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Review of Gene Therapy on Myocardial Ischemia-Reperfusion Injury Treatment

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Abstract: This article aims to summarize decade research which utilizing gene therapy method on myocardial ischemia-reperfusion injury. Relative articles are collected from CNKI retrieval. By analyzing and comparing the relative research articles published decade, the recent research conclusions are summarized. Methods and conclusions are compared on each upside and downside. The recent research is center on the gene transduction or stem cell transplantation. The adenovirus and adeno-associated virus are introduced into the gene transfer. The pretreatment of stem cell transplantation is also researched in recent research. All research conclusions claim that positive results are observed after treatment, including injury reduced or reversed, myocardial protect effects. Due to the complexity of mechanism in myocardial ischemia-reperfusion injury, the works may show conflicting results at the conclusion part. However, the results are roughly positive at the practical application in treatment. Still, the conclusions are limited at laboratory research on pigs and mice, and the safety evaluates are absence in some research, which confined the clinical research and further utilizing.

Keywords: Gene Therapy; Myocardial Ischemia-Reperfusion Injury; Adenovirus; Stem Cell.

1. Introduction

Myocardial ischemia-reperfusion (I/R) injury is the prevalent injury after acute myocardial ischemia reperfusion. Clinically, we suppose timely reperfusion as the first treatment of myocardial ischemia. Reperfusion timely could partly reduce the injury from myocardial ischemia, including the anoxic damage and energy metabolism disturbance. However, the myocardial I/R injury may occur after reperfusion. After a period of hypoxia, the myocardial cells would not relief from the hypoxia effect, contrarily, the cells may engage an outbreak of radical, cause the oxidative damage on cells including cellular structures and metabolize pathway, leading to a programmed necrosis in the end. Calcium overload is also observed during the myocardial I/R injury, which could lead to a mitochondrial apoptotic pathway. Inflammatory reactions are also the reason of the myocardial I/R injury, the leukocyte chemotactic factors increasing causes the leukocytes flow out from blood capillary, histamine causes the swelling of tissue around. These would finally lead to no-reflow phenomenon in which blood does not flow from some of the blood capillaries after reperfusion would causes the constantly anoxic damage. [1]

As for now, there is no specialized medicine or treatment to treat or prevent the myocardial I/R injury. Clinic experience in China suggests selective β-receptor antagonists like metoprolol would partly reduce the injury and myocardial remodeling. But the incidence rate and consequence of myocardial I/R injury would severely influence the prognosis of ever myocardial ischemia patients. By review the past research of myocardial I/R injury, some of the results gives author a new attitude to myocardial I/R injury treatment. Gene therapy is a new technic in clinic application. By transferring the genes or RNA via carriers including adenovirus, gland-related virus and non-viral-carries like lipidosome. The genes would be directly introduced into cells or the in-body genes expression may be enhanced or weakened by the RNA introduced. Unlike the directly chemical drugs effect on receptors, the gene expression regulation could reach more pathway without specific receptor or with conservative receptors which is hard to design chemical drugs.

In recent laboratory research, the adenovirus and stem cell has been introduced treatment on myocardial I/R injury on animal model. An example [2] of gene transferring solution is by transferring vascular endothelial growth factor (VEGF) gene with adenovirus into pigs` myocardial cells by injection, shows a less inflammation result than the control group. Other research [3] on stem cell solution also shows that directly mesenchymal stem cells (MSCs) injection and series pretreatment before injection can influence the effect of myocardial protection and inhibit inflammation rate. By analysis the research method and result, the further research direction could be envisioned.

Some researchers only utilized the similar method of gene therapy as their experiment tools, including knockdown or silent some specific in-body genes to inhibit the pathway of apoptosis. These researches only aiming to reveal the mechanism of myocardial I/R injury, but some of them didn`t cause severe adverse reactions on experiment animals. As author supposed, even those method didn`t aim at treatment at first, the methods and results may be used for reference in further research of myocardial I/R injury treatment. And some of them even given the contradict conclusions. The researches [4,5] on heme oxygenase 1(HO-1) `s effect on myocardial I/R reperfusion supposed HO-1 could increase or decrease the apoptosis of myocardial cells.

By consulting, analyzing and comparing the methods, conclusion of decade researches, this article is aiming to summary the exist results on this title. Author envisions that the gene therapy could become the further keynote of myocardial I/R injury treatment at future.

2. VEGF Gene Transferring Gene Therapy

2.1. adVEGF-DΔNΔC Transferring Gene Therapy on Pig

Pajula et al. (2022) transferred the VEGF-DΔNΔC to pig with adenovirus. A total dose of 1 × 1012 vp diluted in sterile saline to 2 ml were given. AdLacZ was set as the control group to evaluate the transferring success. After the gene transferring was successfully received, multiple evaluate was promoted. By analysis the tissue samples and histology, the increasing of capillary area and lymph angiogenesis were observed, additionally, microsphere measurement for tissue perfusion shown 3.4-flod increase in perfusion near the infarct border zone was observed. Off-target tissue samples and blood samples were collected for analysis of liver function, kidney function, tissue damage, and inflammation, in this test no significant changes were observed, promote the method was safety on animal. [2] The results are positive that body didn`t show resistance to the gene transfer and it was successfully reversed the damage caused by myocardial I/R injury by increase the capillary and adequate reperfusion which decrease the no-reflow phenomenon.

2.2. MSCs Mediated Transferring of Ad5-hERL-IRES-VEGF

Qin et al. (2017) utilized adenovirus 5 to transfer VEGF gene to MSCs and then inject MSCs to coronary arterial branch ligated rat. By Immunohistochemistry measurement, the relative gene expression was successfully observed in the transplantation area, Active angiogenesis was also observed in the transplanted area. Compare with the control group with only MSCs injection, the gene transferred MSCs shown a significant increase of angiogenesis rate.[6]

3. MSCs Therapy and Pretreatment of MSCs

3.1. MSCs Only

Wang et al. (2020) reported that intravenous MSC delivery to pig could reduce the microvascular obstruction (MVO) which related with the no-reflow phenomenon, and improved left ventricular ejection fraction (LVEF). Concurrently, the A decrease in NK cells in the peripheral blood and ischemic heart tissue and reducing of serum concentrations of IL-1β, IL-6, and CRP in the MSC group suggested the effect of MSC therapy may have a systemic anti-inflammatory effect. [3]

In early research by Zhao et al. (2019), the purified MSC-derived exosomes (MSC-Exo), also able to reduced infarct size and alleviated inflammation levels in the heart and serum by intramyocardial injection. Claims that the MSC have multiple effect pathway. In their further research, reducing miRNA-182 partially attenuated the modulation of macrophage polarization by MSC-Exo, which suggest miR-182 is the active ingredient. [7]

3.2. Pretreated MSCs

Yang et al. (2021) reported the Sevoflurane preconditioning increased the expression of TRPC6 and VEGF, and the result supposed to be more positive than the non-preconditioning MSC. [8]

Wei et al. (2019) did a further research on MSC-Exo. By intramyocardially injecting the MSC-EXO carrying miRNA-181a-overexpressing lentiviruses, the miR-181a-overexpressing MSC-Exo shown a significant cardiac function and reduced infarct size effect. Subsequent research revealed the impact of miRNA-181a on Treg polarization. [9]

4. HO-1 Gene Transferring and the Multiple Effect of HO-1 on Myocardial I/R Injury

4.1. Recombinant Adeno-associated Virus Mediated HO-1 Gene Transferring Shown Protection Effect

Li et al. (2011) reported the Recombinant adeno-associated virus mediated HO-1 gene transferring achieved a-l year-long cardinal protection, compare with the control group, the case fatality rate of HO-1 transferring group shown a much decreasing, and the HO-1 expression was increased after the treatment, suggests the treatment was successful and HO-1 shows the protecting effect for myocardial I/R injury. [4]

4.2. HO-1 Expression Promoted the Ferroptosis and Induced Heart Failure

Fang et al. (2019) given a contrast result in their research, by Inhibition of ferroptosis by ferrostatin1 significantly reduced doxorubicin (DOX) cardiomyopathy compared to dexrazoxane. By further researching, the mechanism of HO-1 induct ferroptosis was observed. [5] This result is partly contrast with the above, which suggest the HO-1 decrease the apoptosis of myocardial cells. However, this article also mentioned that the HF model mice was promoted by DOX, this implied that multiple mechanism of apoptosis occur during the myocardial I/R injury.

4.3. Reduced the Expression of HO-1 Shown the Positive on Myocardial I/R Injury.

Tan et al. (2024) reported silencing KCNQ1OT1 with LncRNA KCNQ1OT1 reduced the expression of HO-1, and increased miR-377-3p, decreased ROS levels, and mitigated oxidative stress. Overexpression of miR-377-3p increased cell viability, decreased apoptosis. Further research was promoted on the mice and shown the similar results on cell. [10] This gives a support to the research in 2019 lead by Fang et al.

4.4. MSCs Mediated Transferring of HO-1.

Wu. (2012) introduced transplantation of HO-1 transfected MSCs led to improvements in cardiac function, as evidenced by increased ejection fraction and decreased end-diastolic pressure. [11] This seam supports that HO-1 have the ability to protect myocardial cells. However, the model in this research is dilated cardiomyopathy (DCM), and promoted with DOX, and the MSC should not be ignored in this research. As the above mentioned, MSC pretreatment may increase the effect of its heart protection.

5. Notch Signaling Pathway Regulation

Notch signaling plays an important role in cell development, differentiation, proliferation, and apoptosis. It is a highly conserved signaling pathway.

5.1. Notch1 Over-expression Protected Myocardial Cells

In Zhou et al. (2018) `s research. Recombinant adenoviruses expressing rat N1ICD/Pink1 complementary DNA (cDNA) were constructed and used to modulate Notch1 expression levels in myocardial cells, the results showed that Notch1 overexpression increased the viability of myocardial cells exposed to I/R injury. [12]

5.2. Notch Signaling Pathway and its Importance to Myocardial I/R Injury.

By experiment on H9c2, rat, mice, multiple experiment [13-15] claimed that the drugs protected myocardial cells via regulation and maintain of notch pathway. Dexmedetomidine, Nicotinamide mononucleotide, miR-34a-5p (which repress the notch signaling pathway) inhibition were tested in the experiment and all of them shown a relatively myocardial cell protection effect and the overexpression of notch signaling. Imply the notch signaling pathway would be the candidate target of myocardial I/R injury.

6. Hippo Signaling Pathway Regulation

The Hippo signaling transduction is an evolutionarily highly conserved pathway that can control organ size by regulating cell proliferation, apoptosis, and stem cell self-renewal. The dysregulation of the Hippo pathway can lead to cancer development, which imply the Hippo signaling regulation have the cancer development risk. [16]

6.1. Adeno-associated Virus 9 (AAV9)–based Gene Therapy to Knock Down the Hippo Pathway Gene Salvador (Sav)

Liu et al. (2021) researched the AAV9 knock down the Hippo pathway gene Sav, by treated with AAV9-Sav–short hairpin RNA (shRNA), Pigs treated with AAV9-Sav-shRNA exhibited evidence of cardiomyocyte division, reduced scar sizes, and increased capillary density compared to controls. The safety also be evaluated that the AAV9-Sav-shRNA without inducing mortality or tumor formation in liver and lung tissues. [17]

6.2. Sav1-siRNA Silence Sav1 Expression Blocked Hippo Pathway

Zhou et al. (2023) researched development of nanocomplexes (NCs) reversibly camouflaged with a platelet–macrophage hybrid membrane (HM) for efficient delivery of Sav1 siRNA (siSav1) into cardiomyocytes. siSav1 promotes the regeneration of cardiomyocytes by blocking the Hippo pathway. siSav1 affects not only cardiomyocytes but may also affect other cell types, such as macrophages, endothelial cells, and fibroblasts, by blocking their Hippo pathways, reducing inflammatory responses, oxidative stress, and fibrosis. Multiple safety tests were promoted, the Sav1-siRNA and NCs didn`t show immunogenicity. [18] However, the carcinogenicity didn`t be tested in this research.

6.3. Multiple Compounds Show Cardio Protection through Hippo Signaling Pathway

Echinatin shows the protection effect by inhibiting the Hippo pathway. [19] However, ribonucleotide reductase regulatory subunit M2(RRM2), shikonin shows their cardio protection by active the Hippo-Yap pathway. [20,21] These contrast conclusions should be intentioned in the further research, which imply the Hippo signaling pathway have dual-directional regulation of myocardial cells injury in myocardial I/R injury.

7. Akt Signaling Pathway

7.1. Sfrp4 Knockdown Active the AKT Signaling Pathway Provide Protection to Myocardial I/R Injury.

Zeng et al. (2019) knockdown the Sfrp4 and revealed the protection to myocardial cells. The knockdown of Sfrp4 was achieved through intramyocardial injection of adenoviral particles carrying Sfrp4 small hairpin RNAs (Ad-shSfrp4). [22] However, the safety evaluate was also absence in this research, and the according to Pasha et al. (2024), AKT pathway is suppressed by Phosphatase and tensin homolog (PTEN) [23], which plays a role in tumor suppressor. The active of the AKT signaling pathway may increase the risk of tumor.

7.2. Hmbox1 Knockout and AAV9-mediated Hmbox1 Knockdown Active AKT/mTOR Signaling Pathway Provide Protection to myocardial I/R Injury.

Bei et al. (2024) researched on the role of Hmbox1 in cardiac I/R injury was studied using AAV9 vector-mediated Hmbox1 knockdown and cardiac myocyte-specific Hmbox1 knockout mouse models. Inhibition of Hmbox1 activates the Akt/mTOR/P70S6K signaling pathway and transcriptionally upregulates glucokinase (Gck), thereby reducing apoptosis in cardiomyocytes and improving mitochondrial respiration and glycolysis. [24] However, the safety evaluate was also absence in this research, the risk of tumor should not be ignored.

8. Other Efficient Therapy

8.1. Infection of Adeno-associated Virus (AAV9) for NAT10 Gene

Qu et al. (2024) tested the infection of adeno-associated virus (AAV9) for NAT10 gene interference and overexpression. They found that interference of NAT10 decrease the ferroptosis while overexpression increased the ferroptosis. This implied the NAT10 is highly relative with the ferroptosis. [25] Even the authors didn`t take AAV9 as therapy of myocardial I/R injury, and the safety evaluation was absence. This still revealed the relation between NAT10 and ferroptosis.

9. Conclusion

The recent research has preliminarily progress on the gene therapy of myocardial I/R injury. At the VEGF gene transferring part, the results and conclusions shown many advantages than the other solution which the safety evaluate are complete. And the mechanism of VEGF on myocardial I/R injury has been revealed in recent research as well. MSCs and MSC-Exo also be proved have the myocardial cells protection effect, moreover, due to the low immunogenicity of MSCs, it is also a fine medium of gene transferring, by transplantation into body, the relative gene expression

products could be released as well. However, multiple contrast research results appear at HO-1, Hippo signaling pathway. Author considers these compounds and signaling pathway have the dual-directional regulation of myocardial cells injury in myocardial I/R injury. Some signaling pathway relative with the tumor development, low targeting therapy have the risk of carcinogen such as Hippo and Akt. The relative research in these signaling pathway have less safety evaluate, author considers this might imply the application prospects in these directions are lower than others. But those researches still revealed the mechanism of myocardial I/R injury. Recent research has enumerated the relative miRNA in myocardial I/R injury. Nowadays, the recognition has much progress than decades ago. Author believes the myocardial I/R injury could be systematically resolved in the not-too-distant future.

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