European Review for Medical and Pharmacological Sciences 2019; 23: 5495-5502

22-oxacalcitriol protects myocardial ischemia-reperfusion injury by suppressing NF-κB/TNF-α pathway

C.-N. ZHOU[1], W. YAO[2], Y.-N. GONG[3], Y. LI[4], C.-H. WANG[5], Y.-F. HUO[6]

[1]Department of Public Health, Yantaishan Hospital, Yantai, China
[2]Department of Critical Care Medicine, People's Hospital of Chiping, Chiping, China
[3]Department of Cardiovascularology, the People's Hospital of Zhangqiu Area, Jinan, China
[4]Operating Room, the People's Hospital of Zhangqiu Area, Jinan, China
[5]Department of Pediatrics, the People's Hospital of Zhangqiu Area, Jinan, China
[6]Department of Cardiac Surgery, Qilu Hospital of Shandong University (Qingdao), Qingdao, China

Abstract. – OBJECTIVE: The aim of this study was to explore whether 22-oxacalcitriol could protect inflammatory response induced by ischemia-reperfusion injury (IRI) in rats, and to investigate its underlying mechanism.

MATERIALS AND METHODS: 24 male Sprague Dawley rats were randomly assigned into the sham group, the IRI group and the 22-oxacalcitriol group, with 8 rats in each group. Serum and heart samples of each rat were collected 10 days after the animal procedure. The serum levels of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) in each rat were detected by relative commercial kits. Pathological lesions in rat myocardium were observed by hematoxylin and eosin (HE) staining. Cardiomyocyte apoptosis in rat heart was accessed by TUNEL staining. Meanwhile, the serum levels of tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), and KC-GRO were detected by Real Time-quantitative Polymerase Chain Reaction (RT-qPCR). Also, the protein expression levels of NF-κB, TNF-α, VCAM-1, ICAM-1, and MCP-1 in rat myocardium were detected by Western blot and immunohistochemistry.

RESULTS: The serum levels of CK-MB and LDH in rats of the IRI group were significantly higher than those of the sham group. 22-oxacalcitriol treatment remarkably decreased the serum levels of CK-MB and LDH when compared with the IRI group. However, cardiomyocyte apoptosis of the 22-oxacalcitriol group was markedly less than the IRI group. The activities of SOD, GSH, CAT and T-AOC in the cardiac homogenate of the 22-oxacalcitriol group were significantly elevated than those of the IRI group. Meanwhile, malondialdehyde (MDA) and reactive oxygen species (ROS) levels were remarkably decreased by 22-oxacalcitriol treatment. Furthermore, the serum levels of TNF-α, IL-1β, IL-6 and KC-GRO were significantly downregulated in the 22-oxacalcitriol group. Western blot results showed that the protein expression levels of NF-κB, TNF-α, VCAM-1, ICAM-1 and MCP-1 in the 22-oxacalcitriol group were significantly lower than those of the IRI group.

CONCLUSIONS: 22-oxacalcitriol inhibits the inflammatory response in the myocardium by suppressing NF-kB/TNF-α pathway, thereby protecting myocardial ischemia-reperfusion injury in rats.

Key Words:
22-oxacalcitriol, NF-kB/TNF-α pathway, IRI, Myocardial injury.

Introduction

With the widespread application of intravenous thrombolysis, percutaneous coronary intervention, as well as coronary artery bypass grafting, the therapeutic effect of myocardial ischemia-reperfusion injury (IRI) has been greatly improved[1,2]. Early reperfusion of infarct-related blood vessels is the key to the treatment of IRI[3,4]. However, reperfusion therapy can aggravate tissue damage, characterized by serious cardiac insufficiency, arrhythmia and enlarged myocardial infarction[5,6]. Therefore, it is of great significance to search for effective ways to alleviate IRI, eventually improving the clinical outcomes of affected patients.

As known, myocardial IRI is an extremely complex pathological process. Currently, oxidative stress damage, intracellular calcium overload, cell apoptosis, cellular energy loss and the

Corresponding Author: Yufeng Huo, MM; e-mail: wpgh06@163.com

activation of neutrophil inflammatory response are considered as pathogenic factors of IRI[7-9]. Meanwhile, many signaling pathways are involved in the development of IRI[10-12]. Myocardial ischemia-reperfusion can induce a strong inflammatory response, which mainly includes the activation of corresponding complements, production of reactive oxygen species and infiltration of polymorphonuclear neutrophils[8-10]. Activated polymorphonuclear neutrophils are closely adhered to vascular endothelial cells, resulting in impaired structural integrity of endothelial cells. Besides, activated neutrophils also release a large number of superoxide and enzymes, further aggravating IRI[13,14]. As a key factor in regulating gene transcription, nuclear factor-κB (NF-κB) exerts the characteristics of immediate transcription[15]. Meanwhile, it is involved in the regulation of early defense and inflammatory response[15,16]. Previous studies[17,18] have shown that the NF-κB pathway exerts its biological function in reactive oxygen species (ROS) production and polymorphonuclear neutrophil infiltration in IRI. Other studies have proved that NF-κB stimulates the downstream cytokines that are related to inflammatory response[19,20].

Vitamin D receptor activator (22-oxacalcitriol) has strong anti-inflammatory activity. Previous studies[21,22] have found that 22-oxacalcitriol has anti-oxidation, blood lipid regulation, anti-atherosclerosis and anti-inflammatory effects. However, the specific role of 22-oxacalcitriol in IRI has not been fully elucidated. In the present work, we first constructed the IRI model in rats and further explored the biological effects of 22-oxacalcitriol on myocardial IRI. The aim of this study was to provide new directions for the treatment of IRI.

Materials and Methods

Chemicals and Reagents

22-oxacalcitriol was obtained from Sigma-Aldrich (MO, USA); CK-MB, LDH, MDA, T-AOC, CAT, GSH and SOD determination kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China); Cytokines of tumor necrosis factor-α (TNF-α), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), and KC-GRO were provided by Abcam (Cambridge, MA, USA); Coarse balance, electronic thermometer and 721 type spectrophotometer were obtained from Inesa Analytical Instrument (Shanghai, China).

Animals and Experimental Protocol

24 male Sprague Dawley (SD) rats weighing 200±20 g (Model Animal Research Center of Shandong University, Jinan, China) were maintained in an environment with a 12 h/12 h light/dark cycle. All rats were given free access to food and water. According to the specific procedures, experimental rats were randomly assigned into three groups, including the sham group (saline administration), the IRI group (saline administration) and the 22-oxacalcitriol group (20 μg/kg 22-oxacalcitriol administration). After collection of blood samples from the orbital vein, rats were sacrificed for the following experiments. Body weight and daily activities of each rat were observed regularly. This study was approved by the Animal Ethics Committee of Shandong University Animal Center.

For IRI model construction, rats were first anesthetized by intraperitoneal injection of 60 mg/kg pentobarbital sodium. After tracheotomy and mechanical ventilation, atrial intubation allowed 100% oxygen pass through general circulation from the femoral artery. Myocardial ischemia was induced by ligation of the ascending aorta, followed by the observation of myocardium color. 30 min later, the ligation was removed, and reperfusion was allowed for 3 h. Dark-red myocardium suggested successful ligation. Meanwhile, rats in the sham group received tracheotomy and mechanical ventilation without ligation. Furthermore, for rats in the 22-oxacalcitriol group, 10 mL diluted 22-oxacalcitriol was added to the blood storage tank before reperfusion. Body weight and daily activity of each rat were observed.

Measurement of Myocardial Infarction Area

After the animal procedure, 1% Evans Blue was injected into the left ventricle. Non-ischemic area and ischemic area were stained blue and red, respectively. Rat heart was exposed, and the left ventricle was collected and weighed. After 30 min of frozen at -20°C, the collected left ventricle was sliced into 2 μm sections, followed by incubation with TTC (pH 7.4) at 37°C for 15 min. The infarct size was determined by Image ProPlus 6.0. The ratio of infarcted myocardial weight to ischemic myocardial weight was calculated.

Histological Examination

Heart tissues were fixed with 10% paraformaldehyde and stained with hematoxylin and eosin. Histological changes were assessed by

semi-quantitative detection of myocardial injury and necrosis. 5 randomly selected fields of each sample were captured for histological examination.

Assessment of Cardiomyocyte Function

2 mL blood sample was harvested after the animal procedure. After blood coagulation for 30 min, the collected blood sample was centrifuged at 3500 g/min for 30 min. The serum levels of CK-MB and LDH were detected according to the instructions of relative commercial kits.

Biochemical Measurements

Rat myocardium was perfused with cold normal saline in situ. After the color of the rat heart became pale, the heart was immediately harvested and preserved in liquid nitrogen for subsequent use. The levels of MDA, T-AOC, CAT, GSH, and SOD in heart homogenate were detected in strict accordance with the instructions of relative commercial kits.

TUNEL (Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling) Assay

Cardiomyocyte apoptosis in heart tissue was detected by TUNEL assay according to the instructions of ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Millipore, Billerica, MA). 5-μm paraffin sections were counterstained with hematoxylin. Totally 5 fields were randomly selected for each sample, and the number of TUNEL-positive cells was counted (magnification 200×).

Immuno-Histochemical Staining

Paraffin-embedded heart tissues were incubated with primary and secondary antibodies. Diaminobenzidine (DAB) was added for image exposure and the slices were then counterstained with hematoxylin for 2 min. Subsequently, the slices were hydrated, sealed and observed by an inverted microscope (Nikon, Tokyo, Japan).

Western Blot

Total protein was extracted by the radio-immunoprecipitation assay (RIPA) protein lysate (Thermo, Waltham, MA, USA). The concentration of extracted protein was quantified by the bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). Subsequently, the protein sample was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Merck Millipore, Billerica, MA, USA). After incubation with primary and secondary antibodies, immunoreactive bands were exposed by enhanced chemiluminescence (ECL) method.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 Software (IBM, Armonk, NY, USA) was used for all statistical analysis. Experimental data were expressed as $\bar{x}\pm s$. *t*-test was used to compare the difference between the two groups. Repeated two-way ANOVA was performed to compare the differences among different groups, followed by post-hoc LSD or SNK analysis. $p<0.05$ was considered statistically significant.

Results

Pretreatment of 22-Oxacalcitriol Improved Myocardial Function in IRI Rats

Compared with the sham group, the serum levels of CK-MB and LDH in rats of the IRI group were remarkably increased. This indicated successful construction of the IRI rat model. Besides, the serum levels of CK-MB and LDH in the 22-oxacalcitriol group were significantly lower than those of the IRI group, which were still higher than those of the sham group (Figure 1).

22-Oxacalcitriol Preserved Myocardial Histologic Structure and Mitigated Neutrophil Infiltration

Infarct size of the IRI group was significantly larger than that of the sham group and the 22-oxacalcitriol group (Figure 2A). Irregular cardiomyocytes, abundant inflammation cell infiltration and pink protein mucus exudation were observed in heart tissues collected from the IRI group. Meanwhile, pathological lesions were relatively milder in the 22-oxacalcitriol group, manifesting as a normal histologic structure, a small amount of inflammation infiltration and little mucus exudation (Figure 2B).

22-Oxacalcitriol Decreased the Apoptosis of Cardiomyocyte Tubular Cells After IRI

Cardiomyocyte apoptosis induced by IRI was determined by TUNEL assay. Results showed that the number of TUNEL-positive cells in the IRI group was significantly higher than that of the sham group. However, 22-oxacalcitriol treat-

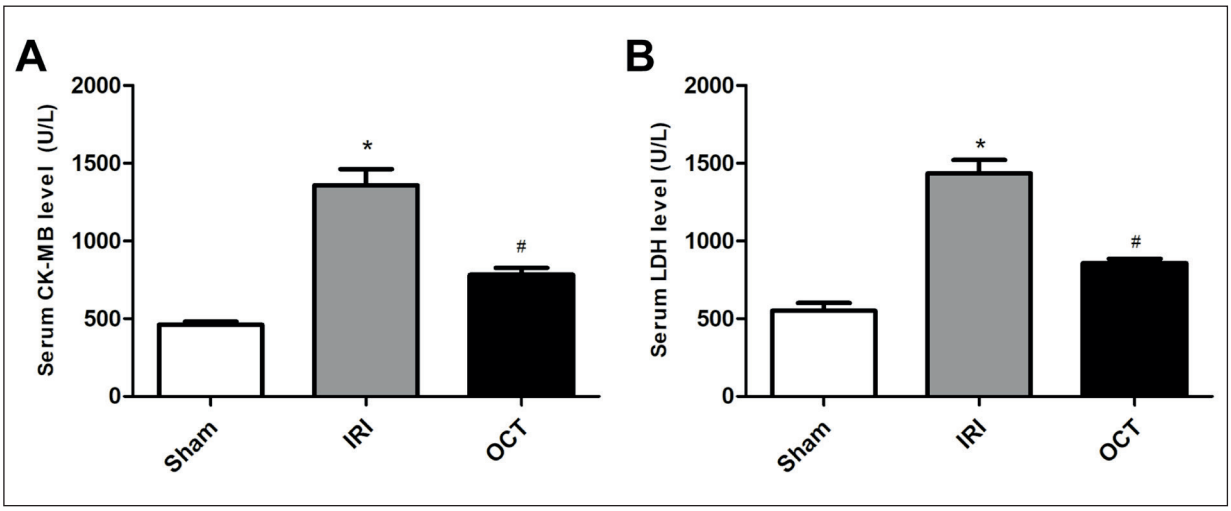

Figure 1. 22-oxacalcitriol conserved myocardial function in IRI rats. ***A,*** Serum level of CK-MB in the sham group (n=8), the IRI group (n=8) and the 22-oxacalcitriol group (n=8); ***B,*** Serum level of LDH in different treatment groups. Data were presented as mean ± SD. *Significant difference *vs* sham group (p<0.05); #Significant difference *vs* IRI group (p<0.05).

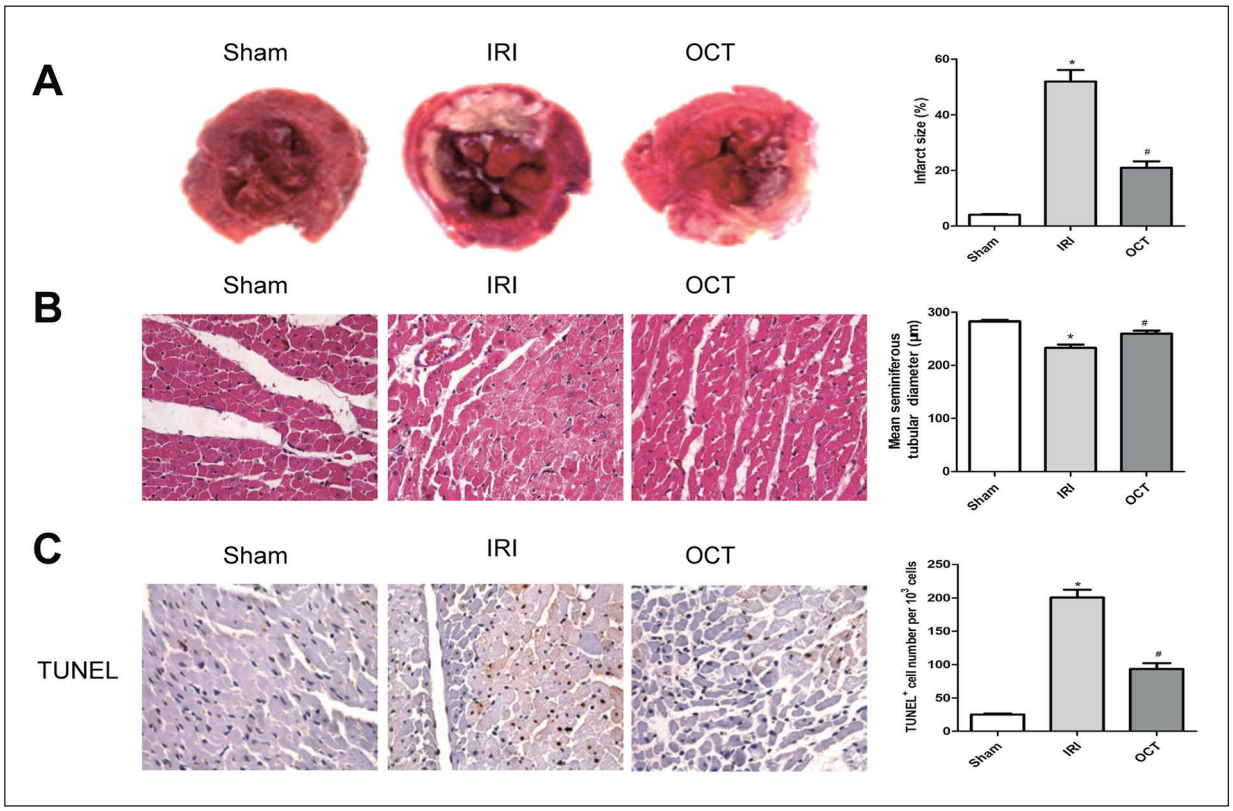

Figure 2. 22-oxacalcitriol prevented myocardial IRI in cardiac morphology. ***A,*** Blue-stained area represented normal tissue, and unstained pale area indicated infarcted tissue. Infarct size was expressed as the percentage of total volume. ***B,*** Myocardial sections were stained with hematoxylin and eosin, and observed under a light microscope (magnification 200×). H&E staining of myocardial tissues in the sham group, the IRI group, and the 22-oxacalcitriol group. ***C,*** Representative images of TUNEL immunostaining in myocardial IRI (magnification 200×); TUNEL-positive cells per 10^3 germ cells of testes in the three groups. Data were expressed as mean ± SD. *Significant difference *vs* sham group (p<0.05); #Significant difference *vs* IRI group (p<0.05).

ment could remarkably decrease the number of TUNEL-positive cells, indicating less cardiomyocyte apoptosis induced by IRI (Figure 2C).

22-Oxacalcitriol Decreased ROS Production and Tissue Impairment by Enhancing Antioxidant Capacity

IRI has been proved to remarkably impair the capacity of anti-oxidation. We then detected the levels of T-AOC, CAT, GSH, SOD and MDA in rat heart homogenate and found that 22-oxacalcitriol treatment could remarkably increase the levels of T-AOC, CAT, GSH and SOD when compared with those of the IRI group (Figure 3C-3F). Meanwhile, the 22-oxacalcitriol group showed significantly less ROS production than that of the IRI group (Figure 3B). In addition, compared with the IRI group, MDA level in heart homogenate of the 22-oxacalcitriol group was remarkably lower (Figure 3A).

22-Oxacalcitriol Downregulated the Expression of NF-κB and Downstream Genes after IRI

To explore whether 22-oxacalcitriol protected against IRI-induced inflammatory response, we collected serum sample from each rat. Results showed that the serum levels of TNF-α, IL-1β, IL-6, and KC-GRO in the 22-oxacalcitriol group were remarkably decreased than those of the IRI group, suggesting the anti-inflammatory effect of 22-oxacalcitriol (Figure 4).

Subsequently, NF-κB expression in rat heart was determined by Western blot and immunohistochemistry, respectively. Compared with the IRI group, NF-κB expression in rat heart of the 22-oxacalcitriol group was significantly downregulated (Figure 5A and 5B). Meanwhile, the protein expression levels of NF-κB, TNF-α, VCAM-1, ICAM-1, and MCP-1 in the 22-oxacalcitriol group were remarkably lower than those of the IRI group (Figure 5C).

Discussion

With the improvement of living standards in recent years, the incidence of coronary atherosclerotic heart disease has greatly increased[23]. Various treatment methods have emerged, such as intravenous thrombolytic therapy, coronary artery resection, atherectomy, percutaneous cor-

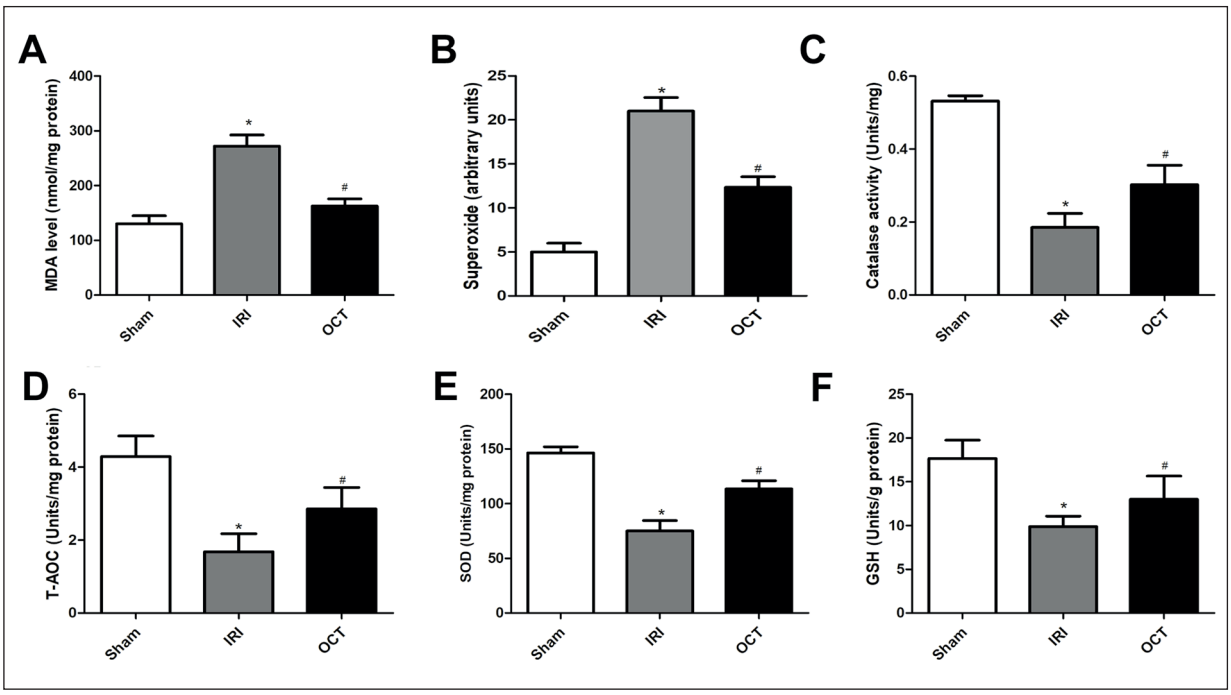

Figure 3. 22-oxacalcitriol attenuated oxidative stress injury by evaluating biochemical parameters. ***A,*** Content of MDA in myocardial tissues. ***B,*** Density of ROS was reported as arbitrary units per millimeter square field. ***C,*** Content of catalase (CAT) activity in myocardial tissues. ***D,*** Content of total antioxidant reduced capacity (T-AOC) in myocardial tissues. ***E,*** Content of superoxide dismutase (SOD) in myocardial tissues. ***F,*** Content of glutathione (GSH) in myocardial tissues. Data were expressed as mean ± SD. *Significant difference *vs* sham group ($p<0.05$); #Significant difference *vs* adenine group ($p<0.05$).

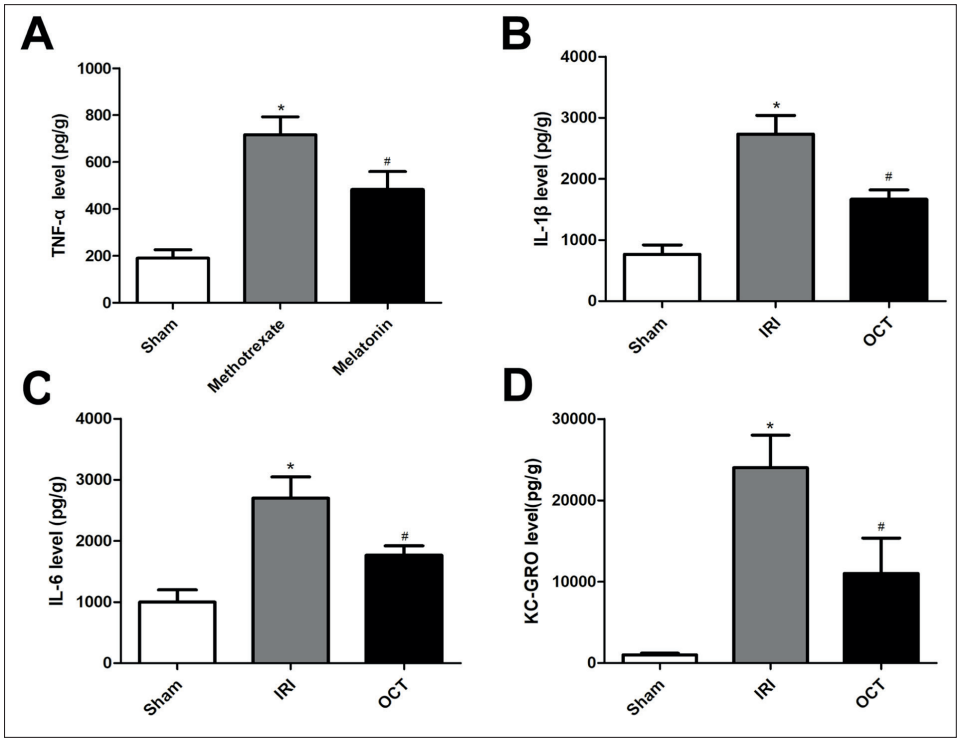

Figure 4. 22-oxacalcitriol decreased NF-κB related cytokines. ***A,*** Content of TNF-α in myocardial tissues. ***B,*** Content of IL-1β in myocardial tissues. ***C,*** Content of IL-6 in myocardial tissues. ***D,*** Content of KC-GRO in myocardial tissues. Data were expressed as mean ± SD. *Significant difference *vs* sham group (p<0.05); #Significant difference *vs* adenine group (p<0.05).

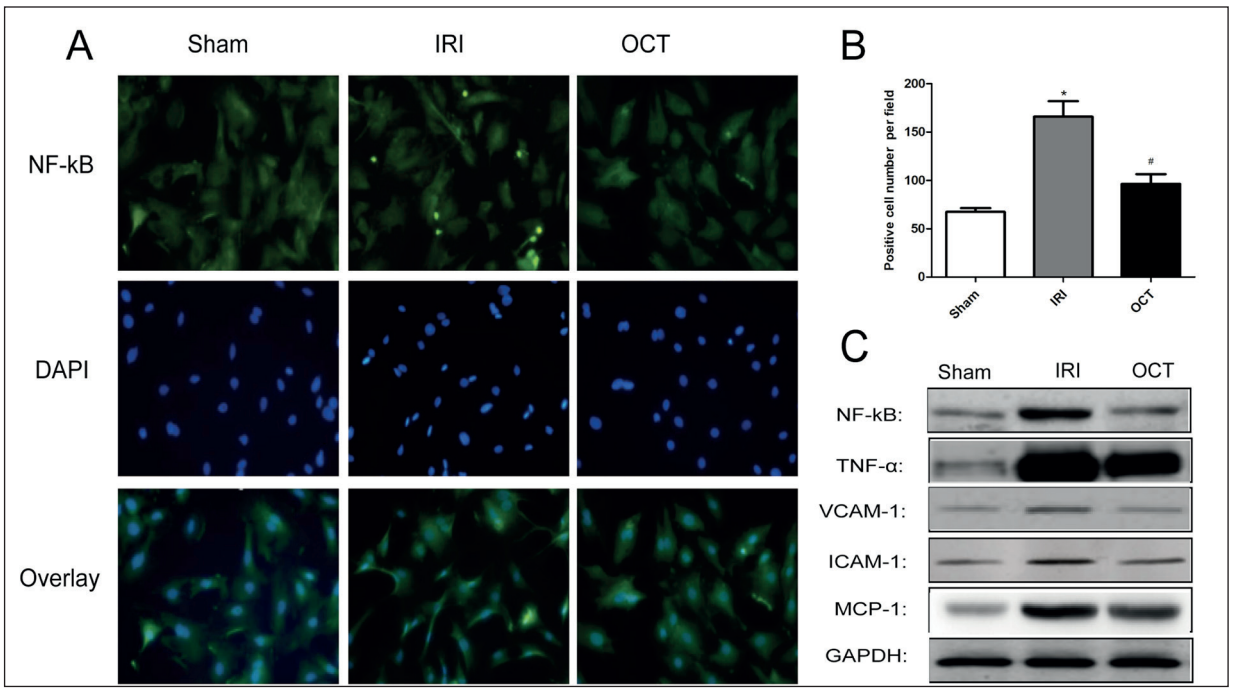

Figure 5. 22-oxacalcitriol treatment decreased the protein expression level of NF-κB/TNF-α. ***A,*** NF-κB expression level in the sham group, the IRI group, and the 22-oxacalcitriol group was detected by immune-histochemical staining. ***B,*** Statistical analysis of NF-κB expression levels in the three groups. ***C,*** Protein expressions of NF-κB, TNF-α, VCAM-1, ICAM-1 and MCP-1 in different groups. GAPDH was used as an internal reference. Data were expressed as mean ± SD. *significant difference *vs* sham group (p<0.05); #significant difference *vs* IRI group (p< 0.05).

onary intervention and surgical coronary artery bypass surgery. These treatments can restore the blood flow of ischemic myocardium, decrease infarct size and improve heart function. However, ischemic myocardium reperfusion may lead to vascular endothelial cell dysfunction, cardiomyocyte apoptosis and necrosis, and even reperfusion arrhythmia, myocardial stunning and sudden cardiac death[3-5]. Therefore, it is urgent to alleviate IRI reduction after reperfusion, to improve heart function[7,8].

22-oxacalcitriol is an effective anti-inflammatory drug, which has been successfully synthesized recently. *In vitro* experiments have confirmed its anti-inflammatory, anti-oxidative and anti-apoptotic biological functions. Through literature review, we speculated that 22-oxacalcitriol could protect against IRI-induced acute myocardial injury[21,22]. Currently, the acute myocardial injury is a severe complication with high mortality clinically. Therefore, it is of great significance to explore the molecular mechanism and to search for new therapeutic targets for IRI[24].

Carrà et al[25] have found that short-term ischemia results in significant degradation of cytosolic IκBα, as well as increased DNA binding activity of NF-κB and TNF-α in the myocardium. This can be explained by the production of ROIs (Reactive Oxygen Intermediates) induced by NF-κB activation, thus stimulating the dissociation of IκBα from the p50-p65/IκBα complex. IκBα is finally degraded, and NF-κB activation further regulates the expression of downstream cytokines[15,17,18,25]. Meanwhile, activated NF-κB, in turn, upregulates the mRNA expression of IκBα, thereby rapidly restoring IκBα expression[16-18]. During the process of reperfusion in ischemic tissues, although the cytoplasmic level of IκBα restores to its original level, a sustained high degree of DNA binding activity of NF-κB still exists. This may be distinguished from NF-κB activation induced by common inflammatory cytokines[26,27]. Abundant ROIs activate PTK and phosphorylates IκBα, which in turn mediates NF-κB activation without degrading IκBα[28,29]. However, different activation pathways mainly depend on the amount of ROIs[29].

Vitamin D receptor activator 22-oxacalcitriol was initially thought to have only the functions of maintaining bone metabolism and mineral metabolism[21,22]. However, in recent years, vitamin D receptor activators are found to exert a wide range of roles, including anti-tumor, inhibiting the renin-angiotensin system, cardio-protection, anti-inflammatory and preventing atherosclerosis. In particular, the relationship between 22-oxacalcitriol and fibroblast factor 23 has attracted much attention[30]. Previous animal experiments have confirmed the protective effect of 22-oxacalcitriol on IRI[30,31]. However, the specific role of 22-oxacalcitriol in myocardial IRI has not been reported. In the present study, we first observed significant pathological lesions in the myocardium, increased oxidative stress level and decreased antioxidant capacity in IRI rats. In addition, 22-oxacalcitriol treatment remarkably decreased the expression level of NF-κB induced by IRI, further indicating its role in alleviating IRI-induced myocardial inflammation.

Conclusions

We showed that 22-oxacalcitriol inhibits inflammatory response by suppressing NF-kB/TNF-α pathway, thereafter protecting myocardial IRI in rats.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Frank A, Bonney M, Bonney S, Weitzel L, Koeppen M, Eckle T. Myocardial ischemia reperfusion injury: from basic science to clinical bedside. Semin Cardiothorac Vasc Anesth 2012; 16: 123-132.
2) Jennings RB. Historical perspective on the pathology of myocardial ischemia/reperfusion injury. Circ Res 2013; 113: 428-438.
3) Binder A, Ali A, Chawla R, Aziz HA, Abbate A, Jovin IS. Myocardial protection from ischemia-reperfusion injury post coronary revascularization. Expert Rev Cardiovasc Ther 2015; 13: 1045-1057.
4) Shi CX, Ding YB, Jin F, Li T, Ma JH, Qiao LY, Pan WZ, Li KZ. Effects of sevoflurane post-conditioning in cerebral ischemia-reperfusion injury via TLR4/NF-kB pathway in rats. Eur Rev Med Pharmacol Sci 2018; 22: 1770-1775.
5) Ibanez B, Heusch G, Ovize M, Van de Werf F. Evolving therapies for myocardial ischemia/reperfusion injury. J Am Coll Cardiol 2015; 65: 1454-1471.
6) Ge L, Zhou X, Ji WJ, Lu RY, Zhang Y, Zhang YD, Ma YQ, Zhao JH, Li YM. Neutrophil extracellular traps in ischemia-reperfusion injury-induced myocardial no-reflow: therapeutic potential of DNase-based reperfusion strategy. Am J Physiol Heart Circ Physiol 2015; 308: H500-H509.

7) Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, Cheng PW, Li CY, Li CJ. Current mechanistic concepts in ischemia and reperfusion injury. Cell Physiol Biochem 2018; 46: 1650-1667.

8) Cadenas S. ROS and redox signaling in myocardial ischemia-reperfusion injury and cardioprotection. Free Radic Biol Med 2018; 117: 76-89.

9) Russo I, Penna C, Musso T, Popara J, Alloatti G, Cavalot F, Pagliaro P. Platelets, diabetes and myocardial ischemia/reperfusion injury. Cardiovasc Diabetol 2017; 16: 71.

10) Li H, Yin A, Cheng Z, Feng M, Zhang H, Xu J, Wang F, Qian L. Attenuation of Na/K-ATPase/Src/ROS amplification signal pathway with pNaktide ameliorates myocardial ischemia-reperfusion injury. Int J Biol Macromol 2018; 118: 1142-1148.

11) Li S, Wu H, Han D, Zhang M, Li N, Yu W, Sun D, Sun Z, Ma S, Gao E, Li C, Shen M, Cao F. ZP2495 protects against myocardial ischemia/reperfusion injury in diabetic mice through improvement of cardiac metabolism and mitochondrial function: the possible involvement of AMPK-FoxO3a signal pathway. Oxid Med Cell Longev 2018; 2018: 6451902.

12) Xuan F, Jian J, Lin X, Huang J, Jiao Y, Huang W, Li J, Shi Z, Huang R. 17-methoxyl-7-hydroxy-benzene-furanchalcone ameliorates myocardial ischemia/reperfusion injury in rat by inhibiting apoptosis and autophagy via the PI3K-Akt signal pathway. Cardiovasc Toxicol 2017; 17: 79-87.

13) Link A, Schwerdt H, Hennen B, Bohm M. [Polymorphonuclear neutrophils in myocardial ischemia and reperfusion injury. Influence of coronary intervention?]. Z Kardiol 2004; 93: 605-611.

14) Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia-reperfusion injury. Cardiovasc Res 1999; 43: 860-878.

15) DiDonato JA, Mercurio F, Karin M. NF-kB and the link between inflammation and cancer. Immunol Rev 2012; 246: 379-400.

16) Shih RH, Wang CY, Yang CM. NF-kappaB Signaling Pathways in Neurological Inflammation: a mini review. Front Mol Neurosci 2015; 8: 77.

17) Latanich CA, Toledo-Pereyra LH. Searching for NF-kappaB-based treatments of ischemia reperfusion injury. J Invest Surg 2009; 22: 301-315.

18) Nichols TC. NF-kappaB and reperfusion injury. Drug News Perspect 2004; 17: 99-104.

19) Xia KP, Ca HM, Shao CZ. Protective effect of notoginsenoside R1 in a rat model of myocardial ischemia reperfusion injury by regulation of Vitamin D3 upregulated protein 1/NF-kappaB pathway. Pharmazie 2015; 70: 740-744.

20) Li T, Yu J, Chen R, Wu J, Fei J, Bo Q, Xue L, Li D. Mycophenolate mofetil attenuates myocardial ischemia-reperfusion injury via regulation of the TLR4/NF-kappaB signaling pathway. Pharmazie 2014; 69: 850-855.

21) Mizobuchi M, Ogata H. Clinical uses of 22-oxacalcitriol. Curr Vasc Pharmacol 2014; 12: 324-328.

22) Goto S, Fujii H, Kono K, Nakai K, Awata R, Yonekura Y, Hirata M, Shinohara M, Nishi S, Fukagawa M. 22-Oxacalcitriol attenuates bone loss in non-obese type 2 diabetes. Bone 2015; 74: 153-159.

23) Schunkert H, von Scheidt M, Kessler T, Stiller B, Zeng L, Vilne B. Genetics of coronary artery disease in the light of genome-wide association studies. Clin Res Cardiol 2018; 107: 2-9.

24) Deng L, Hong T, Lin J, Ding S, Huang Z, Chen J, Jia J, Zou Y, Wang TC, Yang X, Ge J. Histamine deficiency exacerbates myocardial injury in acute myocardial infarction through impaired macrophage infiltration and increased cardiomyocyte apoptosis. Sci Rep 2015; 5: 13131.

25) Carrà G, Crivellaro S, Taulli R, Guerrasio A, Saglio G, Morotti A. Mechanisms of p53 functional de-regulation: role of the IkappaB-alpha/p53 Complex. Int J Mol Sci 2016; 17: 1997.

26) Carlson CG, Dole E, Stefanski C, Bayless D. The effect of specific IKK β inhibitors on the cytosolic expression of IkB-α and the nuclear expression of p65 in dystrophic (MDX) muscle. Am J Transl Res 2015; 7: 670-682.

27) Park J, Min JS, Kim B, Chae UB, Yun JW, Choi MS, Kong IK, Chang KT, Lee DS. Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-kB pathways. Neurosci Lett 2015; 584: 191-196.

28) Wang J, Sun Z, Shen J, Wu D, Liu F, Yang R, Ji S, Ji A, Li Y. Octreotide protects the mouse retina against ischemic reperfusion injury through regulation of antioxidation and activation of NF-kB. Oxid Med Cell Longev 2015; 2015: 970156.

29) Ma L, Liu H, Xie Z, Yang S, Xu W, Hou J, Yu B. Ginsenoside Rb3 protects cardiomyocytes against ischemia-reperfusion injury via the inhibition of JNK-mediated NF-kB pathway: a mouse cardiomyocyte model. PLoS One 2014; 9: e103628.

30) Sueta S, Morozumi K, Takeda A, Horike K, Otsuka Y, Shinjo H, Murata M, Kato Y, Goto K, Inaguma D. Ability of vitamin D receptor activator to prevent pulmonary congestion in advanced chronic kidney disease. Clin Exp Nephrol 2015; 19: 371-378.

31) Franczyk A, Stolarz-Skrzypek K, Wesolowska A, Czarnecka D. Vitamin D and vitamin D receptor activators in treatment of hypertension and cardiovascular disease. Cardiovasc Hematol Disord Drug Targets 2014; 14: 34-44.