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NLRP3 Inflammasome in Cardioprotective Signaling

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(See editorial: *Cardiovascular Pharmacology of the NLRP3 Inflammasome* by Antonio Abbate and George W. Booz, *Journal of Cardiovascular Pharmacology*, 2019;74:173–174).

Abstract: The NLRP3 inflammasome may contribute to infarct development during acute cardiac ischemia-reperfusion (IR). Because infarct size strongly correlates with the degree of heart failure in the long term, therapies that reduce reperfusion injury are still needed as first primary care against heart failure development. Inhibition of the NLRP3 inflammasome is currently viewed as such a potential therapy. However, previous research studies directed at inhibition of various inflammatory pathways in acute cardiac IR injury were often disappointing. This is because inflammation is a double-edged sword, detrimental when hyperactive, but beneficial at lower activity, with activity critically dependent on time of reperfusion and cellular location. Moreover, several inflammatory mediators can also mediate cardioprotective signaling. It is reasonable that this also applies to the NLRP3 inflammasome, although current literature has mainly focused on its detrimental effects in the context of acute cardiac IR. Therefore, in this review, we focus on beneficial, cardioprotective properties of the NLRP3 inflammasome and its components NLRP3, ASC, and caspase-1. The results show that (1) NLRP3 deficiency prevents cardioprotection in isolated heart by ischemic preconditioning and in vivo heart by TLR2 activation, associated with impaired STAT3 or Akt signaling, respectively; (2) ASC deficiency also prevents in vivo TLR2-mediated protection; and (3) caspase-1 inhibition results in decreased infarction but impaired protection through the Akt pathway during mild ischemic insults. In conclusion, the NLRP3 inflammasome is not only detrimental, it can also be involved in cardioprotective signaling, thus fueling the future challenge to acquire a full understanding of NLRP3 inflammasome role in cardiac IR before embarking on clinical trials using NLRP3 inhibitors.

Key Words: NLRP3, ASC, caspase-1 inflammasome, infarct, cardioprotection

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INTRODUCTION

The Nlrp3 inflammasome's primary function is to ward off danger, as is for the entire innate immune system. In general, it can be considered as protective for the organism. From that perspective, it is anticipated that deletion or inhibition the Nlrp3

inflammasome would be detrimental to the overall well-being of the organism, but also likely to the condition of cardiac ischemia-reperfusion (IR) injury. It is therefore somewhat surprising that most of the published preclinical literature on the inflammasome in the field of cardiac I/R injury report positive effects of NLRP3 inflammasome deletion or inhibition.^{1,2} Although it is possible that the positive bias to present positive results in preclinical studies contributes to this,³ the question is begged whether there are any beneficial (or neutral) effects of the NLRP3 inflammasome in the context of cardiac infarction. Indeed, for several other components of the inflammatory-response system, these beneficial effects have been detected. For example, low-level stimulation through TNF α ,^{4,5} IL-6,^{6,7} toll-like receptor 2 (TLR2),^{8,9} and home-mobility group box 1 (HMGB1)¹⁰ have all been reported to generate protection against IR injury. The beneficially low level stimulation seems to be a standard characteristic of many biomolecules in the setting of IR conditions, and fulfills the concept of hormesis.¹¹ This double-edged behavior, together with its strong time and location dependency, probably contributes to the difficulty of obtaining consistent clinical effects with many of the immunomodulatory interventions that have been examined.^{12,13}

It has become clear that gathering the knowledge for when and where beneficial effects of the NLRP3 inflammasome appear is important for the development and testing of Nlrp3 inhibitors in human clinical trials. This will help to avoid conditions wherein treatment may generate possible harm, deplete financial resources for research, and kill valuable hypotheses. In the current review, we focus on the role of the NLRP3 inflammasome in the context of cardioprotective signaling.

NLRP3 INFLAMMASOME IN CARDIOPROTECTIVE SIGNALING

NLRP3 in Cardioprotective Signaling

The first study (Table 1) reporting NLRP3 to be involved in cardioprotective signaling demonstrated loss of ischemic preconditioning (IPC) with NLRP3 deletion in isolated mouse heart studies.⁶ IPC improved recovery of diastolic and systolic function and reduced cell death in isolated wild-type (WT) hearts after 25 minutes of ischemia; these effects were mitigated in NLRP3 knockout (KO) hearts. Considering that small elevations in cytokines may activate cardiac intrinsic protective signaling, cytokines were evaluated in the reperfused hearts. The isolated NLRP3^{-/-} hearts contained significant less cytokine IL-6, with a nonsignificant trend of decreased IL-1 β . Using antibodies against IL-6 and inhibiting the IL-1 β membrane receptor IL-1RA with Anakinra in isolated WT hearts, it was shown that the antibody

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TABLE 1. Summary of Studies Examining Effects of Genetical Deletion of NLRP3 or ASC on Cardioprotective Interventions

KO	Protective Treatment	Effects	Involvement RISK/SAFE Pathway?	Study	Reference
NLRP3	3 × 5 min I	No change in LDH release and cardiac function	Decreased IL-6/STAT3 in reperfused KO heart	Isolated mouse heart (35 min I/45 min R)	Zuurbier et al ⁶
NLRP3	3 × 5 min I	↓ IS	↓ STAT3 baseline ↓ STAT3 activation with IR and IPC	Closed-chest mouse (60 min I/3 h R)	Jong et al ¹⁵
NLRP3	Protective TLR2 ligand activation	No change in IS	↓ p-Akt	Open-chest mouse (30 min I/24 h R)	Sandanger et al ¹⁴
ASC	3 × 5 min I	↓ LDH + ↑ function	IL-6 similar as WT	Isolated mouse heart (35 min I/45 min R)	Zuurbier et al ⁶
ASC	Protective TLR2 ligand activation	No change in IS	p-Akt similar as WT	Open-chest mouse (30 min I/24 h R)	Sandanger et al ¹⁴

I, ischemia; IS, infarct size; LDH, lactate dehydrogenase release during reperfusion; R, reperfusion.

against IL-6 abrogated the IPC effects, whereas Anakinra was without effects on IPC.⁶ The amount of STAT3 protein was also decreased in NLRP3^{-/-} hearts (the survival protein Akt was not examined). These data suggest that NLRP3 somehow partly regulates IPC cardioprotective signaling through a IL-6/STAT3 axis in the isolated heart. Because these effects were only observed for deletion of NLRP3 and not ASC, the loss of IPC effects in this heart model with NLRP3 deletion is not mediated through the inflammasome, ie, it is inflammasome-independent. Cardioprotection through stimulation of TLR2 by Pam3CSK4 was examined in NLRP3^{-/-}, ASC^{-/-}, and WT in vivo mouse models of IR (30 minutes of ischemia; 24 hours of reperfusion) by Sandanger et al.¹⁴ Although TLR2 agonist treatment resulted in significant cardioprotection in WT hearts, no protection was observed in NLRP3^{-/-} and ASC^{-/-} hearts (Table 1). The loss of protection in the NLRP3^{-/-} hearts was associated with diminished phosphorylation of Akt, whereas STAT3 signaling was not examined. Interestingly, there were no alterations in inflammatory signaling (cytokines and chemokines) between the hearts, ruling out that differences in cardioprotective signaling through TLR2 between WT and KO hearts were mediated through changes in inflammatory signaling. In subsequent studies by Jong et al using an in vivo murine model of closed-chest IR,^{15,16} it was observed that deletion of NLRP3 was without effect on infarct size at 3 hours of reperfusion, but that in the in vivo condition, IPC was still effective in the NLRP3^{-/-} animal. Most importantly, baseline STAT3 expression levels and STAT3 activation due to IR and IPC were severely depressed in the NLRP3^{-/-} hearts (Fig. 1). Mizushima et al¹⁷ also showed this interaction between NLRP3 and STAT3: hyperoxia increased the expression and activation of STAT3 in the lungs of WT animals, but not in the lungs of NLRP3^{-/-} animals. The diminished STAT3 signaling in the NLRP3^{-/-} animals was associated with increased hyperoxia-induced mortality.

In summary, the expression of NLRP3, at least partly, regulates the cardioprotective RISK (Akt) and SAFE (STAT3) signaling pathways in an inflammasome-independent fashion. Although IL-6 (or TNF α , because one study observed less TNF α in in vivo NLRP3 KO hearts¹⁶) may mediate this

interaction between NLRP3 and STAT3, how this interaction between NLRP3 on the one side and STAT3 and Akt on the other side is precisely regulated is unknown as of yet, and needs further dedicated research to resolve. NLRP3 deficiency is often, but not always, associated with the loss of effectiveness of cardioprotective interventions to reduce IR injury.

ASC in Cardioprotective Signaling

The Adapter apoptosis-associated Speck-like protein containing a CARD (ASC) provides the scaffold for active caspase-1 with the NLRP3 receptor. To the best of our

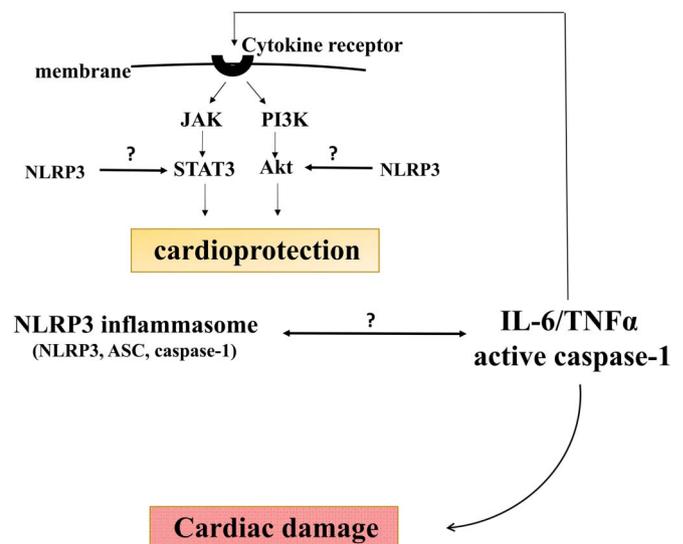


FIGURE 1. Schematic drawing depicting how the NLRP3 inflammasome can contribute to cardiac IR damage and cardioprotection. Activation of the NLRP3 inflammasome generates the production of cytokines that can function as activators of cytokine receptors resulting in the activation of cardioprotective signal molecules such as STAT3 and Akt, whereas the production of cytokines and caspase-1 activation can contribute to cardiac IR damage: it is possible but still unknown that NLRP3 and ASC expression directly determine STAT3 and Akt signaling through a cytokine-independent pathway.

knowledge, only 2 studies have examined to what extent ASC is involved in cardioprotective signaling. In the isolated heart studies of Zuurbier et al,⁶ ASC deficiency was without effect on an IPC protocol. IPC improved diastolic and systolic cardiac function with reductions in cardiac enzyme release following 35 minutes of global ischemia and 45 minutes of reperfusion similarly in WT and ASC^{-/-} hearts. In contrast to the NLRP3^{-/-} hearts that showed a loss of IPC cardioprotection, IL-6 was also not reduced in the reperfused ASC^{-/-} hearts. Using TLR2 activation as means of protective intervention, deficiency of ASC did result in loss of cardioprotection in an in vivo model of cardiac IR injury.¹⁴ The loss of the cardioprotective potential could not be explained by disturbed Akt signaling because TLR2 activation was still able to increase Akt phosphorylation before ischemia in ASC^{-/-} hearts. In conclusion, the available research, albeit sparse, indicates that ASC is needed for TLR2-mediated cardioprotection but not for cardiac IPC.

Caspase-1 in Cardioprotective Signaling

We were unable to find studies that have directly examined whether cardioprotective signaling was affected by genetic manipulations of caspase-1. IL-1 β is the proteolytic product of activated caspase-1, and will partly drive IL-6 and TNF α cytokine production. It is known that caspase-1 knockout mice have reduced production of IL-6 or TNF α when treated with lipopolysaccharide.^{18,19} It is therefore possible that caspase-1 is necessary for cardioprotective signaling, when the signaling pathway of IL-6/TNF α /STAT3 is involved. It may seem confusing or diametrical opposed that caspase-1 inhibition can both reduce IR injury and at the same time also prevent the working of interventions that reduce IR injury. However, such behavior is frequently observed for several phenomena that are involved in IR injury. One such example is the role played by reactive oxidative species: decreasing reactive oxidative species can reduce IR injury, but at the same time will attenuate cardioprotective signaling instigated, for example, by IPC or opening of mitoKATP channels.²⁰

There are, however, several studies that have used pharmacological inhibition of caspase-1, which can be considered as a pharmacological knockout of caspase-1. Do Carmo et al²¹ used pharmacological inhibition of caspase-1 with VX-765 in an isolated rat heart study, showing that VX-765 reduced infarct size to a similar degree as IPC, with IPC ineffective in the presence of this inhibitor.²¹ Congruently with the knowledge that IPC is mediated through the Akt pathway, it was also observed that the protection by caspase-1 inhibition was lost with inhibition of the PI₃K/Akt pathway. These data indicate that caspase-1 activity and IPC protective actions are closely intertwined. It is unclear how Akt inhibition can prevent the protective effects of a caspase-1 inhibitor; maybe VX-765 activates the Akt pathway as an off-target effect. However, in another in vivo rat study with pharmacological caspase-1 inhibition (VX-765), it was shown that the platelet inhibitor cangrelor was still protective in the presence of VX-765.²² Knowing that cangrelor protection is also mediated through the PI₃K/Akt pathway,²³ this study then may suggest that caspase-1 inhibition does not result in the activation of the Akt pathway. However, it is possible that the divergent results

whether Akt signaling is part of the cardioprotective signaling instigated by caspase-1 inhibition relates to the severity of the ischemic insult: with mild ischemia (Do Carmo et al²¹; 35 minutes of ischemia) inhibition of caspase-1 does not add to protection when Akt is already activated, whereas it does add during a more severe ischemic insult (Yang et al²²; 60 minutes of ischemia). It is possible that with short ischemia VX-765 mostly protects because of its activation of Akt, whereas only with extended ischemia activation of caspase-1 occurs, such that then caspase-1 inhibition by VX-765 also starts to contribute to its protection. This dependency of cardioprotective efficacy on ischemic duration is well known,^{24–26} and suggests that depending on ischemia duration, various molecular mechanisms contribute to IR injury. In conclusion, also the caspase-1 component of the Nlrp3 inflammasome seems to be involved in cardioprotective signaling and Akt signaling, although mostly for mild ischemia and not for severe ischemia.

Of note, in the context of caspase-1 inhibition, current studies reporting acute cardioprotection with purportedly specific inhibitors of caspase-1^{21,22} are sometimes interpreted as direct proof that pyroptosis contributes to acute (<3 hours of reperfusion) infarct development.^{2,27} However, older studies showing involvement of caspase-1 in acute cardiac IR injury clearly showed that these effects were not or only partly independent of cytokine production, but related to caspase-1 effects on other caspases, through which for example apoptosis was reduced.^{28,29} Although VX-765 has high selectivity toward caspase-1 (IC₅₀ = 0.2 nM for active component of VX-765), when used at higher dose, it can still inhibit several other caspases such as caspase-4, -5, -8 and -9 (IC₅₀ around 10 nM).³⁰ Although caspase-4 and -5 are absent in rodent hearts, caspase-8 and -9 are present. Therefore, together with possible interactions between caspase-1 inhibitors and the Akt pathway (see above), these inhibitor studies cannot be taken as solid evidence that pyroptosis is involved in acute IR injury.

INFLAMMATION AS A THERAPEUTIC TARGET

Older studies have clearly shown that the proinflammatory response to acute myocardial infarction does certainly not always contribute to ischemic cardiomyocyte death. Experimental studies in closed-chest mice deficient in P-selectin and intercellular adhesion molecule-1,³¹ MCP-1,³² and in animals with defective IL-1 receptor type I signaling³³ all demonstrated similar infarct size when compared to WT, despite having a suppressed inflammatory response after acute myocardial infarction. These studies indicate that several important constituents of the inflammatory pathway induced by acute ischemia do not contribute to infarct size development. This precautions against moving too quickly to clinical models of acute cardiac injury.³⁴

INFLAMMASOME-INDEPENDENT EFFECTS OF THE NLRP3 INFLAMMASOME COMPONENTS

Several studies have reported that cardiac effects of deletion of one NLRP3 inflammasome component was not mimicked by deletion of another component, indicating that Nlrp3, ASC, and caspase-1 can affect protective interventions

and/or I/R injury of the heart independent of each other and the working of an oligomerized Nlrp3 inflammasome complex. Similar noninflammasome findings for NLRP3, ASC, and caspase-1 have been described in models of kidney ischemic injury³⁵ or severe acute pulmonary inflammation.³⁶ It is at the moment unclear through which cellular processes the individual components of the inflammasome components evokes these effects. It does indicate that inflammasome inhibition probably will have differential (inflammasome-dependent and inflammasome-independent) effects depending on which inflammasome component is being targeted. In addition, developing a strategy that specifically inhibits the inflammasome activity without inhibiting the cardioprotective signaling effects would be most promising.

CONCLUSIONS

The available evidence discussed above, although limited, suggests that the NLRP3 inflammasome and its separate components are often intrinsically involved in cardioprotective signaling against acute IR injury. Interactions between the inflammasome and known survival kinases such as Akt and STAT3 have been observed, whereby especially the deficiency of NLRP3 was associated with defective Akt and STAT3 signaling within the heart. The exact nature of how NLRP3 can affect these survival kinases is currently unknown and deserves further attention. This interaction is possibly mediated through cytokine signaling, such as the IL-6/STAT3 axis. Overall, the summarized data warrant against immediate translation of NLRP3 inhibitors during acute cardiac IR conditions in the clinical setting, especially when also other cardioprotective interventions are applied that are mediated through Akt and STAT3 signaling. Such cardioprotective strategies should first be evaluated in preclinical models of cardiac IR injury to avert another “lost-in-translation” therapy and neutral clinical studies.

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