Cardioprotection of Sodium–Glucose Cotransporter 2 Inhibition in Rats With Isoproterenol-Induced Cardiomyopathy

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Research

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Abstract

Background: Sodium-glucose cotransporter 2 inhibitor (SGLT2i) has been reported to improve glycaemic control in patients with type 2 diabetes. The aim of this study was to investigate the effect of SGLT2i Dapagliflozin (Dapa) on cardiomyopathy induced by isoproterenol (ISO) and its potential mechanism.

Methods: Fifty male Sprague Dawley rats were randomly assigned to Control (n = 10) and ISO (2.5 mg/kg/day)-treated groups (n = 40). After 2 weeks, 28 survived rats with obvious left ventricular dysfunction in ISO group were randomized into three groups for medication including ARNI (angiotensin receptor neprilysin inhibitor, 68 mg/kg/day, n = 9), Dapa (3 mg/kg/day, n = 9) and ISO (saline, n = 10) for 4 weeks. After that, electrical programmed stimulation (EPS) was performed in all groups for the evaluation of the susceptibility of ventricular arrhythmias (VAs). Echocardiography was used to evaluate cardiac function.

Results: Echocardiography revealed significant left ventricular (LV) dysfunction in rats with ISO treatment for 2 weeks compared to the control group. Dapa administration for 4 weeks reduced the cumulative risk of death, myocardial fibrosis, plasma angiotensin II level and its functional receptor AT1R protein expression in the heart, and proinflammatory cytokines levels in the cardiac tissue of ISO-treated rats. It also improved cardiac function and inhibited oxidative stress when compared to the ISO group. These effects were similar to ARNI. However, Dapa showed a greater efficacy than ARNI in reducing left ventricular end-diastolic volume, lowing heart rate and VAs, and decreasing body weight and plasma glucose in ISO-treated rats.

Conclusion: Dapa effectively improved the myocardial remodelling and oxidative stress like ARNI in ISO-induced cardiomyopathy in rats, but Dapa may be more effectively in decreasing VAs, and improving cardiac function when compared to ARNI. The mechanisms by which Dapa exerts protective effects on cardiomyopathy may be related to its antioxidant capacity and hypoglycemic action.

Background

Sodium-glucose cotransporter 2 (SGLT2) transporter inhibitors (SGLT2i) such as Dapagliflozin (Dapa), as a new class of anti-diabetic drugs, are proven to have beneficial effects beyond the glucose-lowering effects, such as reducing visceral fat, inhibiting inflammation and oxidative stress, and having cardiac protective effects [1-4]. The Dapagliflozin And Prevention of Adverse-outcomes in Heart Failure (DAPA-HF) Trial outcomes showed that the SGLT2i markedly reduced mortality and impoved heart failure remarkably, these benefits seemed to be similar in people with and without diabetes [5]. Angiotensin receptor neprilysin inhibitor (ARNI), beyond blocking angiotensin II signaling, augments natriuretic peptides by inhibiting their breakdown by neprilysin, and becomes class I drug recommended for the treatment of heart failure in the recent years [6]. At present, more attention was paid to the effects of SGLT2i on cardiovascular system [7-8]. A recent nationwide population-based longitudinal cohort study revealed that patients with type 2 diabetes prescribed with SGLT2i were associated with a lower risk of all-cause mortality and new-onset arrhythmias compared with those not taking SGLT2i in real-world practice [9].

Isoproterenol (ISO) as a synthetic nonselective β-adrenoceptor agonist is well accepted to induce myocardial damage in rats for evaluating cardiac dysfunctions [10]. The pathophysiological and morphological changes of ISO-induced myocardial changes are similar to those observed in human with myocardial infarction or heart

failure. Therefore, ISO-induced myocardial damage is a well-standardized animal model to study the protective effects of many drugs on cardiac dysfunctions. Persistent β-adrenergic stimulation with ISO results in cardiomyocytes injury, generation of reactive oxygen species (ROS), arrhythmias, ventricular hypertrophy and increased fibrosis, inflammation and collagen deposition [11]. Experimental and clinical studies have shown that SGLT2i therapy can prevent or ameliorate cardiac dysfunction through inhibition of oxidative stress, inflammation and so on [12].

The present study was designed to explore the cardiac effects of SGLT2i Dapa in rats with cardiomyopathy induced by ISO and compare the protective effects of ARNI on the heart.

Materials And Methods

Animals

Experimental animal care and use complied with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 8th edition, 2011). All experiment procedures were approved by the Nanjing Medical University. Fifty male Sprague-Dawley rats weighting 200-250 g were purchased from Nanjing Medical University Laboratory Animal Center. All rats were caged in a room with controlled temperature and humidity with a 12-h light/dark cycle and provided a standard chow and drinking water ad libitum. Rats were randomly assigned to Control group (n = 10) and isoproterenol induced cardiomyopathy group (ISO, n = 40). Rats in ISO group were intraperitoneally injected 2.5 mg/kg/d isoproterenol hydrochloride (Sigma, Switzerland) dissolved in normal saline, once a day for 2 weeks [13, 14]. Echocardiography was performed at the end of the 2nd week and the 6th week. After echocardiography measurement at the end of the 2nd week, 28 survival rats in ISO group were randomized into three groups including ARNI (angiotensin receptor neprilysin inhibitor, n = 9), Dapa (Dapagliflozin, n = 9) and ISO (saline, n = 10) groups. ARNI (Novartis Pharma Schweiz AG, Chinese national medicine permission number J20190001) was administered intragastrically at a dose of 68 mg/kg, and Dapa (AstraZeneca Pharmaceuticals LP, Chinese national medicine permission number J20170040) was administered intragastrically at a dose of 3 mg/kg for 4 weeks, respectively. The medication method for ARNI and Dapa was adopted according to previously published literature [15, 16, 17].

Assay of Cardiac Function-related Parameters

Echocardiography was performed at the end of the 2nd week and the 6th week. The rats were anesthetized with ketamine, and then the cardiac function was evaluated using a Vevo 2100 (VisualSonics, Canada) system equipped with a MS-250, 16.0-21.0 MHz imaging transducer.

Electrical Programmed Stimulation

At the end of the 6th week, all rats underwent ventricular electrical programmed stimulation (EPS) for the evaluation of susceptibility of ventricular arrhythmias (VAs) before being sacrificed. They were anesthetized by intraperitoneal injection of 2% sodium pentobarbital (50 mg/kg). Three needle electrodes were placed on the right upper limb and legs to perform electrocardiography. Then EPS was used to stimulate the left ventricular apex of the heart through a bipolar electrode and the incidence of ventricular arrhythmias (VA) was investigated. By a cycle length of 140 ms, the threshold potential for stable pacing was achieved. Pacing was

started with twice as much as the threshold and a cycle length of 140 ms, which was the interval of eight stimuli (S1). An extra stimulus (S2) was applied until it failed to induce ventricular depolarization, while the interval between S1 and S2 was progressively shortened by 10 ms.

Samples and Histopathology

Animals were sacrificed after EPS immediately. Blood was collected from the descending aorta. After being weighed and washed with ice-cold PBS, one part of the heart was cut and fast frozen by liquid nitrogen, then moved to − 80 °C for further detection. The other part fixed in 4% paraformaldehyde was used for staining. Masson's trichrome staining was performed to detect cardiac fibrosis. Five fields of each sample were randomly selected and collagen volume fraction (CVF) was assessed by Image-Pro Plus 6.0.

Measurement of Plasma Angiotensin II (Ang II)

Plasma level of Ang II was measured from the blood collected from the abdominal descending aorta. Blood was collected into tubes containing EDTA, and then centrifuged at 3000 rpm at 4 °C for 15 mins to separate the plasma. Plasma Ang II level was determined using enzyme linked immunosorbent assay (ELISA) kit. All steps were carried out in accordance with the manufacture's specifications (Abcam Inc, UK). The final solution was read by a microplate reader (ELX800, BioTek, Vermont, USA).

Measurement of Plasma Glucose and Cardiac MDA Levels

Plasma level of glucose was detected by the glucose-oxidase method using a commercially available glucose assay kit from Jiancheng Bioengineering (Nanjing, Jiangsu, China). The level of MDA, in the heart tissue, was detected by using a lipid peroxidation (malondialdehyde; MDA) assay kit from Jiancheng Bioengineering (Nanjing, Jiangsu, China). Lipid peroxidation was determined by the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric product, proportional to the MDA present. The intensity of the color was measured spectrophotometrically at 505 nm for glucose and 532 nm for MDA.

Measurement of Superoxide Anions

The lucigenin-derived chemiluminescence method was used to examine superoxide anions level in the cardiac tissue. Superoxide anions can react with dark-adapted lucigenin (5 μM) resulting in photon emission which can be captured once every minute for 10 mins by a luminometer (20/20n, Turner, Sunnyvale, CA, USA). The superoxide anions levels were expressed as the mean light units (MLU) per minute per milligram of protein [18].

Measurement of Nicotinamide Adenine Dinucleotide Phosphate (NADPH) Oxidase Activity

The enhanced lucigenin chemiluminescence method was used to detect NADPH oxidase activity. NADPH oxidase can react with NADPH substrate (100 μM) in the medium to generate superoxide anions which can react with lucigenin (5 μM) to produce light emission. A luminometer (20/20n, Turner, CA, USA) can capture the light emission once every minute for 10 mins. The NADPH oxidase activity could be expressed as the (MLU) per minute per milligram of protein [19].

Western Blotting

Protein expressions of angiotensin II type-1 receptor (AT1R, antibody from Endo Life Science Inc, USA), the superoxide (O_2^-)-generating NADPH oxidase isoforms (NOX2 and NOX4, antibodies from Abcam, Burlingame, CA, USA), and inflammatory markers including TNFα, IL-1β, IL-6 and IL-10 (antibodies from Proteintech, Chicago, IL, USA) in myocardial tissue were detected by Western blotting [20]. Simply, total cardiac proteins in the homogenate were extracted and measured. Antibodies AT1, NOX2, NOX4, TNFα, IL-1β, IL-6 and IL-10 were applied according to the manufacturer's instructions. Horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG were used as secondary antibody. Protein expression level was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH, antibody from Proteintech, Chicago, IL, USA). The signals were quantified by using Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE).

Kaplan-Meier Analysis

Survival over the 6-week experiment was analyzed according to the daily recording of deaths by the standard Kaplan-Meier analysis with the log rank test.

Statistics

Data are expressed as mean ± SEM and analyzed by GraphPad Prism v8.0.2 (GraphPad Software, CA). Comparisons between the two groups performed with two-tailed unpaired *t* test. For multiple-group comparisons, data were performed using one-way ANOVA followed by Bonferroni's post-hoc test. A value of $P<0.05$ was considered statistically significant.

Results

Dapa Ameliorated ISO-induced Cardiac Dysfunction in ISO-treated Rats

At the end of the 2nd week, echocardiography showed significant increases in diastolic left atrial diameter and left ventricular interventricular diameter as shown by the representative tracings of echocardiography (Figure 1A), and a reduction in ejection fraction (EF) and fractional shortening (FS) in ISO group compared with the Control group ($P<0.05$, Table 1). The left ventricular end-diastolic volume (LVEDV) was increased in ISO group compared with the Control group ($P<0.05$, Table 1). These results showed that ISO-induced significant impairment of cardiac function. At the end of the 6th week, EF and FS were notably increased in Dapa and ARNI groups compared with ISO group ($P<0.05$, Table 1), while LAD and LVID increases were significantly improved in ARNI and Dapa groups compared with the ISO group (Figure 1B). But LVEDV and heart rate in Dapa group were lower than in ARNI group ($P<0.05$, Table 1).

Dapa Inhibited the Occurrence of Ventricular Arrhythmias in ISO-treated Rats

Electrical programmed stimulation (EPS) was performed in all groups in order to induce VAs. The original images of electrocardiography were shown in Figure 2A. The mean voltage level of all groups was similar. The incidence of pacing-induced VAs in the ARNI and Dapa groups was greatly reduced than that in ISO group (Figure 2B). It seemed that this effect was more effective in Dapa group than in ARNI group.

Dapa Improved Cardiac Remodeling in ISO-treated Rats

Cardiac fibrosis is well known to increase ventricular stiffness, leading to diastolic dysfunction. In this study, collagen volume fraction (CVF), a critical method to assess organic fibrosis, was evaluated in Masson's Trichrome Staining sections from hearts of rats. After 6 weeks of ISO treatment, the results showed that intraperitoneal injection of ISO resulted in increased myocardial fibrosis significantly. However, the myocardial interstitial fibrosis was obviously improved by ARNI and Dapa medication for 4 weeks ($P<0.05$, Figure 3).

Dapa Reduced Body Weight, Cumulative Risk, and Plasma Glucose and Ang II Levels in ISO-treated Rats

After 6 weeks of ISO treatment, the body weight and the plasma glucose level were significantly decreased ($P<0.05$, Figure 4A, 4C), but the plasma Ang II level ($P<0.05$, Figure 3D) were significantly increased in ISO-treated rats when compared with the control rats. Risk-Function was analyzed according to the daily recording of deaths for 6 weeks by Kaplan-Meier analysis. There were 3 of 10 animals dead in ISO-treated group, but there was only one of 9 animals dead in the Dapa or ARNI group. None of ten died in the Control group ($P<0.05$, Figure 4B). ARNI and Dapa both reduced the Ang II level and cumulative risks of rats induced by ISO ($P<0.05$, Figure 4B, 4D). However, Dapa further markedly reduced the body weight and the plasma glucose level when compared with the ISO group and ARNI group ($P<0.05$, Figure 4A, 4C).

Dapa Inhibited Inflammation and AT1R Protein Expression in ISO-treated Rats

Inflammation is the key mediator for myofibroblast formation and collagen deposition, which lead to cardiac fibrosis [21]. ISO action is partially mediated by inflammation through the activation of β1-adrenergic receptors in the heart [22]. In this study, the rats in ISO group revealed significant cardiac inflammation as shown by the increases in inflammatory factors including TNFα, IL-1β and IL-6 compared to the Control group. However, Dapa treatment significantly reduced myocardial TNFα, IL-1β and IL-6 protein levels ($P<0.05$, Figure 5A, 5B, 5C). ARNI treatment markedly decreased myocardial TNFα and IL-1β protein levels, but not IL-6 (Figure 5C). Dapa and ARNI both significantly upregulated the protein level of anti-inflammatory cytokine IL-10 ($P<0.05$, Figure 5D). AT1R serves as a major mediator of Ang II effects, including fibrogenic effect and increased ROS production [23]. In ISO-treated rats, cardiac AT1R protein level was higher than in the control rats. However, both Dapa and ARNI down-regulated the cardiac protein expression of AT1R ($P<0.05$, Figure 5E), and this may attenuated the pathogenic effects of Ang II via AT1R on the heart.

Dapa Inhibited Cardiac Oxidative Stress in ISO-treated Rats

ISO-induced cardiotoxicity is assumed that it generates highly cytotoxic free radicals in myocytes, which causes oxidative stress involving in the structural and functional myocardial damage. Cytotoxic free radicals can be generated by an activated nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, increased levels of Ang II and proinflammatory cytokines and so on. In this study, the rats in ISO groups revealed significant alteration in cardiac oxidative stress including the increases in ROS level, NADPH oxidase activity, and the main isoform NOX2 of NADPH oxidase but not NOX4 protein expression in the heart when compared to the control group, which were significantly reduced by the treatment with Dapa and ARNI when compared to ISO-treated group ($P<0.05$, Figure 6A, C, D). Cytotoxic free radicals can cause lipid peroxidation (LPO) of intramembranous polyunsaturated fatty acids in the membrane. Malondialdehyde (MDA), as an important LPO by-product, had a significant increase in the cardiac tissue in the ISO-treated rats when compared to in the

control rats. The Dapa and ARNI treatment significantly decreased MDA level when compared to the ISO-treated group ($P<0.05$, Figure 6E).

Discussion

Dapa, a selective inhibitor of SGLT2, is widely used to treat with type 2 diabetes depending on the increase glucose excretion in urine. In addition to its special glycaemic effect, there are many other benefits of Dapa such as weight loss, slowdown of cardiovascular diseases progression and so on [1-4]. In this study, we evaluated the influence of the SGLT2 inhibitor Dapa on cardiac remodeling, function, VAs and oxidative stress in rats with cardiomyopathy induced by ISO. We observed that Dapa had obvious cardiovascular protective roles like ARNI in ISO-treated rats: 1) Dapa and ARNI both effectively improved the cardiac fibrosis and dysfunction, but the increase in left ventricular end-diastolic volume induced by ISO was improved by Dapa more markedly than ARNI; 2) Dapa reduced the incidence of pacing-induced VA, heart rate and body weight more effectively than ARNI; 3) Dapa and ARNI both decreased cumulative risk of death and ameliorated cardiac oxidative stress such as the decreases in ROS level and MDA content in the heart. Dapa may have stronger cardiac protective effects than ARNI in ISO-induced cardiomyopathy.

In recent years, many previous studies have focused on the effects of SGLT2i on the cardiomyopathy in animal models with type 2 diabetes [24]. However, the roles of Dapa on ISO-induced cardiomyopathy have not been explored. ISO, a synthetic nonselective β-adrenergic agonist, is commonly used to activate β1-adrenergic receptors that is associated with deleterious myocardial effects, including ventricular arrhythmia, left ventricular hypertrophy, increased ventricular collagen content and a reduced inotropic response [25]. Therefore, ISO-induced cardiotoxicity is one of the most widely studied model for chronic cardiac injury. Hung-Yi Chen et al [26] has reported that patients prescribed with SGLT2i were associated with a lower risk of new-onset arrhythmias compared with those not taking SGLT2 inhibitors in real-world practice. Therefore, we also studied the role of Dapa on VAs in ISO-treated rats. As well known, ARNI is commonly used in clinical treatment of heart failure. For instance, its effect on VAs prevention has been widely reported [27, 28, 29], but not SGLT2i. Therefore, we also used ARNI as a reference to compare the effects of Dapa in our present study. ISO-induced alterations including cardiac dysfunction, cardiac fibrosis and increase of VAs were found in our present study, and which were effectively improved by application of Dapa and ARNI. Moreover, Dapa was more effective in reducing LVEDV, VAs, heart rate and body weight than ARNI. In the present investigation, the reductions in LVEDV and BW from our results implied the reductions in cardiac preload and afterload, which suggested that Dapa may play an important role in mitigating ventricular loading. The reason for weight loss may be related to its hypoglycemic action. Moreover, the decreases in VAs and heart rate indicated that Dapa application may have greater potential to reduce the risk of ventricular arrhythmias. Therefore, the pharmacological intervention of Dapa to ameliorate ISO induced cardiac abnormalities may have the potential therapeutic value in preventing the initiation and progression of cardiomyopathy.

ISO-induced cardiotoxicityis highly associated cytotoxic free radicals in myocytes, which causes oxidative stress leading to infammation and structural and functional myocardial damage [30]. Oxidative stress is generated due to reactive oxygen species (ROS) and imbalanced antioxidant defence mechanisms. ROS can be generated by an activated NADPH oxidase, increased Ang II and proinflammatory cytokines and so on [31-32]. These major factors for promoting ROS generation were investigated in our present study. Indeed, the

application of ISO produced the obvious increases in ROS level, NADPH oxidase activity and the proinflammatory cytokines production. Moreover, Ang II level in plasma and its functional receptor AT1R protein expression in the heart, and oxidative stress-caused lipid peroxidation (LPO) product malondialdehyde (MDA) content in cardiac tissue were evidently higher than those in the control group. However, these adverse alterations were effectively reduced by the administration of Dapa and ARNI. These results indicated that treatment with Dapa and ARNI both significantly attenuated oxidative stress in cardiac tissue as shown by the decreases in ROS level and MDA content. It may be through reducing NADPH activity, AT1R protein levels and the production of proinflammatory cytokines in the heart. These results also revealed that Dapa had the antioxidant property by protecting cardiac muscle from ISO-mediated oxidative damage.

In conclusion, the protective effects of Dapa observed in this study may be due to its potent antioxidant properties, which protected cardiac tissue from the oxidative damage and helped in maintaining the myocardial cell membrane integrity and function. Our experimental results also provide an effective basis for the further clinical application of Dapa in the prevention and treatment of structural and functional myocardial damage. However, the potent protective mechanisms of Dapa in ISO-induced cardiotoxicity need to be further explored.

Declarations

Authors' Contributions

All authors participated in interpretation of the studies and review of the manuscript. ZZL and YBZ designed the study. FZW, WBW, HYW and PQ conducted the experiments. XL, JYH and WYJ performed the data analysis. ZZL and YBZ wrote the manuscript, and YBZ revised.

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Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed in this study will be made available by the authors on reasonable request.

Consent for publication

This study consists of animal data and is devoid of any human data.

Ethics approval and consent to participate

This study was carried out in accordance with the principles of the Basel Declaration and recommendations of the Experimental Animal Care and Use Committee of Nanjing Medical University, and conformed to the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (NIH publication, 8th edition, 2011).

References

1. Spigoni V, Fantuzzi F, Carubbi C, Pozzi G, Masselli E, Gobbi G, Solini A, Bonadonna RC, Dei Cas A.Sodium-glucose cotransporter 2 inhibitors antagonize lipotoxicity in human myeloid angiogenic cells and ADP-dependent activation in human platelets: potential relevance to prevention of cardiovascular events. Cardiovasc Diabetol. 2020;19(1):46.
2. Hodrea J, Balogh DB, Hosszu A, Lenart L, Besztercei B, Koszegi S, Sparding N, Genovese F, Wagner LJ, Szabo AJ, Fekete A. Reduced O-GlcNAcylation and tubular hypoxia contribute to the antifibrotic effect of SGLT2 inhibitor dapagliflozin in the diabetic kidney. Am J Physiol Renal Physiol. 2020;318(4):F1017-F1029.
3. McMurray JJV, DeMets DL, Inzucchi SE, Køber L, Kosiborod MN, Langkilde AM, Martinez FA, Bengtsson O, Ponikowski P, Sabatine MS, Sjöstrand M, Solomon SD; DAPA-HF Committees and Investigators. A trial to evaluate the effect of the sodium-glucose co-transportor 2 inhibitor dapaglifozin on morbidity and mortality in patients with heart failure and reduced left ventricular ejection fraction (DAPA-HF). Eur J Heart Fail. 2019;21(5):665-675.
4. Maranghi M, Carnovale A, Durante C, Tarquini G, Tiseo G, Filetti S. Pharmacokinetics, pharmacodynamics and clinical efficacy of dapagliflozin for the treatment of type 2 diabetes. Expert Opin Drug Metab Toxicol. 2015;11(1):125-37.
5. Clegg LE, Penland RC, Bachina S, Boulton DW, Thuresson M, Heerspink HJL, Gustavson S, Sjöström CD, Ruggles JA, Hernandez AF, Buse JB, Mentz RJ, Holman RR. Effects of exenatide and open-label SGLT2 inhibitor treatment, given in parallel or sequentially, on mortality and cardiovascular and renal outcomes in type 2 diabetes: insights from the EXSCEL trial. Cardiovasc Diabetol. 2019;18(1):138.
6. Solomon SD, Rizkala AR, Gong J, Wang W, Anand IS, Ge J, Lam CSP, Maggioni AP, Martinez F, Packer M, Pfeffer MA, Pieske B, Redfield MM, Rouleau JL, Van Veldhuisen DJ, Zannad F, Zile MR, Desai AS, Shi VC, Lefkowitz MP, McMurray JJV. Angiotensin Receptor Neprilysin Inhibition in Heart Failure With Preserved Ejection Fraction: Rationale and Design of the PARAGON-HF Trial. JACC Heart Fail. 2017;5(7):471-482.
7. Khan MS, Vaduganathan M. What Makes Sodium-Glucose Co-Transporter-2 Inhibitors Stand out in Heart Failure? Curr Diab Rep. 2020;20(11):63.
8. Rosano G, Quek D, Martínez F. Sodium-Glucose Co-transporter 2 Inhibitors in Heart Failure: Recent Data and Implications for Practice. Card Fail Rev. 2020;6:e31.
9. Chen HY, Huang JY, Siao WZ, Jong GP. The association between SGLT2 inhibitors and new-onset arrhythmias: a nationwide population-based longitudinal cohort study. Cardiovasc Diabetol. 2020;19(1):73.
10. Govindasami S, Uddandrao VVS, Raveendran N, Sasikumar V. Therapeutic Potential of Biochanin-A Against Isoproterenol-Induced Myocardial Infarction in Rats. Cardiovasc Hematol Agents Med Chem.

2020;18(1):31-36.

11. Jannesar K, Abbaszadeh S, Malekinejad H, Soraya H. Cardioprotective effects of memantine in myocardial ischemia: Ex vivo and in vivo studies. Eur J Pharmacol. 2020;882:173277.
12. Zelniker TA, Braunwald E. Mechanisms of Cardiorenal Effects of Sodium-Glucose Cotransporter 2 Inhibitors: JACC State-of-the-Art Review. J Am Coll Cardiol. 2020;75(4):422-434.
13. Zheng A, Yi H, Li F, Han L, Yu J, Cheng X, Su H, Hong K, Li J. Changes in Gut Microbiome Structure and Function of Rats with Isoproterenol-Induced Heart Failure. Int Heart J. 2019;60(5):1176-1183.
14. Simko F, Bednarova KR, Krajcirovicova K, Hrenak J, Celec P, Kamodyova N, Gajdosechova L, Zorad S, Adamcova M. Melatonin reduces cardiac remodeling and improves survival in rats with isoproterenol-induced heart failure. J Pineal Res. 2014;57(2):177-184.
15. Gu J, Noe A, Chandra P, Al-Fayoumi S, Ligueros-Saylan M, Sarangapani R, Maahs S, Ksander G, Rigel DF, Jeng AY, Lin TH, Zheng W, Dole WP. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting angiotensin receptor-neprilysin inhibitor (ARNi). J Clin Pharmacol. 2010;50(4):401-14.
16. Thomson SC, Rieg T, Miracle C, Mansoury H, Whaley J, Vallon V, Singh P. Acute and chronic effects of SGLT2 blockade on glomerular and tubular function in the early diabetic rat. Am J Physiol Regul Integr Comp Physiol. 2012;302(1):R75-83.
17. Huo JY, Jiang WY, Chen C, Chen R, Ge TT, Chang Q, Zhu L, Geng J, Jiang ZX, Shan QJ. Effects of Angiotensin Receptor Neprilysin Inhibitors on Inducibility of Ventricular Arrhythmias in Rats with Ischemic Cardiomyopathy. Int Heart J. 2019; 60(5):1168-1175.
18. Ding L, Kang Y, Dai HB, Wang FZ, Zhou H, Gao Q, Xiong XQ, Zhang F, Song TR, Yuan Y, Liu M, Zhu GQ, Zhou YB. Adipose afferent reflex is enhanced by TNFα in paraventricular nucleus through NADPH oxidase-dependent ROS generation in obesity-related hypertensive rats. J Transl Med. 2019;17(1):256.
19. Sun HJ, Zhou H, Feng XM, Gao Q, Ding L, Tang CS, Zhu GQ, Zhou YB. Superoxide anions in the paraventricular nucleus mediate cardiac sympathetic afferent reflex in insulin resistance rats. Acta Physiol (Oxf). 2014;212(4):267-82.
20. Kang Y, Ding L, Dai H, Wang F, Zhou H, Gao Q, Xiong X, Zhang F, Song T, Yuan Y, Zhu G, Zhou Y. Intermedin in Paraventricular Nucleus Attenuates Ang II-Induced Sympathoexcitation through the Inhibition of NADPH Oxidase-Dependent ROS Generation in Obese Rats with Hypertension. Int J Mol Sci. 2019;20(17):4217.
21. Boarescu PM, Chirilă I, Bulboacă AE, Bocșan IC, Pop RM, Gheban D, Bolboacă SD. Effects of Curcumin Nanoparticles in Isoproterenol-Induced Myocardial Infarction. Oxid Med Cell Longev. 2019;2019:7847142.
22. Attalla DM, Ahmed LA, Zaki HF, Khattab MM. Paradoxical effects of atorvastatin in isoproterenol-induced cardiotoxicity in rats: Role of oxidative stress and inflammation. Biomed Pharmacother. 2018;104:542-549.
23. Tatyana A Meyers, Jackie A Heitzman, Aimee M Krebsbach, Lauren M Aufdembrink, Robert Hughes, Alessandro Bartolomucci, DeWayne Townsend. Acute AT 1 R blockade prevents isoproterenol-induced injury in mdx hearts. J Mol Cell Cardiol. 2019;128:51-61.

24. Ye Y, Bajaj M, Yang HC, Perez-Polo JR, Birnbaum Y. SGLT-2 Inhibition with Dapagliflozin Reduces the Activation of the Nlrp3/ASC Inflammasome and Attenuates the Development of Diabetic Cardiomyopathy in Mice with Type 2 Diabetes. Further Augmentation of the Effects with Saxagliptin, a DPP4 Inhibitor. Cardiovasc Drugs Ther. 2017;31(2):119-132.
25. Zuzana Nichtova, Marta Novotova, Eva Kralova, Tatiana Stankovicova. Morphological and functional characteristics of models of experimental myocardial injury induced by isoproterenol. Gen Physiol Biophys. 2012;31(2):141-51.
26. Chen HY, Huang JY, Siao WZ, Jong GP: The association between SGLT2 inhibitors and new-onset arrhythmias: a nationwide population-based longitudinal cohort study. Cardiovasc Diabetol 2020, 19(1):73.
27. Huo JY, Jiang WY, Chen C, Chen R, Ge TT, Chang Q, Zhu L, Geng J, Jiang ZX, Shan QJ: Effects of Angiotensin Receptor Neprilysin Inhibitors on Inducibility of Ventricular Arrhythmias in Rats with Ischemic Cardiomyopathy. Int Heart J 2019, 60(5):1168-1175.
28. de Diego C, Gonzalez-Torres L, Nunez JM, Centurion Inda R, Martin-Langerwerf DA, Sangio AD, Chochowski P, Casasnovas P, Blazquez JC, Almendral J: Effects of angiotensin-neprilysin inhibition compared to angiotensin inhibition on ventricular arrhythmias in reduced ejection fraction patients under continuous remote monitoring of implantable defibrillator devices. Heart Rhythm 2018, 15(3):395-402.
29. Grabowski M, Ozierański K, Balsam P, Dąbrowski R, Farkowski MM, Gackowski A, Jędrzejczyk-Patej E, Kalarus Z, Leszek P, Nessler J, Sterliński M, Opolski G, Przybylski A. The effect of sacubitril / valsartan on the occurrence of ventricular arrhythmia and the risk of sudden cardiac death in patients with chronic heart failure with reduced left ventricular ejection fraction. Expert opinion of the Heart Rhythm and Heart Failure Sections of the Polish Cardiac Society. Kardiol Pol. 2019;77(10):987-993.
30. Singh PK, Gari M, Choudhury S, Shukla A, Gangwar N, Garg SK. Oleic Acid Prevents Isoprenaline-Induced Cardiac Injury: Effects on Cellular Oxidative Stress, Inflammation and Histopathological Alterations. Cardiovasc Toxicol. 2020;20(1):28-48.
31. Tanriverdi LH, Parlakpinar H, Ozhan O, Ermis N, Polat A, Vardi N, Tanbek K, Yildiz A, Acet A. Inhibition of NADPH oxidase by apocynin promotes myocardial antioxidant response and prevents isoproterenol-induced myocardial oxidative stress in rats. Free Radic Res. 2017;51(9-10):772-786.
32. Liu Q, Zhang Q, Wang K, Wang S, Lu D, Li Z, Geng J, Fang P, Wang Y, Shan Q. Renal Denervation Findings on Cardiac and Renal Fibrosis in Rats with Isoproterenol Induced Cardiomyopathy. Sci Rep. 2015;5:18582.

Tables

Table 1. Echocardiography parameters and heart rate at the end of the 2nd and 6th week.

Week	Group	EF%	FS%	LAD (mm)	LVID (mm)	LVEDV (uL)	HR (bpm)
2	Control	70.17±1.66	41.04±1.67	4.01±0.07	7.86±0.08	341.8±11.03	333±6
	ISO	43.34±1.71*	28.77±1.03*	4.78±0.06*	8.33±0.1*	394.38±4.78*	355±9*
6	Control	69.37±1.37	40.01±1.97	4.22±0.08	8.17±0.19	369.7±9.31	339±7
	ISO	40.07±1.87*	26.21±1.68*	4.97±0.08*	8.81±0.12*	422.6±7.37*	377±6*
	Dapa	60.56±1.73$^{\#}$	36.04±1.72$^{\#}$	4.28±0.04$^{\#}$	8.29±0.12$^{\#}$	341.83±10.37$^{\#,\$}$	356±6$^{\#}$
	ARNI	63.58±0.97$^{\#}$	38.42±1.33$^{\#}$	4.24±0.06$^{\#}$	8.39±0.18#	382.0±6.4$^{\#}$	358±10

ISO: isoproterenol; ARNI: angiotensin receptor neprilysin inhibitors; Dapa: Dapagliflozin; EF: ejection fraction; FS: fractional shortening; LAD: left atrial diameter; LVID: left ventricular internal diameter; LVEDV: left ventricular end-diastolic volume; HR: heart rate.*P<0.05 *vs.* Control, $^{\#}$P<0.05 *vs.* ISO, $^{\$}$P<0.05 *vs.* ARNI.

Figures

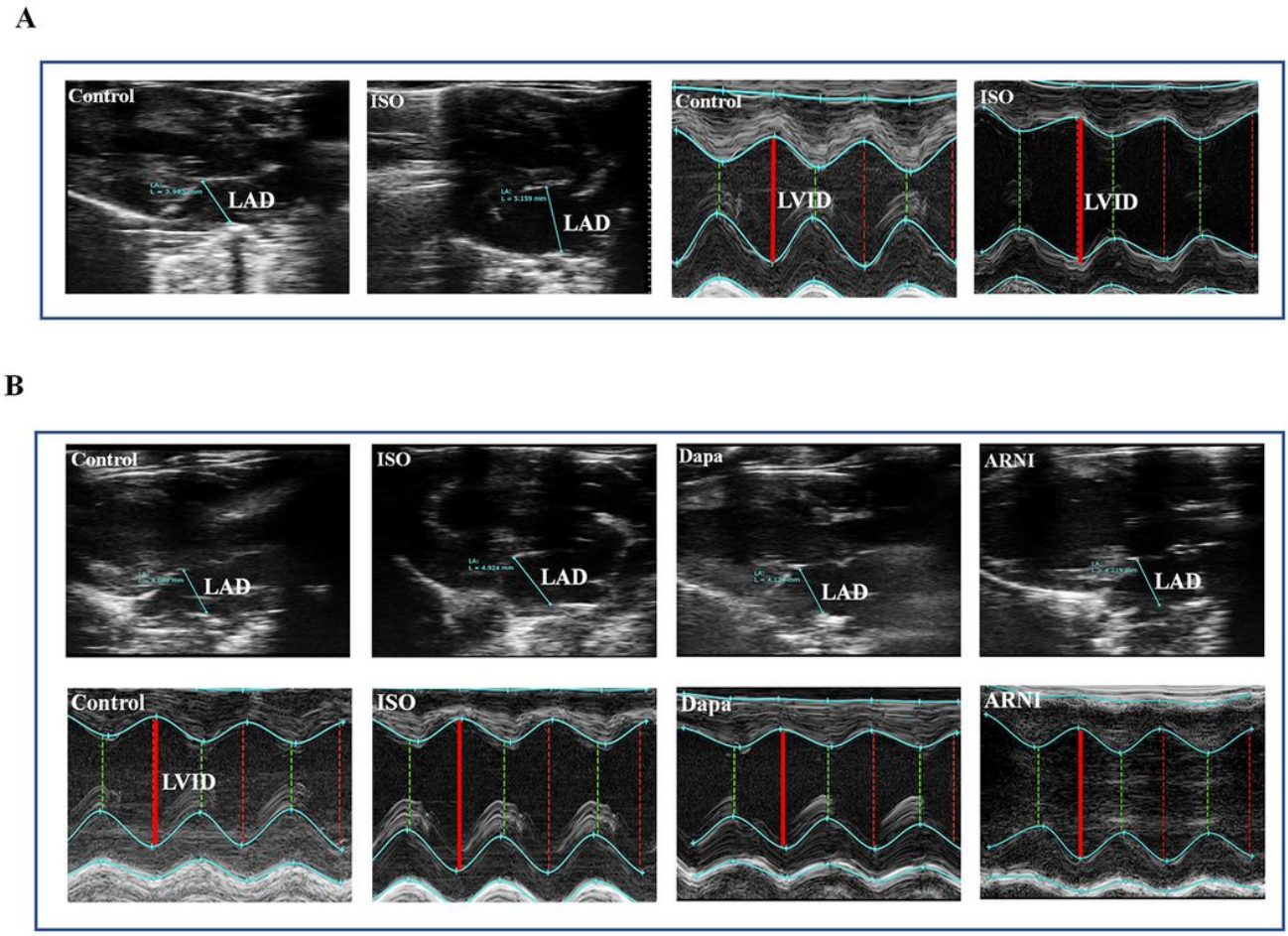
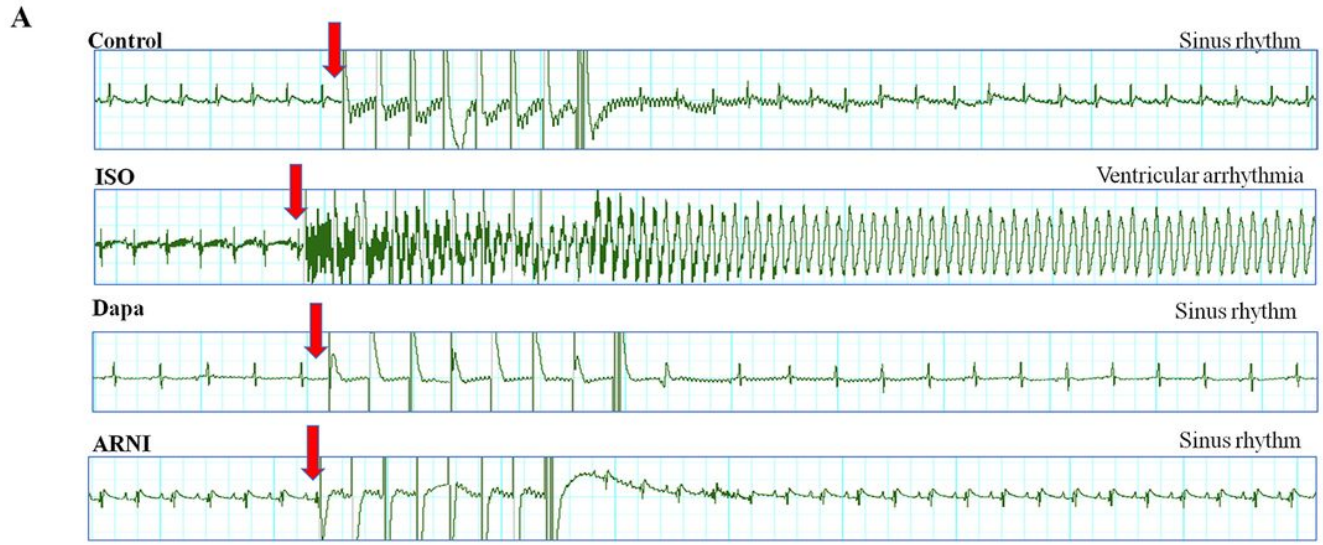


Figure 1

Figure 1

Representative tracings of echocardiography cardiac function at the end of the 2nd week in control and ISO groups and at the end of the 6th week in control, ISO, Dapa and ARNI groups. LA: left atrial diastolic diameter; LVID: left ventricular diastolic interventricular diameter; ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.



B

Group	Incidence of induced VAs	VT/VF duration (second)	Voltage (V)
Control	0/10 rats	/	3.48±0.71
ISO	5/7 rats	1.5 s; 6 s; 10.1 s; 12.5 s; 20.9 s	4.20±1.01
Dapa	0/8 rats	/	4.05±0.73
ARNI	1/8 rats	1.5 s	3.31±0.64

Figure 2

Figure 2

Representative sections showing myocardial remolding (A, black arrow) with Masson's staining with the naked eyes (A) or under the microscope (B). The quatitative myocardial fibrosis analysis was shown in Figure C. n= 3~5 rats. Values represent the means ± SEM. *P<0.05 versus control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.

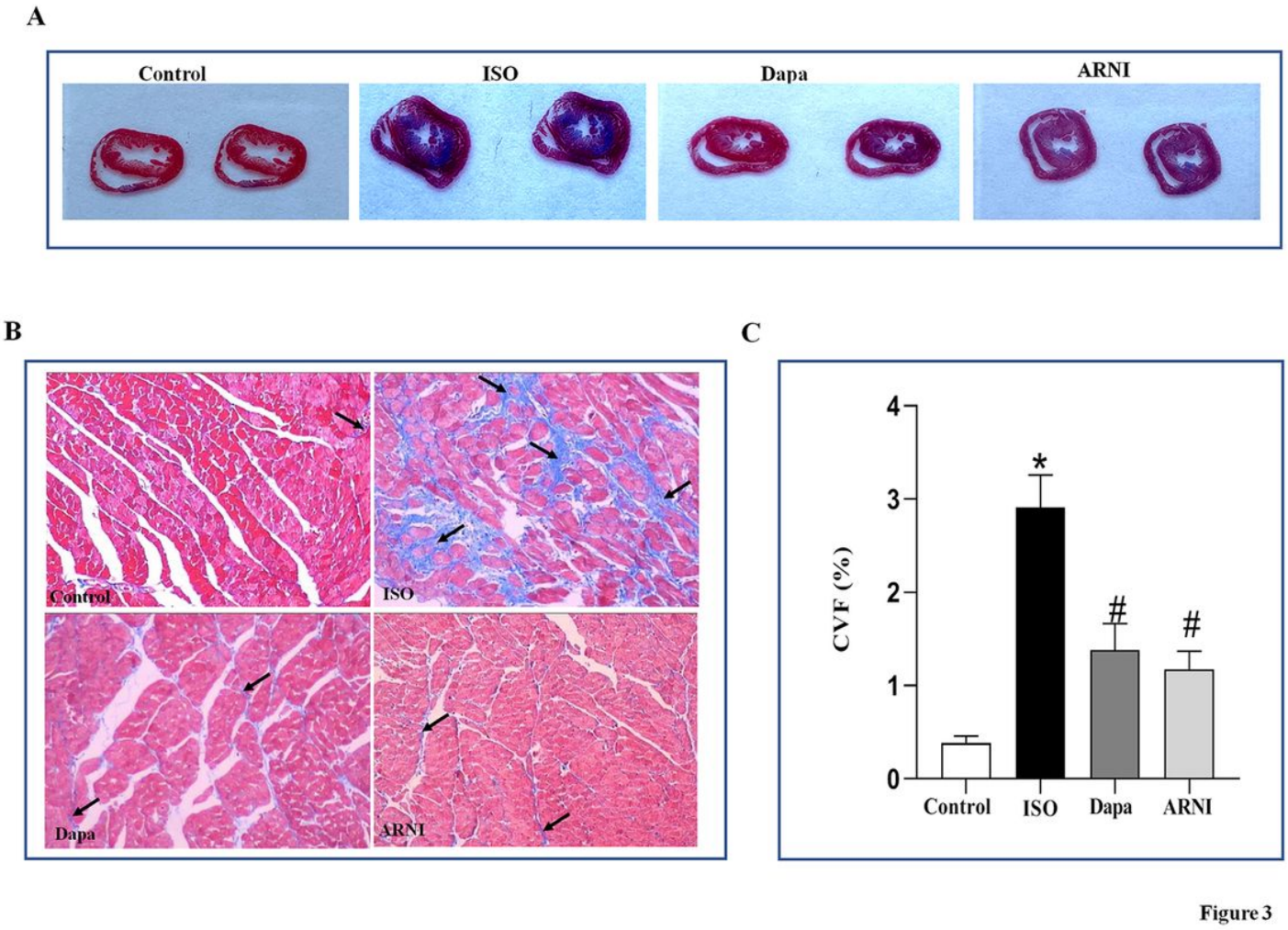


Figure 3

Figure 3

Representative recordings of electrical stimulation (red arrow, A) and the statistic incidence of pacing-induced VAs and Voltage (B) in Control, ISO, ARNI and Dapa groups. n = 7~10 rats. ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.

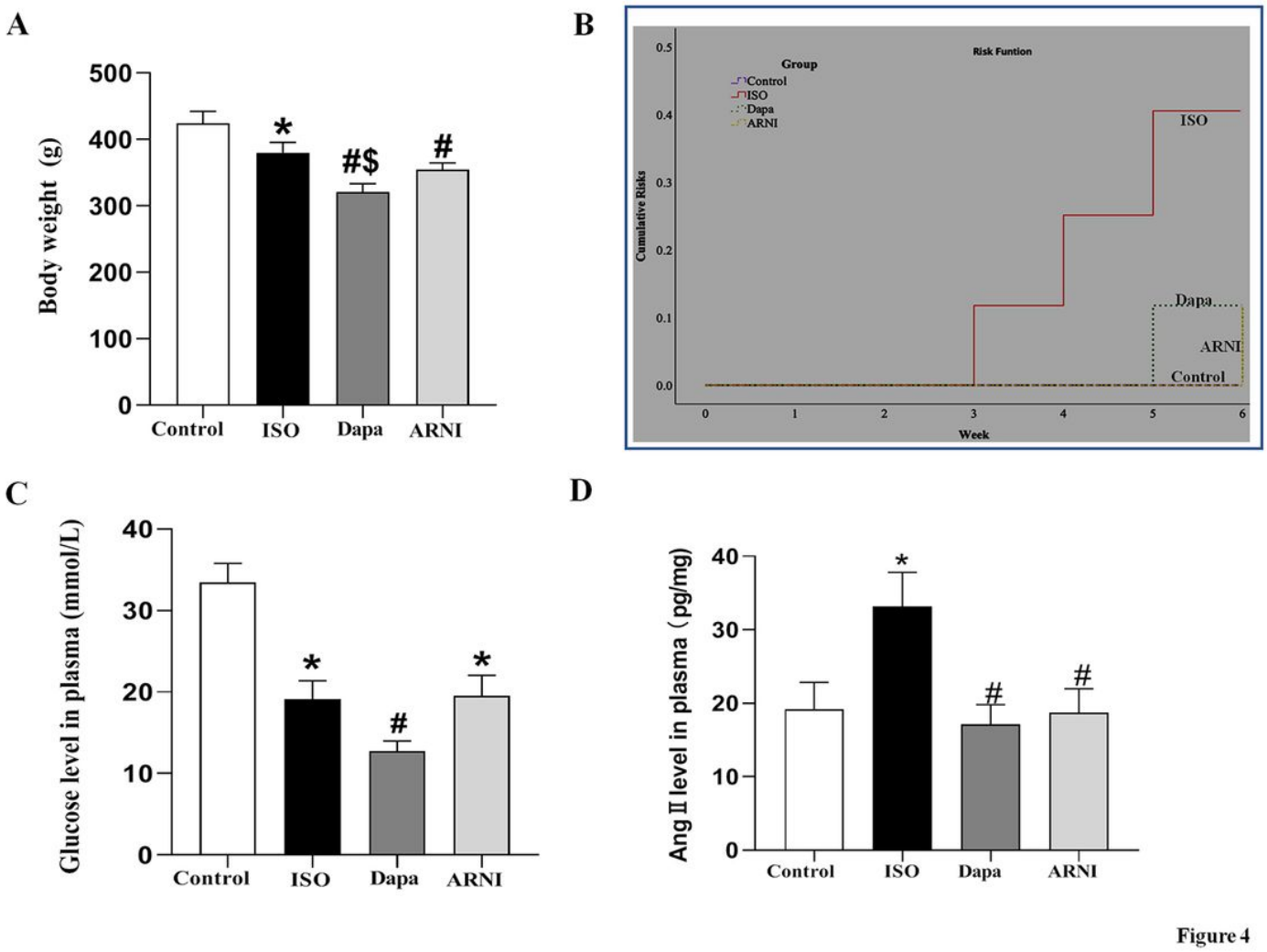


Figure 4

Body weight (A), cumulative risks analysis (B) and the levels of glucose (C) and Ang II (D) in plasma of rats at the end of the 6th week in control, ISO, Dapa and ARNI groups. n = 7~10 rats. Values represent the means ± SEM. *P<0.05 versus control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.

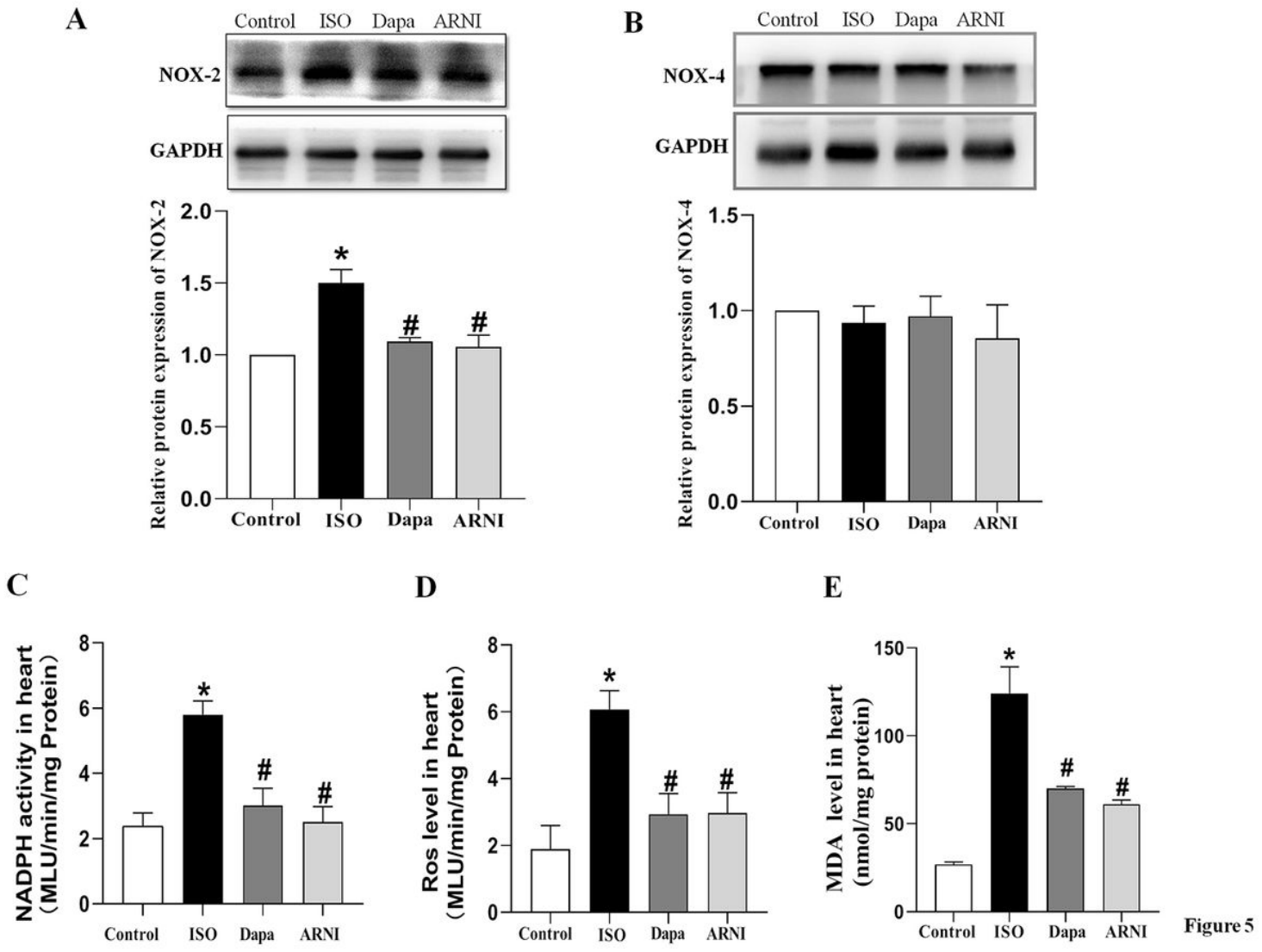


Figure 5

Figure 5

The protein expression levels of the NOX2 and NOX4 of NADPH oxidase isoforms (A, B), NADPH oxidase activity (C), ROS level (D) and MDA content (E) in the cardiac tissue of rats at the end of the 6th week in control, ISO, Dapa and ARNI groups. The values are mean ± SE; n = 4~5 for NOX2 and NOX4 (GAPDH was used as an internal control for Western blotting analysis); n = 7~10 for NADPH oxidase activity, ROS level and MDA content in each group. *P<0.05 versus control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.

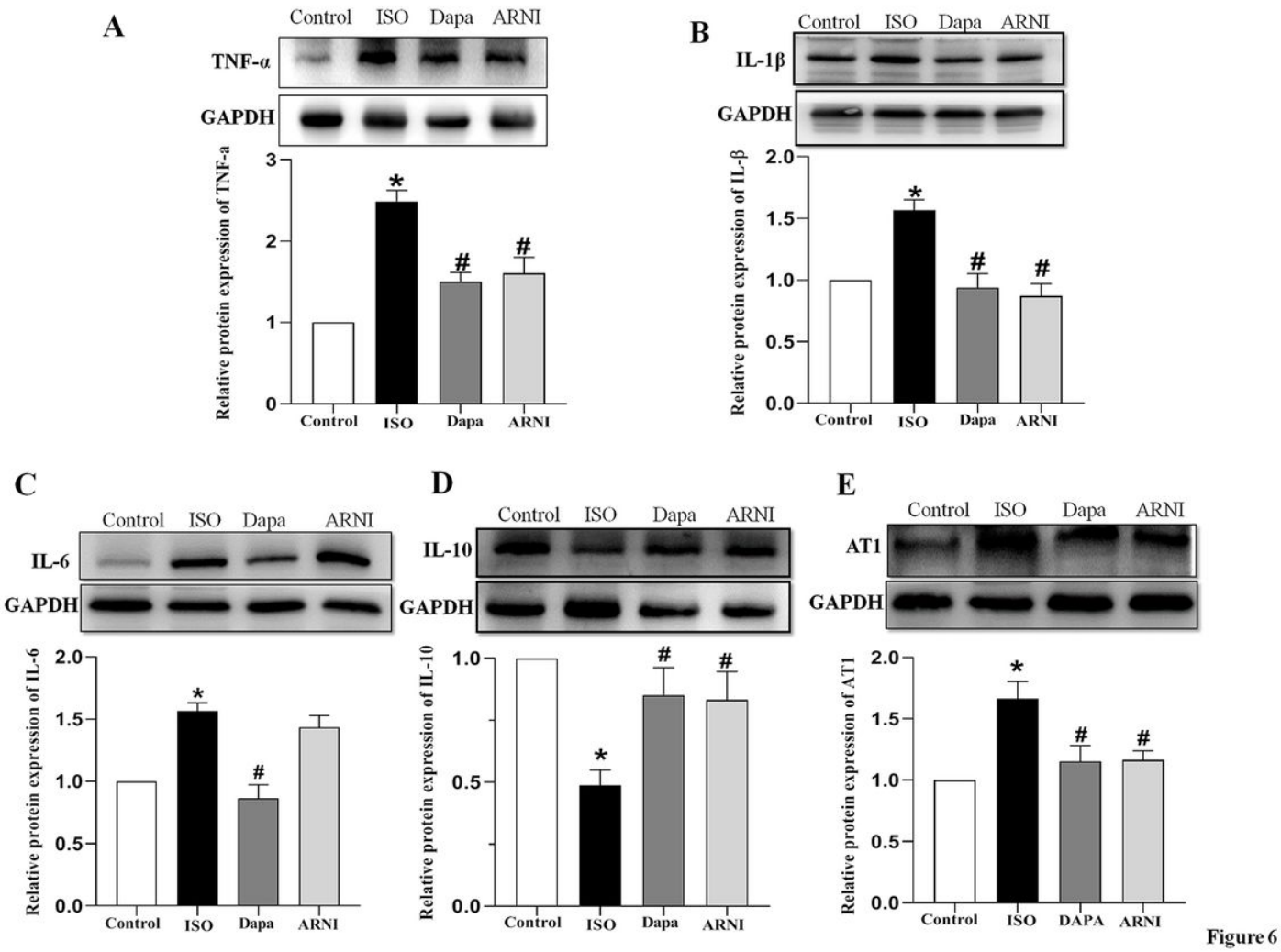


Figure 6

Figure 6

The protein expression levels of inflammatory mediators TNFα, IL-1β, IL-6 and IL-10, and AT1R in the cardiac tissue of rats at the end of the 6th week in control, ISO, Dapa and ARNI groups. GAPDH was used as an internal control for Western blotting analysis. Each value indicates mean ± SEM. n = 4~5 (A-E). *P<0.05 versus Control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: Dapagliflozin; ARNI: angiotensin receptor neprilysin inhibitor.