Research Progress on The Mechanism and Treatment of Inflammatory Response in Myocardial Ischemia-Reperfusion Injury

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ABSTRACT

Acute myocardial infarction can be treated aggressively with intravenous thrombolysis, percutaneous coronary intervention, and coronary artery bypass grafting; however, recanalization can cause myocardial ischemia-reperfusion injury (MIRI). This is an important reason that restricts the treatment effect of patients. After the ischemic myocardium is restored to perfusion, an inflammatory response can occur within minutes and peak within a few days. Many pro-inflammatory cytokines can seriously damage cardiac function. Inflammation can regulate cardiomyocyte apoptosis, autophagy, pyroptosis, and necrosis, and is the main initiating factor leading to MIRI in cardiomyocytes.

This article reviews the mechanism of inflammatory response in the ischemia-reperfusion period after acute myocardial infarction and the clinical value and application prospect of inhibiting inflammatory response in the treatment of acute myocardial infarction.

INTRODUCTION

Acute myocardial infarction (AMI) occurs in tens of thousands of people worldwide every day [Davidson 2019]. Although intravenous thrombolysis, emergency percutaneous coronary intervention and coronary artery bypass grafting and other reperfusion treatment measures are widely used in clinical practice and significantly reduce the mortality of myocardial infarction in hyperacute and acute stages, reperfusion therapy has a significant impact on myocardial infarction. Additional damage to the myocardium, including myocardial stunning, reperfusion arrhythmias, and microcirculation disturbances, seriously affects the long-term prognosis of patients. The pathological process in which this reperfusion therapy restores coronary blood flow and causes additional damage to the ischemic myocardium is called the myocardial ischemia-reperfusion injury (MIRI) [DeFilippis 2019]. Numerous studies have shown that inflammatory response plays an important role in the occurrence and development of MIRI. This article reviews the mechanism of inflammatory response in MIRI and the clinical application value of inhibiting inflammatory response in AMI reperfusion therapy.

Myocardial inflammatory response: The rupture of the plaque or the shedding of the thrombus leads to acute blockage of the coronary artery, causing acute ischemic necrosis of the myocardium and leading to myocardial infarction. Myocardial ischemia and hypoxia can induce immune cells to secrete pro-inflammatory cytokines and induce an inflammatory response. The myocardial inflammatory response can lead to myocardial cell damage and deterioration of cardiac function, while inhibition of inflammatory response can improve cardiac function [Lafruse 2020]. There mainly are chronic and acute inflammatory responses in various pathophysiological processes of myocardial tissue. Chronic inflammatory responses mostly exist in the formation stage of atherosclerosis (AS). The immune dysfunction of vascular endothelial cells leads to activation of inflammatory pathways, resulting in endothelial dysfunction, regulating downstream inflammatory responses, and promoting the formation of AS. On the contrary, acute inflammatory response mainly occurs in cardiomyocytes necrotic due to acute ischemia and hypoxia, mainly due to pro-inflammatory factors, such as ruptured cell fragments of acutely necrotic cardiomyocytes, which activates acute inflammatory response [Pluijmert 2020]. After myocardial reperfusion, ion channel function disorder is caused, resulting in cell edema and rupture, and finally release of insufficiently hydrolyzed immune complexes and inflammatory factors, such as high mobility group box-1 protein (HMGB1), tumor necrosis factor (TNF), NLR (nucleotide-binding and oligomerization domain-like receptor, NLR) family proteins, Caspases1/4/5/11 and interleukin 1β (IL-1β), etc., the above inflammatory factors can trigger an inflammatory response of innate immune cells. Antigen-presenting cells transmit the information of inflammatory factors in AMI to B-lymphocytes and T-lymphocytes. These two cells have different antigen receptors and can recognize and target related molecules through pattern recognition receptors (PRRs) to clear [McKernan 2020]. The above evidence indicates that chronic inflammatory response and acute inflammatory response in the myocardium jointly mediate...
the expression of inflammation in various stages and various injuries of the myocardium.

**Mechanism of inflammatory response in MIRI**: Inflammation plays an important role in MIRI and inhibiting the inflammatory response can reduce the scar tissue area in the infarcted area and improve the function of the infarcted myocardium. There are many protein families in MIRI that are involved in the regulation of inflammatory responses, including the NLR family, Caspase family, Interleukin (IL) family, etc. Among them, the NLR protein family has 14 members, namely NLRP1-NLRP14. The immune-related ones include NLRP1, NLRP3, NLRP6, NLRP10, and NLRP12, NLR protein family can regulate inflammatory response through different pathways [Nagyőssi 2015]. NLRP1 mainly regulates the downstream inflammatory response by activating Caspase1 and then inducing the secretion and release of various inflammatory factors [Tenthorey 2017]. Myocardial infarction can damage the autophagy pathway, induce this pathway to fail or only partial autophagy occurs, and unrecovered organelles or cellular debris can induce the activation of the NLRP3 inflammasome, which ultimately aggravates myocardial injury [Wang 2021]. The death properties of cardiomyocytes in MIRI are determined by the properties of Caspase family proteins, which are divided into inflammatory caspases and apoptotic caspases, according to their functional differences. Inflammatory caspases include caspase 1, 4, 5, 11, and 12 et al and apoptotic caspases mainly include Caspase 2, 3, 6, and 7 et al. In MIRI, inflammatory caspases 1, 4, 5, and 11 specifically cleave Gasdermin D (GSDMD), thereby inducing pyroptosis. When cells are subjected to different stimuli, two pyroptotic pathways can be induced, namely the classical pyroptotic pathway dependent on caspase 1 and the non-canonical pathway dependent on caspase 4/5/11. IL is a potent pro-inflammatory cytokine produced by cells of the innate immune system. Previous studies have found that IL-1, IL-2, IL-10, and IL-18 play important roles in defense responses and infection immunity [Munjal 2020]. Lu et al. [Lu 2019] showed that in the early stage of blood flow recovery after myocardial infarction, cardiomyocytes, endothelial cells, and fibroblasts produce excess reactive oxygen species (ROS). ROS directly can damage cardiomyocytes and even lead to cardiomyocyte death. The stage also can damage the lipid structure of the cell membrane and induce neutrophils to secrete a large amount of tumor necrosis factor α (TNF-α). The study found that TNF-α can induce the activation of NF-κB, regulate the inflammatory response, and regulate the pathological changes of MIRI myocardial tissue [Huang 2018]. In addition, TNF-α can activate xanthine oxidase,

![Figure 1. Activated pathways of inflammatory responses.](image-url)
promote the generation of ROS, leading to the up-regulation of inflammatory factor expression, and aggravate the inflammatory response. The above process not only aggravates myocardial MIRI, but also promotes the remodeling of ventricular structure, which eventually leads to the deterioration of cardiac function [Chen 2017]. Experiments have shown that in the early stage of myocardial ischemia-reperfusion, damaged myocardium can secrete and release various chemokines and ROS, activate the complement system, induce the recruitment of neutrophils to the infarcted area, and under the regulation of cell adhesion molecules, neutrophils migrate to cardiomyocytes, leading to blockage of microcirculation, stimulating the production of proteolytic enzymes and ROS, enhancing inflammatory response, and aggravating MIRI [Fan 2019]. Recent studies have shown that in the MIRI model of diabetic rats, myocardial tissue can secrete endogenous inflammatory factors, amplify the inflammatory response, and aggravate myocardial tissue damage [Frati 2017]. The above studies have shown that various related node proteins are involved in regulating the occurrence of inflammatory responses, and ultimately regulate the occurrence and progression of MIRI, as shown in Figure 1 and Figure 2. (Figure 1) (Figure 2) In addition, mitochondria, as an important organelle, are involved in the relevant regulation during the occurrence and progression of MIRI inflammatory response. Mitochondria provide Adenosine triphosphate (ATP) to cells by degrading glucose and other pathways, and it is an energy supply site, which is particularly important [de Dios 2019]. During reperfusion, although acute ischemia and hypoxia are recovered, due to the restoration of blood flow, Ca2+ overload and sudden increase of free radicals cannot be eliminated in time and effectively, which leads to mitochondrial dysfunction [Jia 2022]. At the same time, mitochondrial membrane selectivity is dysregulated, due to Ca2+ overload, which causes mitochondria to swell. Additionally, due to the disorder of mitochondrial ion channels, a large amount of Ca2+ is accumulated in the cell, and the high level of intracellular Ca2+ can easily promote the activation of certain enzymes (for example phospholipase A and proteinase C), and the activation of such enzymes will promote the production of fatty acids. These substances can cause the adhesion, accumulation, and infiltration of neutrophils and release a series of inflammatory mediators, inducing the occurrence of inflammatory responses and aggravating MIRI [Tan 2020]. Mitochondrial damage of cardiomyocytes during reperfusion will promote the release of mtDNA, which in turn induces inflammatory injury and local inflammatory response of cardiomyocytes by activating the toll-like receptors 9 (TLR9) signaling pathway in cardiomyocytes, ultimately leading to MIRI [Li 2021]. Studies have shown that mitochondrial quality control can regulate cell

![Figure 2. After acute myocardial infarction, the myocardial tissue is reperfused through timely measures, but this also causes a large number of oxygen-free radicals, Ca2+, and various dead cell fragments to be generated and activates the Caspase family through the corresponding pathways, inducing inflammatory responses and cells. Death, and ultimately to regulate reperfusion injury.](image-url)
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... death, and the regulation of mitochondrial quality control by pupils can lead to cell death, thereby reducing the occurrence of cellular inflammation, and ultimately reducing MIRI [Wang 2020; Zhu 2021; Zhou 2021]. Some team studies have pointed out that the expression of Phosphoglycerate mutase 5 is up-regulated during reperfusion, which will lead to the increase of oxygen free radicals, which will induce inflammation and aggravate MIRI [Zhu 2021]. The above studies have shown that mitochondria have an important regulatory role in MIRI, and it complements the related protein molecules mentioned above to jointly interfere with MIRI.

Therapeutic strategies for inflammatory responses – regulatory strategies of the NLR family: The NLR family is a key regulator of MIRI inflammatory response. There is more and more research on the development of targeted inhibition of the NLR family. After decades of efforts, in the past, due to technical limitations and insufficient understanding of the nature and structure of the NLR family, the target of the NLR family was the slow development of drugs has made new breakthroughs in research in recent years. Ma et al. [Ma 2020] found that propofol could inhibit the increase in the expression of NLRP1 inflammasome induced by low glucose and hypoxia, while propofol inhibited the activation of Caspase-1 by inhibiting NLRP1 in cortical neurons. Furthermore, in primary macrophages deficient in Raf kinase inhibitor protein (RKIP), up-regulation of NLRP1 inflammasome expression enhances Caspase-1 activation and IL-1β secretion [Qin 2021]. In recent years, MCC950 has attracted extensive attention as a novel small molecule drug that inhibits NLRP3. MCC950 is a highly selective NLRP3 inhibitor. Previous studies have found that MCC950 exerts a protective effect on MIRI by regulating the expression of NLRP3 inflammasome to inhibit pyroptosis. This study confirmed that MCC950 is a potential drug for the treatment of NLRP3 inflammasome-mediated MIRI and verified the effectiveness of this therapeutic target in a rat model, further providing a theoretical basis for MCC950 in the treatment of human MIRI [van Hout 2016]. In addition, Mastrocola et al. [Mastrocola 2016] conducted experiments by establishing a mouse MIRI model and found that compared with the control group, the pretreatment group with INF4E (a newly synthesized small-molecule inhibitor of the NLRP3 inflammasome) significantly reduced the area of MI and the release of lactate from cells. Hydrogenase (lactate dehydrogenase, LDH) release, and further found that INF4E attenuated the formation of NLRP3 inflammatory complex induced by MIRI, negative feedback attenuated MIRI, proving that INF4E can effectively inhibit the expression of NLRP3 in MIRI. Recent studies have shown that metformin enhances cell viability and reduces LDH levels. Part of the pathway is through the activation of AMPK pathway by metformin to regulate the expression of NLRP3, which ultimately reduces the expression of inflammation in MIRI and exerts a protective effect on the heart [Zhang 2020]. Babamale et al. [Babamale 2021] found that NLRP12 acts as a key factor of NF-KB regulating TLRs pathway, so it induces mature protein-1 (Blimp-1) through transcriptional silencing factor B lymphocytes involved in the transcriptional repression of NLRP12 after activation of TLRs and ultimately involved in regulating the inflammatory response in MIRI. The above studies collectively show that regulating the expression of related proteins at the drug or gene level can regulate the activation of NLR family inflammasomes, thereby controlling the occurrence of inflammatory responses, and ultimately exerting MIRI cardio-protective effects. However, there are relatively few related studies on the regulation of NLR family proteins by drugs, and a large amount of research still is needed to explore the mechanism of drugs and related targets that can reduce NLR-like inflammasomes, in order to achieve clinical translation as soon as possible.

Therapeutic strategies for inflammatory responses – regulatory strategies of the Caspase family: In clinical studies, it has been found that Caspase 1 inhibitors effectively can inhibit cell osmotic swelling and cell death, among which VX-765, ac-YVAD-cmk and Pralnacasan have significant effects [Audia 2018; Liang 2019; Rudolphi 2003]. VX-765 currently is widely used to inhibit Caspase 1. Upon reperfusion, VX-765 reduced myocardial infarct size by inhibiting Caspase 1 activity in rats with acute myocardial ischemia. VX-765 attenuated MIRI, indicating that inhibition of Caspase 1 is a rational approach for the treatment of inflammation-response-induced myocardial reperfusion injury [Do 2018]. In addition, Belnacasan, the improver of Pralnacasan, also can effectively inhibit Caspase 1 and has a milder effect on Caspase 8. It can regulate the activation and expression of Caspase 8 upstream and achieve the purpose of reducing the inflammatory response. It inhibited the release of IL-1β more effectively than Pralnacasan in an LPS-treated cellular model. It also has shown significant efficacy in animal models of osteoarthritis, epilepsy, and myocardial infarction [Jacotot 2020]. Studies have shown the effect of the caspase 3 inhibitor Ac-DEVD on functional recovery, myocardial infarct size, and apoptosis in isolated rat hearts subjected to 30 minutes of global ischemia and 120 minutes of reperfusion. Inhibitors were added to the perfusion medium during the first 10 min of ischemia and the first 30 min of reperfusion. Although Ac-DEVDs did not alter post-ischemic function, they significantly reduced infarct size and positive TUNEL staining in cardiomyocytes. The results of this study indicate that the Caspase 3 inhibitor Ac-DEVD can protect the heart, but its role in mitigating MIRI and reducing inflammatory response is unclear, and further research is needed [Perrin 2003]. Previous reports indicate that Caspase-6 is upregulated in injured retinal ganglion cells and that its inhibition promotes the survival and regeneration of these adult CNS neurons. Treatment of the whole rat retina with Z-VEID, a selective inhibitor of Caspase-6, improved the survival rate of ganglion cells, however, whether this process reduces the inflammatory response, and the specific mechanism is unclear [Monnier 2011]. Mocanu et al. [Mocanu 2000] found that Caspase 8 plays an important role in the mediation of inflammation. The model was established by hypoxia/reoxygenation, and the selective Caspase 8 inhibitor Z-IEETD was added before hypoxia/reoxygenation. The results showed that Z-IEETD effectively can reduce the inflammatory response and reduce MIRI. In MIRI, macrophages secrete a large number of pro-inflammatory factors in the cardiomyocyte inflammatory...
response pathway. The pan-Caspase inhibitor Z-VAD can induce macrophage death under specific conditions, thereby reducing the inflammatory response [Li 2019]. The above studies collectively show that although there has been great research progress on inhibitors against Caspase family proteins, further experimental exploration is needed for clinical application, and it is expected to provide effective drug support for reducing inflammatory responses in the future.

Therapeutic strategies for inflammatory responses – regulatory strategies of the interleukin family: Inflammatory responses play a key role in MIRI. One of the most potent pro-inflammatory mediators is IL-1, which includes IL-1α and IL-1β, which mediate leukocyte chemotaxis, macrophage chemotaxis, cell activation, endothelial cell dysfunction, and cardiomyocyte apoptosis [Dabouz 2020]. Fearon et al. [Fearon 2008] found that IL-1 receptor antagonist (IL-1ra) is a natural protein that inhibits the effects of IL-1α and β by competing with IL-1 receptors, thereby significantly reducing inflammation. Recombinant human IL-1Ra (rhIL-1Ra) has been shown to have cardioprotective properties. Meanwhile, induction of upregulation of IL-1Ra expression in a rat model was cardioprotective, attenuated inflammation responses, and reduced infarct size [Zhu 2019]. In view of the important role of inflammatory response in MIRI, the anti-inflammatory thrombosis prognosis study (CANTOS) in the previous exploration found that directly targeting the inhibition of inflammation has become a potential therapeutic option. Part of the mechanism is that Canakinumab binds IL-1β with high affinity and selectivity and neutralizes the activity of IL-1β by blocking its interaction with the receptor, and the final result shows that it can significantly reduce inflammation reaction [Shah 2019]. Recently, it was reported that a total of 117 patients with myocardial infarction in a clinical trial were given the IL-6 selective inhibitor Tocilizumab and placebo within two days after myocardial infarction, and the first time after myocardial infarction. Blood was collected on the second day and the third day to detect C-reactive protein and TNT protein, and it was observed that the expression levels of C-reactive protein and TNT protein in the blood of the Tocilizumab group were significantly lower than those of the placebo group, suggesting that Tocilizumab can significantly reduce the level of inflammatory response in MIRI and improve heart function [Kleveland 2016]. The above studies have proved that the interleukin family plays a key role in the inflammatory response in MIRI and can significantly reduce MIRI and improve cardiac function by selectively inhibiting key targets. At present, some interleukin inhibitors have been put into use, but the scope is too small, and the actual clinical effect is far from the expected value. Therefore, strengthening the exploration of interleukin inhibitors is expected to be a reliable solution to increase clinical benefits.

CONCLUSION

Inflammatory response plays a key role in MIRI, and the inflammatory response is not regulated by an independent mechanism, but involves multiple mechanisms, which are independent and interrelated, and ultimately contribute to the occurrence of MIRI inflammatory response. This article describes the inflammatory response and its mechanism of action in MIRI and analyzes the exploratory drugs and clinical drugs that regulate the inflammatory response as shown in Table 1. (Table 1) Although the inflammatory response has been explored for a long time, some mechanisms still are unclear, and many clinical selective inhibitors still need to be supported by evidence, especially the specific mechanism of action of selective inhibitors, their effects, and their side effects. There only are a handful of inflammatory response inhibitors currently approved to reduce MIRI. Therefore, future research still needs to further explore the specific mechanism of inflammatory response in MIRI and more clinical trials are needed to prove the efficacy and safety of selective target inhibitors.

Table 1. Synoptic table reporting the main literature data dealing with anti-inflammatory agents employed in the treatment of reperfusion injury

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Research object</th>
<th>Target protein</th>
<th>Inhibitor</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Hout</td>
<td>2016</td>
<td>Pig</td>
<td>NLRP3</td>
<td>MCC950</td>
<td>Effective</td>
</tr>
<tr>
<td>Matrocola</td>
<td>2016</td>
<td>Male Wistar rats</td>
<td>NLRP3</td>
<td>INF4E</td>
<td>Effective</td>
</tr>
<tr>
<td>Zhang</td>
<td>2020</td>
<td>Rat</td>
<td>NLRP3</td>
<td>metformin</td>
<td>Effective</td>
</tr>
<tr>
<td>Audia</td>
<td>2018</td>
<td>Rat</td>
<td>Caspase 1</td>
<td>VX-765</td>
<td>Effective</td>
</tr>
<tr>
<td>Jacotot</td>
<td>2020</td>
<td>Rat</td>
<td>Caspase 8</td>
<td>Belnacasan</td>
<td>Effective</td>
</tr>
<tr>
<td>Perrin</td>
<td>2003</td>
<td>Rat</td>
<td>Caspase 3</td>
<td>Ac-DEVD</td>
<td>Effective</td>
</tr>
<tr>
<td>Monnier</td>
<td>2011</td>
<td>Rat</td>
<td>Caspase 6</td>
<td>Z-VEID</td>
<td>Effective</td>
</tr>
<tr>
<td>Mocanu</td>
<td>2000</td>
<td>Rat</td>
<td>Caspase 8</td>
<td>Z-IETD</td>
<td>Effective</td>
</tr>
<tr>
<td>Li</td>
<td>2019</td>
<td>Rat</td>
<td>Pan-Caspase</td>
<td>Z-VAD</td>
<td>Effective</td>
</tr>
<tr>
<td>Shah</td>
<td>2019</td>
<td>Rat</td>
<td>IL-1b</td>
<td>Canakinumab</td>
<td>Effective</td>
</tr>
<tr>
<td>Kleveland</td>
<td>2016</td>
<td>Humanity</td>
<td>IL-6</td>
<td>Tocilizumab</td>
<td>Effective</td>
</tr>
</tbody>
</table>
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