspase-1 (though not of caspase-3)[52]. Furthermore, they showed *in vitro* that AAT reduced NLRP3 inflammasome-mediated caspase-1 activition[52]. Crucially the therapy did not alter the number of infiltrating leukocytes suggesting the effect was independent of the circulating immune system[52]. The authors hypothesized that the mechanism of protection of AAT was that it interfered with the formation of the NLRP3 inflammasome[52]. They repeated their experiment in a mouse model of ischemia without reperfusion and found the results were similar, suggesting the effect was independent of reperfusion[52]. This is in keeping with findings from Mezzaroma et al, who showed that the inflammasome is active in ischemic cells as well as during reperfusion and therefore there is a theoretical benefit of giving an inflammasome inhibitor even prior to reperfusion[43]. The study lacked evidence to show that AAT inhibited the NLRP3 inflammasome directly to bring about the reduction of caspase-1. Other mechanisms could have been implicated, for example, by reducing infarct size, AAT would be expected to reduce the overall leukocyte burden in the heart after I/R. Given that leucocytes have significant levels of caspase-1 expression, this may have resulted in lower levels of active caspase-1 in the heart.

Marchetti et al[53] synthesized a novel compound (16673-34-0 (5-chloro-2-methoxy-N-[2-(4-sulfamoylphenyl)ethyl]benzamide) based on glyburide, which was expected to inhibit inflammasome function without causing hypoglycemia. The drug was administered to mice, which then underwent an *in vivo* procedure of coronary artery ligation followed by 24 h reperfusion. Treatment significantly reduced infarct size as a percentage of AAR by over 40%[53]. Significantly less caspase-1 activity was measured in the heart[53], although since this was measured after 24 h reperfusion, it is hard to be certain whether this is a primary effect of inflammasome inhibition, or secondary to a diminished recruitment of caspase-1 containing leukocytes. However, the inhibitor also suppressed caspase-1 activation and decreased cell death in HL-1 cardiomyocytes subject to hypoxia and reoxygenation *in vitro*[53], which supports the suggestion that cardioprotection by NLRP3 inflammasome inhibitors may partly by via the suppression of caspase-1 activation[53].

The exploration of caspase-1 has been expanded with the development of VX-765, an orally absorbed prodrug of VRT-043198, which is a potent and selective inhibitor of caspase-1[54]. Initially developed for use as an orally administered drug for diseases in which caspase-1 is implicated such as rheumatoid arthritis and epilepsy, it has allowed researchers to conduct studies of the effect of specific caspase-1 inhibition in animal models of I/R. Yang et al first showed that it was highly protective against infarction when administered prior to the onset of ischemia in a rat, open-chest coronary artery occlusion/ reperfusion model [55]. Audia et al[56] subsequently showed it was also protective when administered at reperfusion. The platelet inhibitor cangrelor in addition to VX-765 in the active group in order to better simulate real world conditions, given that most patients with acute myocardial infarction undergoing percutaneous intervention have P2Y12 platelet inhibitors administered prior to reperfusion. In

the presence of cangrelor, VX-765 significantly attenuated the infarct size[55,56]. The reduction in infarct size was greater than that seen with either cangrelor or VX-765 alone, which suggest that the two compounds do not share a common mechanism for cardioprotection[56]. The study was further supported by experiments using in an *ex vivo* rat Langendorff model of I/R[56,57]. The benefit of this model is the lack of circulating blood so the infarct is independent of immune cells which also contain intracellular inflammasome complexes and platelets and clotting factors which are also known to contribute to compounding effects such as microvascular obstruction and endothelial cell dysfunction. This "cleaner" model allows a direct examination of the effects of I/R on the myocardium. These experiments showed that, while the pro-drug VX-765 only protected isolated hearts when started prior to ischemia[56,57], its active derivative VRT-043198 provided the same amount of protection when started at reperfusion[56]. Importantly, caspase inhibition was found to greatly attenuate lactate dehydrogenase (LDH) release in the first 10 minutes of reperfusion, suggesting it is rapidly activated and is an important cause of early infarct formation[56]. Despite this, the combination of VX-765 and IPC was not found to provide additive benefit in infarct size reduction[57].

The above studies clearly demonstrate that targeting caspase-1 can reduce myocardial I/R injury. However further work is yet to be undertaken to identify a clinically translational caspase inhibitor that has the combined attributes of being effective when given at reperfusion, and which is shown to provide added benefit above and beyond that which is obtained through use of conventional therapies administered during acute MI[7,8].

What is the mechanism of protection by caspase-1 inhibition?

Cardioprotection can clearly be obtained following inhibition of caspase-1, and this seems likely to occur via the prevention of pyroptosis and reduced release of the pro-inflammatory

cytokines IL-1□ and IL-18. However, there is also a degree of variability in expression and activation of the NLRP3 inflammasome in different cardiac cell types[21,58]. For example, NLRP3 inflammasome and caspase-1 activation in cardiac fibroblasts leads to significantly increased production of IL-1β[40,59], but not in cardiomyocytes where activation of the NLRP3 inflammasome and caspase-1 is thought to result in pyroptotic death of the cell independently of IL-1β production[30,40,43]. Therefore, a clearer understanding of the mechanism of protection at the cellular level needs to be elucidated.

The kinases of the pro-survival RISK pathway were first shown by Schulman and Yellon to confer powerful cardioprotection when activated at the point of reperfusion following a period of ischemia[60]. This seminal study has prompted a wealth of research aiming to identify clinically translatable means of activating this pathway when reperfusion occurs following myocardial infarction. Interestingly, in a relatively recent study by Mastrocola et al, it is postulated that inhibition of the NLRP3 inflammasome may also confer benefit via activation of the RISK pathway[61]. Using a novel NLRP3 inhibitor (INF4E) they demonstrated significantly reduced caspase-1 expression, which correlated with reduced infarct size and lactate dehydrogenase release, as well as improvements in post-ischemic left ventricular pressure[61]. Furthermore, they demonstrated increased phosphorylation (a measure of activity) of key members of the RISK pathway including ERK1, ERK2 and GSK-3β in hearts that were pretreated with INF4E. Thus, they suggested that inhibition of the NLRP3 inflammasome complex results in enhanced activity of the pro-survival RISK pathway. However, whether activation of these kinases is mechanistically involved the protection seen was not studied.

It is important to recognize that neither Toldo et al and Mastracola et al. found evidence for NLRP3 inflammasome activation or GSDMD cleavage until a relatively late time-point after 1 h of reperfusion[52,61]. This contrasts with the observation that caspase-1 is activated within 10 min of reperfusion[56], and argues for the existence of an inflammasome-independent pathway of caspase-1 activation.

The aforementioned study by Do Carmo et al also implicated a role for the RISK pathway in the cardioprotective effects seen with the caspase-1 inhibitor VX-765[57]. By administering wortmannin, a RISK pathway inhibitor that acts by inhibiting PI3-kinase, for 15 min during early reperfusion, the protective effect of VX-765 was abolished[57]. This suggests that caspase-1 inhibition does offer protection through the RISK pathway. The authors did not investigate, however, whether the entirety of the RISK pathway was upregulated with caspase-1 inhibition to show a clear causal effect, implying that at this stage this is an incidental finding and not evidence of mechanistic certainty. Further, it should be mentioned that in contrast to the findings of Do Carmo et al[57], the earlier study Audia et al[56] saw a synergistic effect of ischemic post conditioning with the caspase inhibitor VX-765, suggesting activation of separate protective pathways.

Controversies

Despite a significant body of evidence suggesting a role for the NLRP3 inflammasome and caspase-1 in I/R injury, several studies using knockout mice suggest that the inflammasome is either not involved or is even protective against reperfusion injury[62-64]. It is possible that some of these discrepancies relate to possible compensatory upregulation of other inflammatory pathways seen in some experimental models[65,66]. It must be recognized that the

expression levels of the NLRP3 inflammasome components is usually low in the hearts of healthy organisms, increasing when primed by the presence of infection, inflammation, or metabolic dysfunction (metaflammation)[15]. In this regard, it is interesting to note that recent studies have shown that the calcium activated protease calpain can rapidly liberate caspase-1 bound to the actin fibers[67].

Conclusions

The mechanism of action through which caspase-1 inhibition is cardioprotective needs further clarification. NLRP3 inflammasome and caspase-1 activation in the various cardiac cell types leads to differing downstream effects depending on cell type[30,40]. In cardiomyocytes, activation of the NLRP3 inflammasome leads to increased caspase-1-mediated cell death but not to increased IL-1β production. In fibroblasts, the main effect is IL-1β-mediated myofibroblast differentiation and collagen synthesis. In leukocytes, NLRP3 inflammasome activation leads to significant levels IL-1β and IL-18 production leading to an overall amplification of the inflammatory response and further pyroptotic and apoptotic cell death[59,68]. It is not clear whether inhibiting caspase-1 provides a protective effect by inhibiting NLRP3 inflammasome activity in all of these cell types, or whether inhibiting activity in one particular cell type is more important.

Furthermore, the studies suggesting activation of the RISK pathway have highlighted an alternative interaction of caspase-1 with this pro-survival pathway and this needs further investigation. This again should be undertaken with agents given at reperfusion, since the earlier experiments examining the RISK pathway activation were undertaken using agents

given prior to onset of ischemia. This will ascertain whether the mechanism of any beneficial effect in terms of upregulating RISK pathway activation could be clinically relevant.

In conclusion, further studies should aim to use very specific caspase-1 inhibitors given at reperfusion and have precise measures of activity to ensure that the drug being given is indeed having the intended effect, i.e. measurement of caspase-1 activity or downstream products of the pathway such as IL-1□. Finally, studies should aim to elucidate the true mechanisms of protection, e.g. is this limited to certain cardiac cells, is the presence of inflammatory cells in the circulation necessary, and are other pathways such as the RISK pathway involved?

Figure legends

Figure 1. The general mechanism of NLRP3 inflammasome activation and pyroptosis. During the priming step, damage associated molecular patterns (DAMPs) released from dying cells in the vicinity stimulate the toll-like receptors (TLRs), leading to the NF-□B-dependent transcription of NLRP3 inflammasome components. In the second, activation, step, a diverse range of compounds (eg: crystals, ATP or mitochondrial reactive oxygen species - mtROS) cause the inflammasome to activate caspase-1 mediated cleavage of Pro-Il-1□ to Il-1□, pro-Il-18 to Il-18 (not shown) and gasdermin D (GSDMD) to N-terminal gasdermin D (GSDMD-NT). GSDMD-NT causes the formation of pores in the plasma membrane, which cause pyroptotic cell death and the release of Il-1□ and Il-18. The extent to which this pathway represents what occurs during ischemia and reperfusion remains to be precisely determined.

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Study	Method of Caspase inhibition	Experimental Model	Caspase inhibition Results
Mocanu et al 2000	Pharmacological inhibitors of Caspase 3,8 and 9	Ischemia reperfusion in Langendorff perfused, isolated rat hearts.	Reduction in Infarct Size.
Pomerantz et al 2001	Pharmacological inhibitors of Caspase-1	Ischemia reperfusion model of superfused human atrial appendage muscle	Reduction in ischemia induced myocardial dysfunction.
Frantz et al 2003	Caspase 1 knockout Mice	*In vivo* permanent coronary artery ligation in mice	Reduction in ventricular dilatation. Reduction in MMP-3.
Syed et al 2005	Transgenic mice HEK293 Cells expressing Pro-caspase 1	*In vivo* coronary artery ligation and reperfusion in a mouse.	Pro-caspase 1 overexpression was associated with 50% larger infarct size.
Merkle et al 2007	Caspase-1 Knock out Mice. Isolated murine cardiomyocytes	*In vivo* coronary artery ligation and reperfusion. Primary cardiomyocytes. ELISA	Reduction in cardiomyocyte hypertrophy. Reduction in post-ischemia LV deterioration.
Mezzaroma et al 2011	Inhibition of Cryopyrin and P2X7	*In vivo* permanent coronary artery ligation in mice. Murine HL-1 cells.	Reduction in infarct size. Increased survival of HL-1 cells. Reduced expression of Caspase 1.
Toldo et al 2011	Alpha-1-antitrypsin (AAT)	*In vivo* coronary artery ligation and reperfusion in mice. HL-1 cardiomyocytes.	AAT treatment – Reduced infarct size, Reduced Caspase-1 activity, Reduced LVEDD and LVESD.
Marchetti et al 2014	Pharmacological inhibitor of NLRP3 inflammasome	*In vivo* coronary artery ligation and reperfusion in mice. HL-1 cardiomyocytes.	Reduction in infarct size.
Do Carmo et al 2018	Pharmacological inhibitor of Caspase-1	Ischemia reperfusion in Langendorff perfused, isolated rat hearts.	Reduction in infarct size. Not additive with IPC. Protection abolished by PI3K inhibitor.
Audia et al 2018	Pharmacological inhibitor of Caspase-1	*In vivo* coronary artery ligation and reperfusion in rats. Ischemia reperfusion in Langendorff perfused, isolated rat hearts.	Reduction in infarct size. Further reduction in infarct size when combined with $P2Y_{12}$ receptor antagonist.

Table 1. Key experimental studies illustrating the effect of caspase-1 inhibition on infarct size. Experiments included use either direct pharmacological or genetic inhibition of caspase-1, or indirect prevention of caspase activation via inhibition of the NLRP3 inflammasome.