Indian Journal of Experimental Biology
Vol. 60, July 2022, pp. 549-556

Notes

Protective effect of ivabradine on mice with viral myocarditis and its mechanism

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Received 19 January 2022; *revised 10 June 2022*

Viral myocarditis (VMC) is a type of cardiovascular disease caused by viral infection of myocardial cells characterized by myocardial interstitital inflammatory cell infiltration, myocardial fiber necrosis or figrinolysis. Ivabradine (IVA) is a commonly known drug used to control heart rate, resist inflammation and oxidative stress, particularly in VMC. Here, we have tried to evaluate the protective effects of IVA on mice with VMC and understand the possible mechanism behind this process. Eighty male mice aged 6 weeks old were randomly divided into normal control, VMC model, low-dose IVA (L-IVA) and high-dose IVA (H-IVA) groups. Half an hour after modeling, IVA aqueous solution was administered intragastrically into L-IVA and H-IVA groups at 5 $mg \cdot kg^{-1} \cdot d^{-1}$ and 20 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively for 14 consecutive days. Another 120 mice of the same batch were grouped and treated as described above. At 7 and 14 d, 6 mice in each group were sacrificed to obtain blood and heart samples. Body wt./heart wt. (BW/HW) was calculated, hematoxylin-eosin staining was performed to observe the pathological changes of myocardium, and the level of cardiac troponin I (cTnI) was measured by ELISA. Related kits were employed to measure superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and catalase (CAT) activities in myocardial homogenate, and the levels of interleukin-6 (IL-6), IL-18, IL-1β and tumor necrosis factor-α (TNF-α) were determined by double-antibody sandwich ELISA. TUNEL assay was performed to detect the apoptosis of myocardial cells. The protein expressions of Bcl-2, Bax and Caspase-3 in myocardial cells were measured by Western blotting. Compared to the VMC model group, the heart/body weight ratio, myocardial pathological score, cTnI level, MDA activity, and levels of IL-6, IL-18, IL-1β and TNF-α were found decreased, while survival rate and activity of SOD, GSH-Px and CAT increased in L-IVA and H-IVA groups (P <0.05). The apoptosis rate of myocardial cells declined in L-IVA and H-IVA groups (P <0.05), especially in H-IVA group. IVA downregulated the protein expressions of Bax and Caspase-3 and upregulated that of Bcl-2 (P <0.05). Thus, it has been found that IVA elevates the survival rate of VMC mice and relieves myocardial damage possibly by enhancing antioxidant capacity and modulating apoptosis-related proteins to suppress apoptosis.

Keywords: Apoptosis, Inflammatory response, Oxidative stress

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Viral myocarditis (VMC) is a cardiovascular disease caused by viral infection of myocardial cells, which is characterized by focal or diffuse myocardial interstitial inflammatory cell infiltration and myofibrosis cordis, myocardial fiber necrosis or fibrinolysis[1]. Its global prevalence rate is estimated to be 0.12-12%. In China, VMC shows an increasing trend in the prevalence rate (about 5-15%) in recent years, and it is one of the leading causes of sudden death in adolescents[2]. Acute VMC may develop into dilated cardiomyopathy on progression, with a fatality rate of 50%[3]. Viral infection is one of the important causes of VMC that can be induced by enteroviruses, adenoviruses, influenza viruses and cytomegaloviruses. VMC caused by enteroviruses, especially coxsackie virus group B type 3 (CVB3), is the most common type[4], accounting for over half of the patients. Recent studies have manifested that the early innate immune response of the body to viruses and the direct damage to the myocardium by viruses are vital determining factors of VMC associated myocardial damage and disease progression, which jointly lead to pathological changes mainly characterized by focal or diffuse inflammatory cell infiltration and myocardial cell necrosis. However, the mechanism remains unclear.

Increased heart rate is one of the important independent risk factors for various cardiovascular diseases, so effective control of heart rate is essential in the case of cardiovascular diseases. Ivabradine (IVA), a novel drug for heart rate control, is able to reduce exercise and resting heart rates, notably improve the cardiac function level in VMC mice, remarkably lower the mRNA and protein expression levels of inflammatory mediators (MCP-1, TNF-α and IL-16), and decrease the expression of inducible nitric oxide synthase (iNOS) protein in the myocardium, thus repressing the excessive increase in nitric oxide (NO) level, and an excessively high NO level will result in systolic dysfunction[5,6]. Besides, IVA is also capable of inhibiting myocardial remodeling and atherosclerotic plaque formation and resisting inflammation and oxidative stress[7].

In this study, we have made an attempt to evaluate the protective effect of ivabradine on viral myocarditis mice and understand its specific mechanism behind the process.

Materials and Methods

To conduct the above study, we constructed animal models of VMC by multiple inductive infection of low-dose cardiotropic CVB3 strain (CVB3 Nancy) in mice, the protective effect of IVA on the myocardium of VMC mice was observed, and the mechanism of action of IVA in protecting the myocardium of VMC mice was investigated in combination with the effects of IVA on oxidative stress response, inflammatory factors and apoptosis-related proteins in the myocardium of VMC mice, hoping to provide a theoretical basis for clinical treatment of VMC.

Experimental animals, cells and viral strain

BALB/c male mice (batch No. SCK Beijing 2002-0003) aged 6 weeks old and weighing 19-20 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., and raised in the SPF-grade animal room in our hospital. The cardiotropic CVB3 Nancy strain was bought from the Chinese Center for Disease Control and Prevention, and subcultured on human cervical cancer HeLa cells (ATCC No. CCL-2). The 50% tissue culture infective dose ($TCID_{50}$) detected was 10^7.

Main reagents and apparatus

The materials used in this study included IVA (molecular formula: $C_{27}H_{36}N_2O_5$, Servier, France), DMEM (GIBCO, USA), anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) and pro-apoptotic proteins Bcl-2-associated X protein (Bax) and cysteinyl aspartate specific proteinase-3 (Caspase-3) primary antibodies (Santa Cruz), mouse anti-β-actin antibody (Sigma, USA), horseradish peroxidase labeled goat anti-rabbit IgG antibody (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd.), bicinchoninic acid (BCA) protein kit, enhanced chemiluminescence (ECL) chemiluminescence kit (Thermo Fisher, USA), radioimmunoprecipitation assay (RIPA) lysis buffer, anti-penicillin-streptomycin double antibody (Beyotime Biotechnology), cardiac troponin I (cTnI), interleukin-6 (IL-6), IL-18, IL-1β and tumor necrosis factor-α (TNF-α) detection kits (Nanjing Jiancheng Bioengineering Institute), enzyme-linked immunosorbent assay (ELISA) kits for glutathione peroxidase (GSH-Px), catalase (CAT), malondialdehyde (MDA) and superoxide dismutase (SOD) (Sangon Biotech (Shanghai) Co., Ltd.), eosin and hematoxylin (Sigma, USA) and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay kit (ROCHE).

The apparatus used included a CO2 incubator (Thermo, USA), an inverted microscope (UFX-ILA, Olympus, Japan), a 4°C high-speed refrigerated centrifuge (Eppendrof, German), a virus incubator (Binder, German) and a CX9 automatic biochemical analyzer (Beckman, USA).

Detection of survival rate

Eighty BALB/c mice were adaptively fed for one week and randomly assigned into normal control group (n=20), VMC model group (n=20), low-dose IVA treatment group (L-IVA group, n=20) and high-dose IVA treatment group (H-IVA group, n=20). Then, 0.2 mL of Eagle's solution containing no CVB3 was intraperitoneally inoculated into mice in normal control group, while 0.2 mL of 103 $TCID_{50}$ CVB3 was intraperitoneally injected into those in VMC model group, L-IVA group and H-IVA group to prepare the VMC model. 0.5 h later, IVA aqueous solution was administered intragastrically into mice in L-IVA and H-IVA groups at 5 $mg\cdot kg^{-1}\cdot d^{-1}$ and 20 $mg\cdot kg^{-1}\cdot d^{-1}$, respectively. Besides, an equivalent volume of PBS was intragastrically administered into mice in normal control group. The above intragastric administration was conducted every day for 14 consecutive days. During the experiment, the eating, drinking and activity of mice in each group were observed and recorded, and the death of mice was recorded.

Experimental grouping and model establishment

Another 120 BALB/c mice of the same batch were selected, grouped (n=30) as above, and administered the drug as described above.

Sample collection

On the 7^{th} and 14^{th} day after inoculating the virus, 6 mice were randomly taken from each group and weighed. Then, blood was collected from the inner canthus and centrifuged to separate the serum. Next, the serum was harvested and cryopreserved at –80°C for later use. After that, the mice were killed via cervical vertebra disconnection, and the heart was obtained in a sterile manner and weighed, followed by calculation of the body weight/heart weight (BW/HW). Next, the heart was cut in half along its long axis for hematoxylin-eosin (HE) staining and ELISA, respectively.

HE staining

The mouse heart specimens were conventionally fixed in 4% paraformaldehyde for 24 h, dehydrated with gradient ethanol (75, 85, 95 and 100%) and embedded in paraffin. Then, the paraffin was serially sectioned into 4 μm thick slices and stained with hematoxylin and counterstained with eosin. Next,

they were dehydrated with gradient ethanol, permeabilized with xylene, and sealed with neutral resin. 5 fields of view of each slice were selected to observe the pathological changes of mouse myocardium under the optical microscope. The ratio of the area of inflammatory cell infiltration and necrosis in each high-power field to the area of the entire field of view was calculated. The pathology of mouse myocardial tissues was scored according to the method of Rezkalla[8]. Specifically, no lesions were scored as 0 points, and lesions accounting for <25, >25-50, >50-75 and >75% of the area of the field of view of each heart slice were scored as 1 point, 2 points, 3 points and 4 points, respectively.

Detection of virus titre

The mouse heart tissues were cut into pieces (about 1 mm^3), washed with Hank's solution containing 1% double-antibody per milliliter four times, and fully digested with trypsin-EDTA for 5 min. Then, they were resuspended in growth-promoting media with 10-fold volume of the tissues, subjected to repetitive freezing and thawing for thrice, and centrifuged at 3000 rpm for 20 min. Next, the precipitation was removed, and the supernatant was diluted to different dilutions (10^{-6}-10^{-1}) by the successive 10-fold dilution method and infected Hela cells, followed by observation for 5 consecutive days. $TCID_{50}$ of CVB3 was calculated according to the Reed-Muenc method[9].

Measurement of serum cTnI level

A total of 50 μL of serum was collected to detect the serum cTnI level of mice in each group by chemiluminescence method according to the kit instructions.

Measurement of oxidative stress factors in myocardial homogenate

The myocardial tissues of each group of mice were cut into pieces, homogenized and centrifuged, followed by collection of the supernatant. Then, the BCA protein assay kit was adopted to detect the level of proteins, and the activity of SOD, MDA, GSH-Px and CAT in the myocardial homogenate was determined using relevant kits.

Measurement of inflammatory factors in myocardial homogenate

The mouse myocardial tissues in each group were homogenized and centrifuged. Then, the supernatant was harvested, and the protein level was detected using the BCA protein assay kit. Next, double-antibody sandwich ELISA was performed to measure the levels of IL-6, IL-18, IL-1β and TNF-α in the myocardial homogenate.

Detection of cell apoptosis by TUNEL assay

The cell apoptosis in paraffin sections of mouse myocardial tissues in each group was detected using TUNEL assay kit. 6 fields of view were randomly selected from each slice to count apoptotic cells and total cells, and the Image Pro Plus 6.0 software was employed to analyze images. Apoptosis index = number of apoptotic cells/total number of cells ×100%.

Detection of protein expressions by Western blotting

After the last administration, the myocardial tissue samples of mice in each group were taken, ground in liquid nitrogen, and lyzed with RIPA lysis buffer on ice for 30 min, followed by centrifugation in a low-temperature refrigerated centrifuge for 10 min (12500 rpm). Then, the supernatant was collected to detect the level of proteins by BCA method. The total proteins were subjected to SDS-PAGE (5% concentrated gel + 10% separation gel, 80 V, 100 min) and transferred onto a PVDF membrane. Next, the membrane was washed with TBS for 10 min, blocked with 5% blocking protein dry powder diluent for 2 h, and incubated with Bax, Bcl-2 and Caspase-3 primary antibody diluents at 4°C overnight. The next day, the corresponding secondary antibodies were added and incubated for 1-2 h, followed by colour development with ECL luminescent solution. Afterwards, the image analysis system ImageJ2x was utilized to analyze the grey value of protein bands, with β-actin as an internal reference.

Statistical analysis

SPSS 22.0 software was utilized for statistical analysis. The survival rate was analyzed through the Kaplan-Meier method. All variables were subjected to one-way analysis of variance. The t test was employed for comparison between groups. P <0.05 indicated that the difference was statistically significant.

Results

Basic state and survival rates

Symptoms such as hair loss, reduced food intake and water intake, hair standing on end, malaise, irritability and biting each other were observed in CVB3-infected mice from the 2^{nd}-3^{rd} day. On the 4^{th} day, mice began to die, suggesting that the modeling

was successful. The above symptoms were obviously relieved in L-IVA and H-IVA groups. The mice in normal control group displayed strong activity, good food intake, uniform and glossy hair and increased weight. At the end of experiment on the 14th day, no death was found in the mice in normal control group. Compared with that in normal control group, the survival rate was significantly lowered in VMC model group (40%, 8/20) (P <0.01) and significantly raised in H-IVA group (75%, 15/20) (P <0.05). Besides, there was no statistically significant difference in the survival rate of mice between L-IVA group and normal control group ((50%, 10/20) *vs*. (100%, 20/20), P >0.05) (Fig. 1).

HW/BW of mice was significantly higher in VMC model group than that in normal control group (P <0.05). After treatment with IVA, HW/BW of mice declined in L-IVA and H-IVA groups compared with that of VMC model group, and a statistically significant difference was detected in the ratio between H-IVA group and VMC model group (P <0.05) (Table 1).

Effects of IVA on myocardial pathological changes and pathological scores

Pathological indices are most direct and important for the diagnosis of VMC. It was found by HE staining that normal control group exhibited normal myocardial structure, regular cell morphology, neatly arranged tissues, and no inflammatory cell infiltration. VMC model group displayed visible focal myocardial necrosis, myocardial fiber disintegration and rupture, interstitial edema around necrotic myocardial cells, massive inflammatory cell infiltration, and atrophy of some myocardial cells. In comparison with VMC model group, L-IVA and H-IVA groups had relieved myocardial inflammatory cell infiltration and myocardial necrosis. In L-IVA group, there were necrosis and disintegration of some myocardial tissues, disordered cell arrangement and inflammatory cell infiltration. In H-IVA group, fractional necrotic myocardial tissues, more neatly arranged cells and inflammatory cells infiltrated in a small amount were observed (Fig. 2).

The myocardial pathological score of mice was significantly higher in VMC model group than that in normal control group (P <0.01), while it was reduced after IVA intervention. Besides, the myocardial pathological score of mice showed a statistically significant difference between L-IVA group and VMC model group and between H-IVA group and VMC model group (P <0.05, P <0.01) (Table 2).

Effects of IVA on serum cTnI level

On the 14th d after administration, the serum cTnI level of mice dropped in all groups compared with that on the 7th day. The serum cTnI level of mice was significantly higher in VMC model group than in normal control group (P <0.01), whereas it was lower

Table 1 — Heart weight/Body weight (HW/BW) ratios (mg/kg) of experimental mice during the study period

Groups	7th day	14th day
Normal control	4.47±0.22	5.17±0.09
VMC model	6.49±0.12*	6.84±0.37*
L-IVA	5.98±0.47	6.45±0.23
H-IVA	5.18±0.15**	5.79±0.07**
F	5.25	3.91
P	<0.05	<0.05

[*,** P <0.05 *vs.* normal control group and *vs.* VMC model group, respectively]

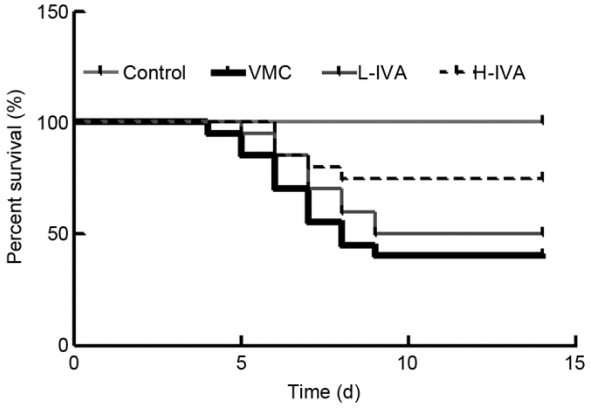

Fig. 1 — Survival rates of experimental groups of mice during the study period.

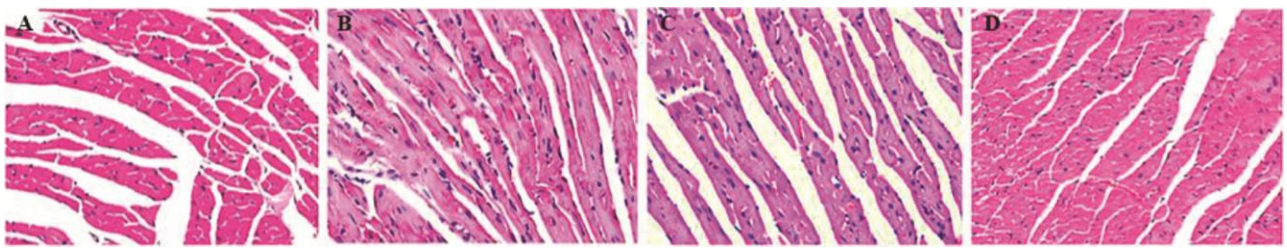

Fig. 2 — Effect of ivabradine (IVA) on pathological changes of myocardial tissues in VMC mice. (A) Normal control group; (B) VMC model group; (C) L-IVA group; and (D) H-IVA group. (HE staining: 400X)

Table 2 — Myocardial pathological scores

Groups	7th day	14th day
Normal control	0.00	0.00
VMC model	0.80±0.45*	2.79±0.67*
L-IVA	0.58±0.21**	2.01±0.23**
H-IVA	0.4±0.15***	1.27±0.38***
F	10.19	12.77
P	<0.01	<0.01

[*P <0.05 *vs.* normal control group, **P <0.05 and ***P <0.01 *vs.* VMC model group]

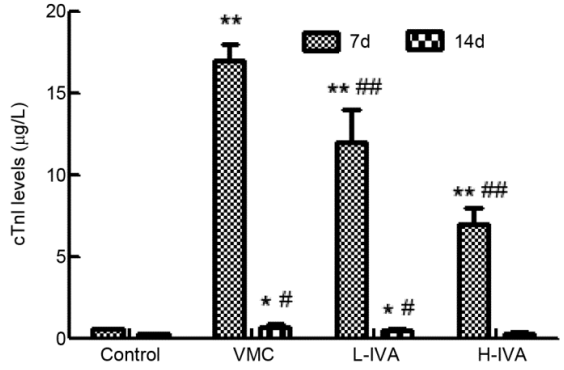

Fig. 3 — Effect of ivabradine (IVA) on serum cTnI level in VMC mice. **P <0.01 *vs.* normal control group, $^{\#}P$ <0.05 and $^{\#\#}P$ <0.01 *vs.* VMC model group.

in L-IVA and H-IVA groups than that in VMC model group (P<0.05, P<0.01) (Fig. 3).

Effects of IVA on levels of oxidative stress factors in myocardial homogenate

In comparison with the normal control group, the VMC model group had significantly enhanced MDA activity and significantly weakened activity of SOD, GSH-Px and CAT in mouse myocardium (P <0.01). Further, compared with VMC model group, both L-IVA group and H-IVA group exhibited significantly decreased MDA activity and significantly enhanced activity of SOD, GSH-Px and CAT (P <0.05, P <0.01) (Table 3).

Effects of IVA on levels of inflammatory factors in myocardial homogenate

Compared with that in normal control group, the levels of IL-6, IL-18, TNF-α and IL-1β in mouse myocardium were significantly elevated in VMC model group (P <0.01). After treatment with IVA at different doses, such levels reduced dose-dependently (P <0.05, P <0.01) (Table 4).

Effects of IVA on myocardial cell apoptosis

All myocardial nuclei were stained blue with 4',6-diamidino-2-phenylindole (DAPI), and the positive nucleus of apoptotic cells was compact and round, with typical morphological characteristics of apoptotic cells. The number of TUNEL-positive cells

Table 3 — Changes in myocardial activities of MDA, SOD, GSH-Px and CAT

Group	n	MDA (nmol/mg)	SOD (U/mg)	GSH-Px (U/mg)	CAT (U/mg)
Normal control	30	71.86± 9.92	66.42± 7.42	25.79± 4.61	36.75± 6.52
VMC model	19	137.28± 16.13*	26.54± 4.98*	9.45± 1.42*	17.27± 2.38*
L-IVA	23	114.16± 9.28**	38.62± 4.76**	14.37± 2.68**	25.37± 3.68**
H-IVA	27	85.43± 8.48***	52.49± 6.76***	21.28± 3.64***	34.05± 3.64***

[*P <0.05 *vs.* normal control group, $^{\#}$**P <0.05 and $^{\#\#}$***P <0.01 *vs.* VMC model group]

Table 4 — Changes in myocardial IL-6, IL-18, TNF-α and IL-1β levels

Groups	n	IL-6	IL-18	TNF-α	IL-1β
Normal control	30	61.39± 8.45	28.54± 8.39	6.95± 0.86	24.01± 3.67
VMC model	19	139.17± 12.41*	55.72± 7.89*	26.46± 6.49*	62.38± 7.31*
L-IVA	23	114.26± 10.15$^{\#}$	43.66± 4.82$^{\#}$	16.55± 3.81$^{\#}$	50.14± 6.67$^{\#}$
H-IVA	27	84.29± 6.93$^{\#\#}$	32.43± 4.96$^{\#\#}$	10.79± 1.32$^{\#\#}$	37.58± 4.99$^{\#\#}$

[*P <0.05 *vs.* normal control group; **P <0.05; and ***P <0.01 *vs.* VMC model group]

was significantly larger in VMC model group than that in normal control group, and the percentage of apoptotic myocardial cells in mice was significantly lower in L-IVA and H-IVA groups than that in VMC model group (P <0.05) (Fig. 4).

Expressions of apoptosis-related proteins in myocardial tissues

The results of Western blotting revealed that compared with normal control group, VMC model group showed significantly increased protein expression levels of Bax and Caspase-3 (P <0.05) and a significantly reduced protein expression level of Bcl-2 in mouse myocardial tissues (P <0.05). In comparison with VMC model group, L-IVA and H-IVA groups had significantly lowered protein expression levels of Bax and Caspase-3 (P <0.05) and a significantly raised protein expression level of Bcl-2 in mouse myocardial tissues (P <0.05) (Fig. 5).

Discussion

VMC is a leading cause of sudden death and heart failure in adolescents, with CVB3 as the most common etiological agent. The pathogenesis of VMC is not fully understood, but it is mainly caused by the direct damage to myocardial cells by viruses, immune damage, apoptosis and cytokines[10]. A study showed that IVA, a drug used to slow down heart rate

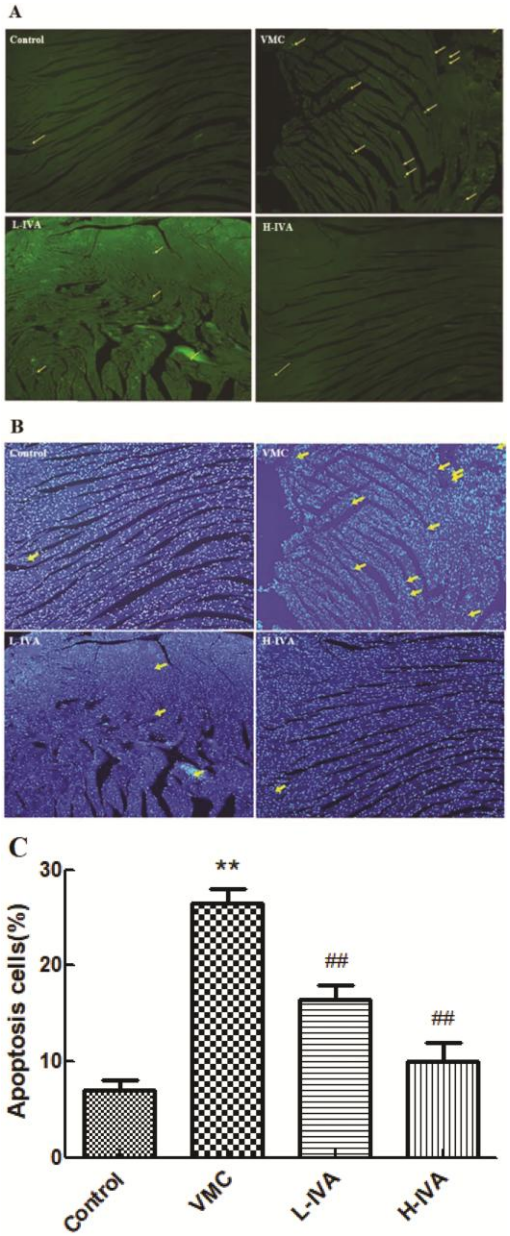

Fig. 4 — Apoptosis of myocardial cells. (A) Apoptotic cells marked green with yellow arrows; (B) myocardial cells marked blue with DAPI; and (C) apoptosis rates.

in routine clinical practice, is able to significantly reduce the cardiovascular death in patients with chronic heart failure, reverse myocardial remodeling, alleviate cardiac dilatation and increase the left ventricular ejection fraction[11]. In this study, BALB/c mice were enrolled as the research objects, CVB3 was inoculated into mice to establish the VMC model, and VMC mice were treated with low- and high-dose IVA, so as to explore the protective effect and relevant mechanism of IVA on the myocardium of VMC mice.

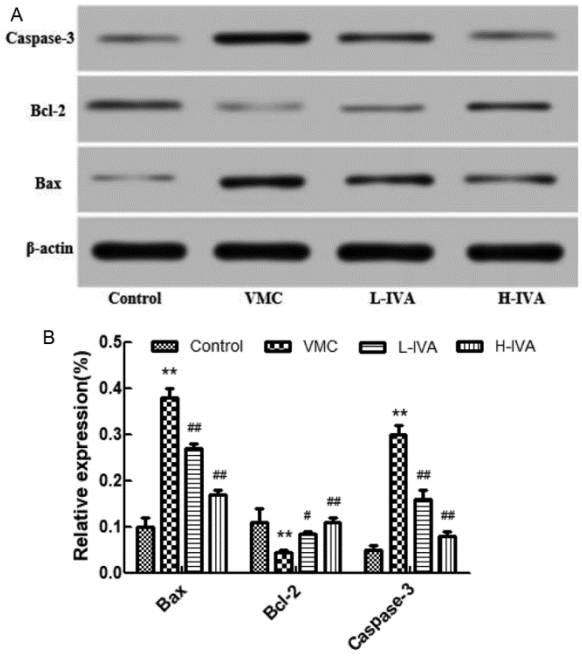

Fig. 5 — (A) Expressions of apoptosis-related proteins in myocardial tissues; and (B) Western blotting

As a myocardial structural protein, cTnI is released from myocardial cells into the peripheral blood when myocardial cells are destroyed, and it is the most sensitive specific marker currently known for detecting myocardial damage[12]. In this study, the results of survival curve analysis showed that the survival rate of mice was significantly higher in L-IVA and H-IVA groups than that in VMC model group. The results of HE staining for the myocardium of mice in each group uncovered that the myocardial inflammatory cell infiltration and necrosis were significantly alleviated, and the myocardial pathological score, HW/BW and serum cTnI level were significantly lowered in L-IVA and H-IVA groups compared with those in VMC model group, and H-IVA group had a better effect than L-IVA group. The above results suggest that IVA can relieve myocardial damage and protect mouse myocardium to some extent.

Oxidative stress response is a vital player in the pathogenesis of VMC. Viral infection of myocardial cells disrupts the cell membrane lipid microenvironment, production of massive free radicals and enhancement of lipid peroxidation, thereby leading to myocardial damage[13]. MDA, the final product of lipid peroxidation, is capable of indirectly reflecting cell damage by reflecting the degree of lipid peroxidation in the body. SOD, GSH-

Px and CAT constitute the first-line antioxidant enzyme defense system. The results of this study uncovered that the activity of MDA in mouse myocardium was significantly lower in L-IVA and H-IVA groups than that in VMC model group, whereas the activity of SOD, GSH-Px and CAT was higher in L-IVA and H-IVA groups than that in VMC model group. It indicates that IVA is capable of increasing the activity of antioxidant enzymes and scavenging free radicals in VMC mice, thus inhibiting lipid peroxidation-induced myocardial damage.

Inflammatory factors play crucial roles in the development and progression of VMC, and immune dysfunction in organism is a major pathogenesis of VMC. IL-6, IL-18, TNF-α, IL-1β and other pro-inflammatory factors play crucial roles in the development and progression of VMC[14]. A study of Becher et al. showed that IVA could inhibit the expressions of myocardial inflammatory factors TGF-β, IL-6, TNF-α and IL-1β in mice with hypertensive heart failure, relieve myocardial fibrosis and improve the cardiac function of mice[15]. It was discovered in this study that the levels of IL-6, IL-18, TNF-α, and IL-1β in the myocardial homogenate of VMC mice were obviously lower in L-IVA and H-IVA groups than in VMC model group, and H-IVA group displayed a better inhibitory effect on inflammatory factors. These results suggest that IVA is able to alleviate myocardial inflammatory response in mice by suppressing the production of myocardial inflammatory factors.

Abnormal apoptosis of myocardial cells is the third pathogenesis of VMC following direct damage by viruses and indirect autoimmune injury. TUNEL results revealed that the number of apoptotic myocardial cells was significantly larger in VMC model group than that in normal control group, while it was clearly smaller in L-IVA and H-IVA groups than that in VMC model group. Among members of the Bcl-2 family, both Bax (a pro-apoptotic protein) and Bcl-2 (an anti-apoptotic protein) can regulate cell apoptosis. Both Bcl-2 and Bax are involved in the infiltration and apoptosis of myocardial cells in mice with CVB3-induced myocarditis[16]. Besides, Caspase-3, a protease closely associated with cell apoptosis, acts as an ultimate pivot in programmed cell death. In this study, the expression of Bcl-2 in mouse myocardial tissues was downregulated, while the expressions of Bax and Caspase-3 were upregulated in VMC model group. After treatment with IVA, the expression of Bcl-2 rose, whereas the expressions of Bax and Caspase-3 declined, and the Bcl-2/Bax ratio was increased. The above results imply that IVA can repress the apoptosis of myocardial cells by down-regulating the protein expressions of Bax and Caspase-3 and upregulating the protein expression of Bcl-2 therein, thereby protecting the myocardium of VMC mice.

Conclusion

Results of the above study demonstrate that ivabridine (IVA) could increase the survival rate of viral myocarditis (VMC) mice (mention the optimal dose here as observed in the study) and relieve their myocardial pathological changes possibly by reducing free radical damage and suppressing inflammation response and apoptosis of myocardial cells.

Conflict of interest

Authors declare no competing interests.

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