

Research Progress on the effect of calcium overload on myocardial ischemia-reperfusion injury

Zhenrun Zhan^{1,2}, Xiaodan Bi^{1,2}, Jinpeng Yang^{1,2}, Tingting Zhao^{1,2,*}

¹ Changzhi Medical College, Changzhi, Shanxi, 046000, China

² Heping Hospital Affiliated to Changzhi Medical College, Changzhi, Shanxi, 046000, China

* Corresponding author: Tingting Zhao (Email: 649823325@qq.com)

Abstract: In the case of acute myocardial infarction, after coronary angioplasty, thrombolytic therapy and cardiac arrest and rebound surgery, the ischemic myocardium of patients may suffer from blood reperfusion injury. However, this is an inevitable complication of treatment, mainly manifested in arrhythmia, myocardial stunning, heart failure and so on, and even death in severe cases. The main mechanisms of myocardial ischemia-reperfusion injury include inflammatory response, autophagy, apoptosis, oxidative stress response, calcium overload, mitochondrial dysfunction and so on. What makes me curious is the calcium overload mechanism, which is the main inducement of reperfusion injury, and can act with other inducing mechanisms to further aggravate reperfusion injury. It is an important cause of myocardial injury and provides a new idea for myocardial protection. This paper comprehensively discusses calcium overload from the perspective of the mechanism of ischemia-reperfusion injury.

Keywords: Calcium overload; Myocardium; Ischemia-reperfusion injury.

1. Introduction

In clinical work, myocardial infarction and other diseases with insufficient myocardial perfusion seriously affect the life and health of the people, but with the development of medical technology, the development and improvement of coronary intervention and heart bypass, the lives of patients with myocardial infarction are increasingly better protected. Some studies have shown that after myocardial ischemia for more than a certain period of time, myocardial cells will begin to die from damage, so in clinical practice, we are often asked to give reperfusion to ischemic myocardium as soon as possible to reduce the damage caused by ischemia. Surprisingly, after extensive clinical studies and basic research, a related article [1] pointed out that myocardial ischemia-reperfusion is not always beneficial to protect cardiomyocytes, but instead can cause further damage to ischemic tissue cells in some cases, namely myocardial ischemia-reperfusion injury (MI-RI), whose main mechanisms of occurrence are inflammatory response, autophagy, apoptosis, oxidative stress response, calcium overload, and mitochondrial dysfunction [2]. Therefore, the prevention and treatment of ischemia-reperfusion injury has become a research hotspot in many fields, including heart and cerebrovascular. In recent years, many articles in the cardiovascular field have indicated that calcium overload is not only the main inducement of reperfusion injury, but also can promote other inducing mechanisms, such as mitochondrial dysfunction, oxidative stress response, etc. It can be seen that calcium overload plays an important role in the occurrence and development of MI-RI. Based on the recent research on the mechanism of MI-RI and the effective measures to reduce MI-RI, this paper summarizes the relevant research materials on MI-RI, and provides convenience for Chinese people to carry out the research on the occurrence mechanism and protection mechanism of MI-RI.

2. The Mechanism of Calcium Overload in Cardiomyocytes

2.1. Na-H exchanger (NHE)

As the name implies, sodium hydrogen exchanger is an ion transport protein located on the cell membrane that can exchange hydrogen ions and sodium ions in equal amounts. Its main role is to maintain the pH stability of cells. During myocardial ischemia, the ischemic cells perform anaerobic respiration due to insufficient oxygen supply, and generate a large amount of H⁺ to accumulate in the cells, leading to cell acidosis [3]; When the blood is reperfusion, the blood flow takes away the H⁺ accumulated during the extracellular ischemia, which makes the pH of the extracellular fluid rise rapidly. Under the combined action of the two mechanisms, the pH gradient on both sides of the cell membrane further increases, rapidly activating the sodium hydrogen exchanger. With the massive inflow of sodium ions, the sodium gradient difference formed inside and outside the cell expands and activates the reverse transport of sodium calcium exchanger protein, which can eventually cause the accumulation of calcium ions in the cell, namely calcium overload. The article published by Inserte et al. mentioned the following viewpoint: after myocardial ischemia, the body will stimulate cGMP/PKG pathway, which can reduce myocardial ischemia reperfusion injury by inhibiting sodium hydrogen exchanger [4]. Michiel ten Hove et al. [5] made myocardial ischemia model in mice to verify the effect of NHE inhibitor on reperfusion injury. The results showed that NHE inhibitors could reduce the ischemic sodium overload by blocking NHE, thereby reducing the intracellular Ca²⁺ concentration, and ultimately reducing the heart damage. Therefore, we can conclude that sodium hydrogen exchanger is an important part of calcium overload mechanism, and we can reduce reperfusion injury through it.

2.2. Sodium Calcium Exchange Protein (NCX)

Under normal physiological conditions, sodium calcium exchange proteins transfer sodium ions into cells and calcium ions out of cells, and the transport ratio is 3:1. Its main function is to use the sodium concentration gradient potential energy on both sides of the membrane established by the sodium pump activity to expel calcium ions from the cells, so as to maintain a low concentration of free calcium ions in the cells. However, when sodium hydrogen exchanger is activated and sodium potassium ATPase dysfunction is caused by insufficient energy supply due to myocardial ischemia, the intracellular sodium concentration increases significantly, leading to the activation of reverse sodium calcium exchanger and the pathological state of calcium overload [6]. Maddaford et al. [7] designed the experiment by cloning shRNA into adult rat cardiomyocytes through adenovirus mediated cloning to reduce the expression of sodium calcium exchange protein (NCX). When the treated cardiomyocytes are exposed to simulated ischemia solution, they will be protected from the increase and damage of cytoplasmic Ca^{2+} detected in control normal cells during ischemia and reperfusion, indicating that cells with NCX deficiency are obviously protected. It can be concluded that NCX of sarcolemma contributes to calcium overload of cytosol during IRI.

2.3. Calcium Pump of Endoplasmic Reticulum/Sarcoplasmic Reticulum (ER/SR SERCA)

SERCA is a transmembrane transport protein rich in sarcoplasmic reticulum, which plays an important role in the regulation of calcium homeostasis in cardiomyocytes. SERCA belongs to the P-type pump, which can pump Ca^{2+} from the cytoplasmic matrix to the sarcoplasmic reticulum using the energy released by ATP hydrolysis. When the myocardial perfusion is insufficient, there is not enough energy supply, and its active transport ability to drain Ca^{2+} from cytoplasm into the sarcoplasmic reticulum by consuming ATP reverse concentration gradient will be significantly reduced [8]. When ATP concentration reaches a critical low threshold (lower than 100mmol/L), myocardial cells begin to appear stiff contracture, which marks the beginning of the increase of cytosolic Ca^{2+} and represents a key event in the progress of ischemic injury [9]. Low ATP concentration will damage the activities of NCX and ER/SR SERCA in the sarcolemma, thus triggering cytosolic Na^{+} and Ca^{2+} overload [10].

2.4. L-type Ca^{2+} channels (LTCC)

L-type calcium channel is a voltage dependent calcium channel. It can be seen from the above mechanism that when cells are ischemic, the cell membrane potential will depolarize, and then activate the L-type calcium channel to open. Next, a large amount of calcium ions will stimulate another synergistic mechanism, namely calcium triggered calcium release (CICR). Arreola et al. [11] described the characteristics of Ca^{2+} current in guinea pig ventricular myocytes using voltage clamp experiment simulating physiological action potential. The Ca^{2+} current generated by action potential consists of L-type and T-type calcium channels. T-type calcium current participates in the fast component of Ca^{2+} current, while L-type calcium current participates in the fast component and slow component of

Ca^{2+} current. By measuring cytosolic Ca^{2+} transients through fura-2, they concluded that calcium ions flowing into sarcoplasmic reticulum can trigger the release of calcium ions from sarcoplasmic reticulum when L-type calcium channels open. In another article [12], the mechanism of sarcoplasmic reticulum releasing calcium ions was not triggered by the administration of drugs to reduce the activity of L-type calcium channel, thus the calcium overload of ischemic cells was reduced. Therefore, we can more clearly understand the existence of calcium triggered calcium release (CICR) mechanism caused by L-type calcium channel.

2.5. Ryanodine receptor (RyR)

RENOTIN RECEPTOR is a calcium releasing channel located in the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). It can rapidly release calcium ions from ER/SR, and is an important link of cardiac excitation contraction mechanism [13]. Toit et al. [14] verified whether "at the beginning of reperfusion, the reduction of calcium flow through the sarcolemma or sarcoplasmic reticulum can reduce the subsequent mechanical stunning". The isolated active rat hearts were subjected to ischemia-reperfusion treatment, and the cardiac output of the experimental group added with RyR (a pharmacological inhibitor of RyR) was significantly higher than that of the control group without drug inhibition. It shows that during the period of ischemia-reperfusion, pharmacological inhibitors can inhibit RyR, reduce the calcium release of sarcoplasmic reticulum, reduce the intracellular Ca^{2+} overload, and significantly facilitate the recovery of contraction.

2.6. Ca^{2+} /calmodulin dependent protein kinase II (CaMKII)

CaMKII is a key mediator, which regulates excitation contraction coupling of myocardial cells through phosphorylation of RyR2, PLB/SERCA2a and LTCC [15]. Of course, its effect on calcium transport is mainly through the activation of voltage gated calcium channels to make calcium ions flow in, thus triggering a series of mechanisms to increase the intracellular calcium concentration. At the same time, the overexpression of calmodulin dependent protein kinase II will also prolong the calcium current time and increase its amplitude, thus increasing calcium overload [16].

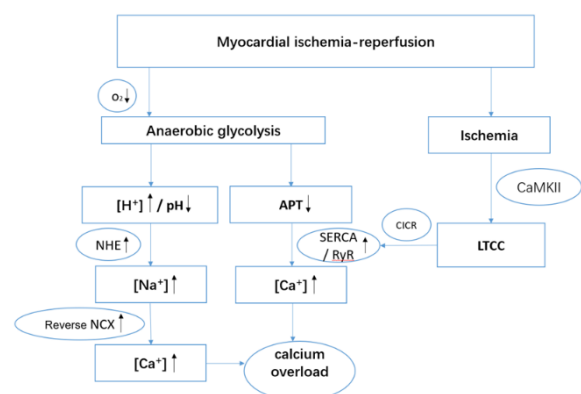


Fig.1 Flow chart of calcium overload mechanism

2.7. Generalization

The oxygen supply of cells in the myocardial ischemic area is insufficient, and ATP can only be produced by anaerobic glycolysis. Anaerobic glycolysis will produce a large amount

of lactic acid, H⁺ and NADH⁺. When they accumulate in the cells to a certain extent, the acid-base balance of the cytoplasm will be broken, and the body will exchange hydrogen ions and sodium ions in the cells by activating NHE, increasing the pH value in the cells, and rebuilding the acid-base balance. At the same time, the influx of sodium ions activates the pathological state of NCX, in which NCX exchanges sodium ions inside cells with calcium ions outside cells. During blood flow reperfusion, the hydrogen ions accumulated outside the cells in the ischemic period are taken away, thus increasing the gradient difference of hydrogen ion concentration inside and outside the cell membrane, which will further stimulate the activity of sodium hydrogen exchanger and indirectly promote the further increase of calcium ion concentration in the cytoplasm. In addition, the ability of calcium pump of endoplasmic reticulum/sarcoplasmic reticulum to process Ca²⁺ was impaired by ischemia/reperfusion, and the ability to reuptake Ca²⁺ from cytoplasm was severely reduced. At the same time, L-type calcium channel opens, a large number of calcium ions enter the cell and activate the calcium triggered calcium release mechanism, causing the rapid release of calcium ions from ER/SR by the ryanodine receptor. These two mechanisms together lead to a further lethal increase in the level of cytoplasmic Ca²⁺ in myocardial cells [17]. To sum up, cells in the ischemic region have multiple mechanisms working together to promote a significant increase in intracellular calcium concentration, which in turn activates a variety of processes, which lead to cell death after ischemia-reperfusion. Next, the second part of this paper will discuss these processes.

3. The Role of Calcium Overload in MIRI

3.1. Mitochondrial permeability transition (MPT)

Mitochondrial injury is considered as a sign of the change of myocardial cells from reversible injury to irreversible injury [18]. After the ischemic myocardium was reperfused by blood flow, the cytoplasmic calcium concentration of ischemic cells fluctuated strongly. In order to reduce the concentration of calcium ions in the cytoplasm, the body will use the potential difference between the two sides of the mitochondrial membrane caused by ischemia and reperfusion to transport calcium ions in the cytoplasm to the mitochondrial matrix by using the one-way calcium transporter protein on the mitochondrial membrane, but at the same time, it will also increase the level of calcium ions in the mitochondria, damage the mitochondria, and trigger the mitochondrial permeability transition (MPT), which is mainly manifested by the opening of mPTP. This is a material channel with large conductance and high flux, which is mainly composed of adenine nucleotide transporter in mitochondrial inner membrane, cyclophilin D in matrix and blood pressure dependent anion channel (VDAC). When opened, substances with molecular weight up to 1.5kD can freely pass through [19]. With the formation and opening of mPTP, the mitochondrial inner membrane potential began to depolarize and the mitochondrial matrix began to swell. Finally, the outer membrane of the mitochondria broke, and the content flowed out of the cytoplasm. The proteins in the content, such as cytochrome C, jointly activated the caspase cascade reaction, and started the cell lysis process, leading to cell death. Baines

et al. [20] created mice that cut off the Ppif gene encoding cyclophilin D and mice that overexpress cyclophilin D in the heart. Ppif negative mice could avoid ischemia/reperfusion induced cell death *in vivo*, while cyclophilin D overexpression mice showed mitochondrial swelling and spontaneous cell death. From this group of experiments, we can draw a conclusion that cyclophilin D and mitochondrial permeability transition are important mediating mechanisms of calcium overload induced cell death.

3.2. Hypercontract

At present, the mechanism of excessive contraction of cardiomyocytes is controversial, mainly including energy dependence and induction of intracellular Ca²⁺ overload. Nuclear magnetic resonance spectroscopy shows that after transient ischemia, energy recovery is rapid and nearly complete, but the heart still has excessive contraction and verification necrosis [21]. This shows that the excessive contraction of myocardial cells is not caused by low ATP level. However, Piper et al. said that cytoplasmic Ca²⁺ oscillations can cause sustained and uncontrolled activation of the contractile apparatus, resulting in excessive contraction [22]. In tissue, the mechanical force generated by the excessive contraction of adjacent cells will lead to the decomposition and necrosis of the interacting adjacent cells [23]. Therefore, we can further confirm the mechanism that calcium overload induces excessive contraction of myocardial cells, and induces cell lysis and necrosis.

3.3. Calpain Mediated Protein Hydrolysis

Calpain is an enzyme that degrades proteins. Calpain activity is affected by the concentration of calcium ions in muscle cells. Calcium ions can activate this protein. It can degrade proteins in myofibrils, mainly including cytoskeleton, mitochondrial proteins and endoplasmic reticulum. The increase of Ca²⁺ level induced by tissue ischemia/reperfusion can also lead to pathological activation of calpain [17]. Yoshikawa et al. conducted experiments on the effects of rat heart ischemia reperfusion on left ventricular mechanical work and energetics, which showed that calpain hydrolyzed the lining protein (α -fodrin) and anchor protein (ankyrin) from the sarcolemma and cytoskeleton, reduced the tolerance of the sarcolemma to the mechanical stress related to excessive contraction and acute cell swelling during reperfusion, and increased the vulnerability of the sarcolemma [24]. Anchoring proteins have a central domain that binds to the lining protein and an N-terminal domain that interacts with a variety of receptors and channels, including Na⁺/K⁺ pump subunits. Na⁺/K⁺ pump can be connected to the membrane cytoskeleton based on fodrin by binding with anchoring protein. In addition, activated calpain can induce the release of a variety of pro apoptotic factors, including cytochrome C. Huss R et al. [26] proved through experiments that inhibition of calpain activity can reduce the infarct size of different models, and the pharmacological inhibition of calpain can play a protective role in the myocardium.

3.4. Apoptosis and necrosis

Apoptosis, also known as programmed cell death, is caused by the enzymatic hydrolysis of cells due to the activation of apoptosis proteins by a variety of apoptosis factors. Calcium overload is also an important factor that can stimulate programmed cell death through a series of signal transduction. It can induce the generation of death ligands such as tumor

necrosis factor, which can activate the apoptosis process by binding with corresponding receptors. At the same time, calcium overload can also induce the programmed cell death by activating calmodulin kinase and stimulating mitochondria to release apoptosis promoting proteins [27]. In ischemia-reperfusion tissues, the increase of intracellular calcium concentration can also lead to the formation of calcium pyrophosphate complex and urea in cells. These components can activate inflammatory bodies to make IL-1 β And TNF α and other cytokines begin to be produced, which aggravates the tissue ischemia-reperfusion injury. These factors can also reverse activate a variety of transcription factors, including NF κ B, and promote the expression of chemokines and cytokines, leading to further inflammation and further cell damage, thus forming a vicious circle and causing necrosis [17].

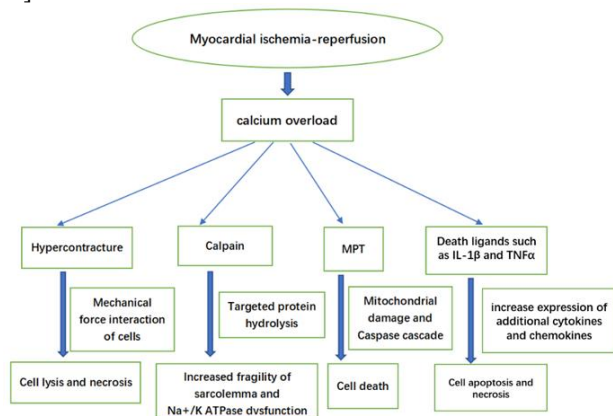


Fig 2. Diagram of calcium overload in MIRI

4. Conclusion

By studying the mechanism of MI-RI and better understanding the potential signaling pathways, we will have a greater possibility to develop therapeutic methods to prevent MI-RI. We look forward to the development of new therapies to reduce myocardial infarction/reperfusion injury and bring them into clinical trials as soon as possible. In a word, understanding and further studying the mechanism of calcium overload and the mechanism of causing MI-RI is the basis for our in-depth study of the pathogenesis of MI-RI. A large number of literatures have proved that calcium overload occurs during myocardial ischemia reperfusion, and calcium overload can further aggravate myocardial ischemia reperfusion injury.

Therefore, we can study the mechanism of calcium overload and the mechanism of calcium overload aggravating ischemia-reperfusion injury. Inhibiting the mechanism of calcium overload and blocking the mechanism of calcium overload aggravating injury can reduce reperfusion injury and protect myocardial cells. The mechanisms reviewed in this article provide multiple potential targets for the prevention and treatment of clinical myocardial infarction. At the same time, the clinical demand is urging researchers to make greater efforts to find more effective and safer drugs to treat MI-RI. Multi target prevention and treatment of MI-RI has potential application value. Therefore, to study the multiple signal pathways involved in myocardial ischemia-reperfusion injury; To clarify the interaction between various mechanisms related to myocardial ischemia-reperfusion and calcium regulation; To test the effect of different calcium channel drug combinations or multi target chemosynthetic drugs in MI-RI

treatment, in order to seek the therapeutic effect of 1+1>2. It is the trend and direction of future research.

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