Am J Transl Res 2021;13(9):10950-10961
www.ajtr.org /ISSN:1943-8141/AJTR0135157

Original Article

The cardioprotective effect of the sodium-glucose cotransporter 2 inhibitor dapagliflozin in rats with isoproterenol-induced cardiomyopathy

Fang-Zheng Wang[1], Wen-Bo Wei[2], Xin Li[2], Jun-Yu Huo[3], Wan-Ying Jiang[3], Hong-Yu Wang[1], Pei Qian[1], Zhen-Zhen Li[2], Ye-Bo Zhou[1]

[1]Department of Physiology, Nanjing Medical University, Nanjing 211166, Jiangsu, China; [2]Department of Cardiology, Nanjing BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing 210021, Jiangsu, China; [3]Department of Cardiology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210006, Jiangsu, China

Received May 11, 2021; Accepted August 7, 2021; Epub September 15, 2021; Published September 30, 2021

Abstract: Sodium-glucose cotransporter 2 inhibitor (SGLT2i) has been reported to improve glycemic control. This study was designed to investigate the effects of SGLT2i dapagliflozin (dapa) on cardiomyopathy induced by isoproterenol (ISO) and its potential mechanisms. Fifty male Sprague Dawley rats were randomly assigned to the control (n=10) and the ISO (2.5 mg/kg/day)-treated groups (n=40). After 2 weeks, the 28 surviving rats with obvious left ventricular dysfunction in the ISO group were randomized into three medication groups, including the angiotensin receptor neprilysin inhibitor (ARNI) sacubitril/valsartan group (S/V, n=9), the dapa group (n=9), and the ISO group (n=10) for 4 weeks. Next, electrical programmed stimulation was performed in all the groups to evaluate their susceptibility to ventricular arrhythmias (VAs). Compared to the ISO rats, the dapa administration not only effectively reduced the cumulative risk of death, the myocardial fibrosis, the plasma angiotensin II levels and its functional receptor AT1R protein expressions in the heart, and the proinflammatory cytokine levels in the cardiac tissue of the ISO-treated rats, but it also improved their cardiac function and inhibited oxidative stress. These effects were similar to S/V. However, dapa showed a greater efficacy than S/V in reducing the left ventricular end-diastolic volumes, lowing the heart rates and VAs, and decreasing the body weights and plasma glucose levels. The mechanisms by which dapa exerts protective effects on cardiomyopathy may be related to its indirect antioxidant capacity and direct hypoglycemic action.

Keywords: Sodium-glucose cotransporter 2 inhibitors, ventricular arrhythmias, cardiac function, fibrosis, oxidative stress, inflammation

Introduction

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

Isoproterenol (ISO), a synthetic nonselective β-adrenoceptor agonist, is well accepted as a method of inducing myocardial damage in rats for evaluating cardiac dysfunction [10]. The

pathophysiological and morphological changes resulting from ISO-induced myocardial changes are similar to those observed in humans with myocardial infarction or heart failure. Therefore, ISO-induced myocardial damage is a well-standardized animal model for studying the protective effects of many drugs on cardiac dysfunction. Persistent β-adrenergic stimulation with ISO results in cardiomyocyte injury, the generation of reactive oxygen species (ROS), arrhythmias, ventricular hypertrophy and increased fibrosis, and inflammation and collagen deposition [11]. Experimental and clinical studies have shown that SGLT2i therapy can prevent or ameliorate cardiac dysfunction by inhibiting oxidative stress, inflammation, and so on [12].

This study aimed to explore the cardioprotective effects of SGLT2i dapagliflozin (dapa) on cardiomyopathy in rats induced by ISO and to compare its effects with the protective effects of ARNI S/V on the heart.

Materials and methods

Animals

The experimental animal care and use in this experiment was carried out in compliance with the *Guidelines for the Care and Use of Laboratory Animals* (National Institutes of Health publication, 8th edition, 2011). All the experimental procedures were approved by the Animal Experimental Ethics Committee of the Animal Core Facility of Nanjing Medical University (1601149-6, November 15, 2018). Fifty male Sprague-Dawley rats weighing 200-250 g were purchased from the Nanjing Medical University Laboratory Animal Center. All the rats were caged in a room with controlled temperature and humidity with a 12-h light/dark cycle and were provided a standard chow and drinking water ad libitum. The rats were randomly assigned to the control group (n=10) and the ISO induced cardiomyopathy group (ISO group, n=40). The rats in the ISO group were intraperitoneally injected with isoproterenol hydrochloride (2.5 mg/kg/day, Sigma, Switzerland) dissolved in normal saline, once a day for 2 weeks [13, 14]. Echocardiographies were performed at the end of the 2nd and 6th weeks. After the echocardiography measurement at the end of the 2nd week, the 28 surviving rats with obvious left ventricular dysfunction in the ISO group were randomized into three groups, including the ARNI S/V group (n=9), the dapa group (n=9), and the ISO group (n=10). S/V (Novartis Pharma Schweiz AG, Chinese national medicine permission number J20190001) was administered intragastrically at a dose of 68 mg/kg/day, and dapa (AstraZeneca Pharmaceuticals LP, Chinese national medicine permission number J20170040) was administered intragastrically at a dose of 3 mg/kg/day for 4 weeks, respectively. An equivalent amount of saline was administered intragastrically in the ISO group for 4 weeks. The S/V and dapa medication methods we used were based on those described in previously published articles [15-17].

Assay of cardiac function-related parameters

Echocardiography was performed at the end of the second and sixth weeks. The rats were anesthetized with ketamine, and then their 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 the rats underwent ventricular electrical programmed stimulation (EPS) to evaluate their susceptibility to ventricular arrhythmias (VAs) before they were sacrificed. They were anesthetized using intraperitoneal injections of 2% sodium pentobarbital (50 mg/kg). Three needle electrodes were placed on their right upper limbs and legs to perform the electrocardiography. Then EPS was used to stimulate the left ventricular apex of the heart through a bipolar electrode, and the incidence of ventricular arrhythmias (VAs) was investigated. By a cycle length of 140 ms, the threshold potential for stable pacing was achieved. The pacing was started at twice as much as the threshold and a cycle length of 140 ms, which was the interval of the eight stimuli (Stimulation 1). An extra stimulus (Stimulation 2) was administered until it failed to induce ventricular depolarization, while the interval between Stimulations 1 and 2 was progressively shortened by 10 ms.

Samples and histopathology

The animals were sacrificed immediately after the EPS. Blood was collected from their aortas. After they were weighed and washed with ice-cold PBS, one part of each of their hearts was cut and fast frozen using liquid nitrogen, then they were brought down to -80°C for further measurements. The other parts were fixed in

4% paraformaldehyde, and were used for staining. Masson's trichrome staining was performed to determine the cardiac fibrosis levels. Five fields from each sample were randomly selected, and the collagen volume fractions (CVF) were assessed using Image-Pro Plus 6.0.

Measurement of the plasma Ang II

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

Measurement of the plasma glucose and cardiac malondialdehyde (MDA) levels

The plasma glucose levels were measured using the glucose-oxidase method with commercially available glucose assay kits from Jiancheng Bioengineering (Nanjing, Jiangsu, China). The MDA levels in the heart tissues were measured using lipid peroxidation assay kits from Jiancheng Bioengineering (Nanjing, Jiangsu, China). The lipid peroxidation (LPO) levels were determined by measuring the reaction of MDA with thiobarbituric acid (TBA) to form a colorimetric product, proportional to the MDA present. The intensities of the colors were measured spectrophotometrically at 505 nm for glucose and 532 nm for MDA.

Measurement of the superoxide anions

The lucigenin-derived chemiluminescence method was used to examine superoxide anions levels 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 minutes using 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 the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity

The enhanced lucigenin chemiluminescence method was used to measure the 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 is expressed as the (MLU) per minute per milligram of protein [19].

Western blotting

The protein expressions of Ang 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 the inflammatory markers including TNFα, IL-1β, IL-6, and IL-10 (antibodies from Proteintech, Chicago, IL, USA) in the myocardial tissue were determined using Western blotting [20]. Briefly, the total cardiac proteins in the homogenate were extracted and measured. The antibodies AT1, NOX2, NOX4, TNFα, IL-1β, IL-6, and IL-10 were used according to the manufacturer's instructions. Horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG were used as a secondary antibody. The protein expression levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH, antibody from Proteintech, Chicago, IL, USA). The signals were quantified using an Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE). The uncropped western blot results of the NAD(P)H oxidase subunits (NOX-2 and NOX-4), the inflammatory mediators TNFα, IL-1β, IL-6, and IL-10, AT1R and GAPDH in the rats' cardiac tissue at the end of the 6th week in the control, ISO, Dapa, and S/V groups were shown in Figures S1 and S2.

Kaplan-Meier analysis

The survival rate over the 6-week experiment was analyzed according to the daily recording of the deaths using a standard Kaplan-Meier analysis with a log rank test.

Statistical analysis

The survival rate over the 6-week experiment was analyzed using a standard Kaplan-Meier analysis with a log rank test. The data are expressed as the mean ± SEM and analyzed using GraphPad Prism v. 8.0.2 (GraphPad Software, CA). The comparisons between the two groups were done using two-tailed unpaired t tests, such as the echocardiography parame-

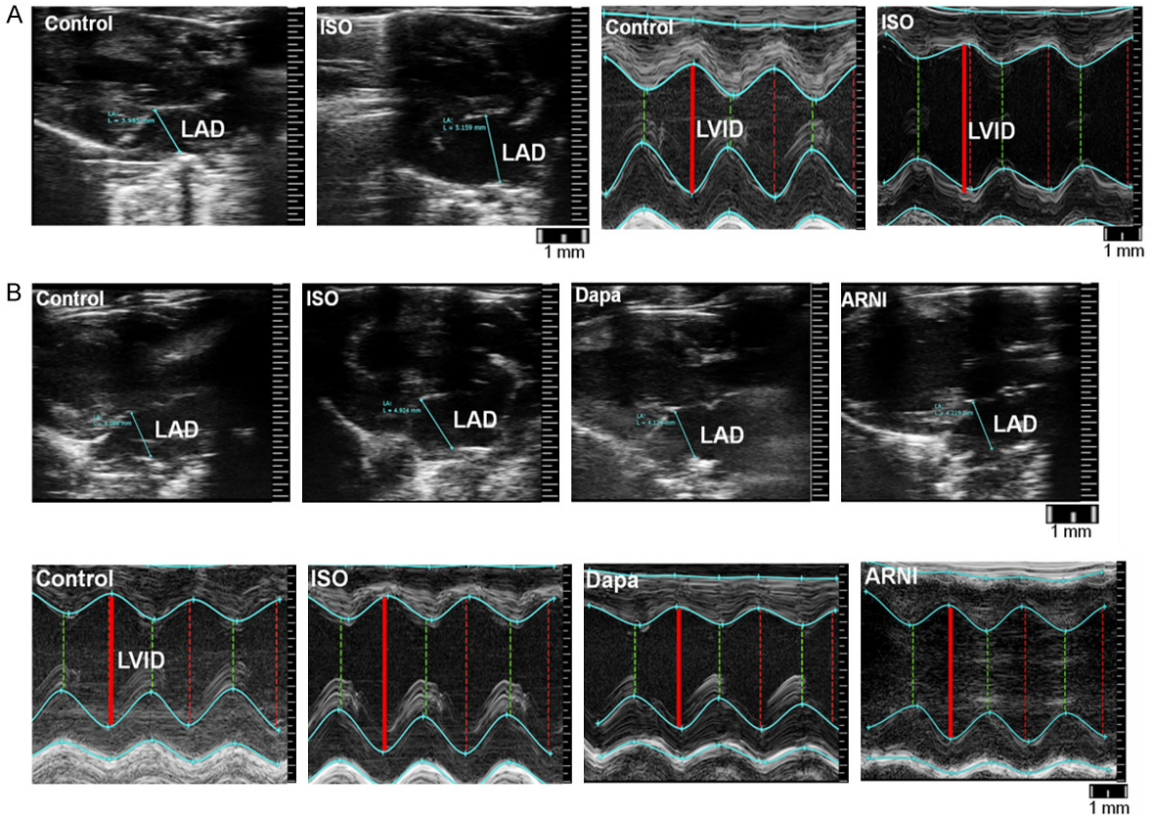


Figure 1. Representative tracings of the echocardiographic cardiac function at the end of the second week in the control and ISO groups (A) and at the end of the sixth week in the control, ISO, dapa, and S/V groups (B). LA: left atrial diastolic diameter; LVID: left ventricular diastolic interventricular diameter; ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan.

ters and the heart rates at the end of the 2nd week. For the multiple-group comparisons, including the quantitative myocardial fibrosis analysis (CVF%), the body weight, and the plasma glucose and Ang II levels, western blot, NADPH oxidase activity, and the ROS and MDA levels in the cardiac tissues, one-way ANOVA followed by Bonferroni's post-hoc tests were used. A value of $P<0.05$ was considered statistically significant.

Results

Dapa ameliorated the ISO-induced cardiac dysfunction in the ISO-treated rats

At the end of the second week, the echocardiography showed significant increases in the diastolic left atrial diameters and the left ventricular interventricular diameters as shown by the representative tracings of the echocardiography (**Figure 1A**), and a reduction in the ejection fractions (EF) and the fractional shortening (FS) levels in the ISO group compared with the control group (P<0.05, **Table 1**). The left ventricular end-diastolic volumes (LVEDV) were increased in the 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, the EF and FS levels were notably increased in the dapa and S/V groups compared with the ISO group (P<0.05, **Table 1**), while the increases in LAD and LVID were significantly improved in the S/V and dapa groups compared with the ISO group (**Figure 1B**). But the LVEDV and heart rates in the dapa group were lower than they were in the S/V group (P<0.05, **Table 1**).

Dapa inhibited the occurrence of ventricular arrhythmias in the ISO-treated rats

EPS was performed in all groups in order to induce VAs. The original electrocardiography images are shown in **Figure 2A**. The mean voltage levels of all the groups were similar. The incidences of pacing-induced VAs in the S/V and dapa groups were greatly reduced compared with the ISO group (**Table 2**). It seemed

Table 1. The echocardiographic parameters and heart rates at the end of the second and sixth weeks

Group	EF%	FS%	LAD (mm)	LVID (mm)	LVEDV (uL)	HR (bpm)
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*
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#	341.8±10.37#,$	356±6#
S/V	63.58±0.97#	38.42±1.33#	4.24±0.06#	8.39±0.18#	382.0±6.42#	358±10

ISO: isoproterenol; S/V: sacubitril/valsartan; 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. S/V.

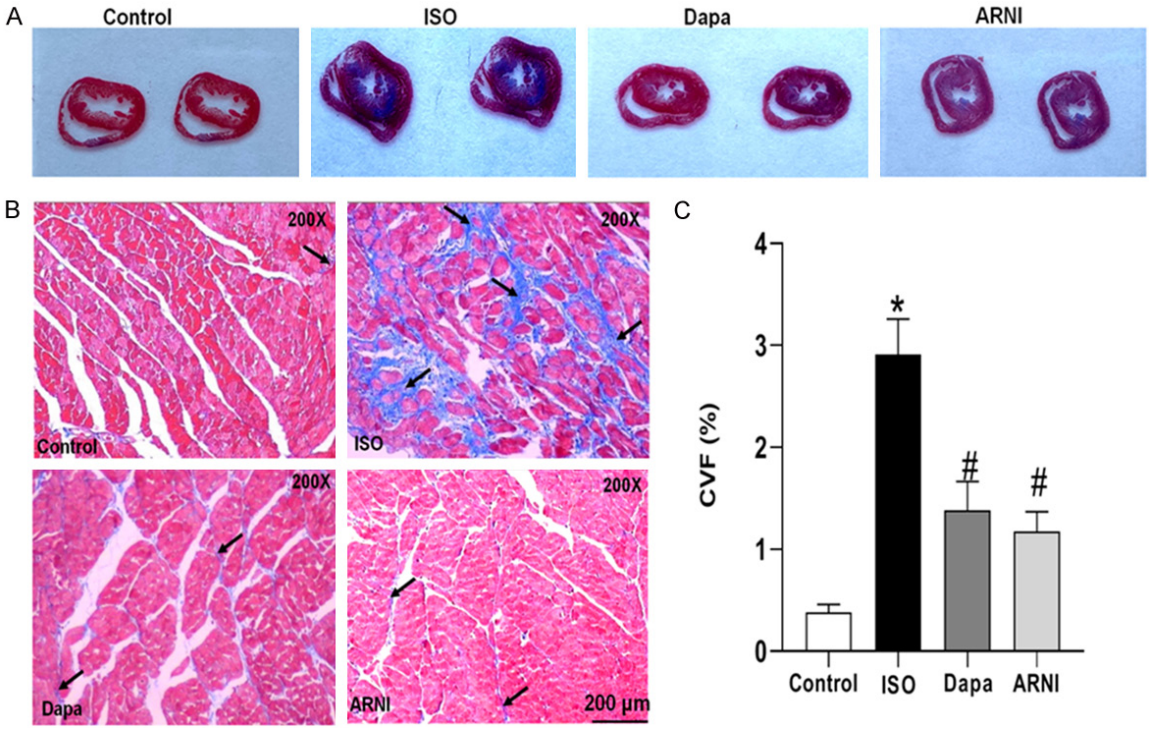


Figure 2. Representative sections showing the myocardial remolding (A, B) and the quantitative myocardial fibrosis analysis (C). (A) Masson's staining using the naked eye; (B) observation under a microscope (200×); (C) The quantitative myocardial fibrosis results. n=3~5 rats. The values represent the means ± SEM. *P<0.05 versus the control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan. CVF: collagen volume fraction.

that this effect was more effective in the dapa group than in the S/V group.

Dapa improved cardiac remodeling in the ISO-treated rats

Cardiac fibrosis is well known to increase ventricular stiffness, leading to diastolic dysfunction. In this study, the collagen volume fraction (CVF), a critical method of assessing organic fibrosis, was evaluated using Masson's Trichrome Staining with sections taken from the rats' hearts. The ISO treatment for 6 weeks significantly resulted in increased myocardial fibrosis. However, the myocardial interstitial fibrosis was significantly improved using the S/V or dapa medication for 4 weeks (P<0.05, **Figure 3**).

Dapa reduced the body weights, the cumulative risk, and the plasma glucose and Ang II levels in the ISO-treated rats

After 6 weeks of ISO treatment, the body weights and the plasma glucose levels were significantly decreased (P<0.05, **Figure 4A**,

Table 2. The incidences of the pacing-induced VAs and the voltages in the control, ISO, dapa, and S/V groups

Group	Incidence of induced VAs	VT/VF duration	Voltage
Control	0/10	/	3.48±0.71
ISO	5/7	1.5S; 6S; 10.1S; 12.5S; 20.9S	4.20±1.01*
Dapa	0/8	/	4.05±0.73#
S/V	1/8	1.5S	3.31±0.64#

ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan; VAs: ventricular arrhythmias; VT: ventricular tachycardia; VF: ventricular fibrillation. n=7~10 rats. *P<0.05 versus the control; #P<0.05 versus ISO.

4C), but the plasma Ang II levels (P<0.05, **Figure 4D**) were significantly increased in the ISO-treated rats when compared with the control rats. The risk-function was analyzed according to the daily recording of deaths for 6 weeks using the Kaplan-Meier analysis. Three rats died in the ISO-treated group, but only one died in the dapa and S/V groups. None died in the control group (P<0.05, **Figure 4B**). The S/V and dapa both reduced the Ang II levels and the cumulative risks of the ISO-induced rats (P<0.05, **Figure 4B**, **4D**). However, dapa further significantly reduced the body weights and the plasma glucose levels when compared with the ISO and S/V groups (P<0.05, **Figure 4A**, **4C**).

Dapa inhibited cardiac oxidative stress in the ISO-treated rats

ISO-induced cardiotoxicity is assumed to generate highly cytotoxic free radicals in the myocytes, which causes oxidative stress involving structural and functional myocardial damage. Cytotoxic free radicals can be generated using activated NADPH oxidase, increased levels of Ang II, proinflammatory cytokines, and so on. In this study, the rats in the ISO group revealed significant alterations in their cardiac oxidative stress levels including increases in their ROS levels, their NADPH oxidase activity, and the main isoform NOX2 of NADPH oxidase, but not the NOX4 protein expressions (**Figure 5B**) in the heart when compared to the control group, which were significantly reduced by the treatment with dapa and S/V when compared to the ISO-treated group (P<0.05, **Figure 5A**, **5C**, **5D**). Cytotoxic free radicals can cause LPO of the intramembranous polyunsaturated fatty acids in the membranes. MDA, an important LPO byproduct, was significantly increased in the cardiac tissue in the ISO-treated rats when compared to the control rats. The dapa and S/V treatment significantly decreased the MDA levels when compared to the ISO-treated group (P< 0.05, **Figure 5E**).

Dapa inhibited the inflammation and AT1R protein expression in the ISO-treated rats

Inflammation is the key mediator for myofibroblast formation and collagen deposition, and it leads to cardiac fibrosis [21]. ISO action is partially mediated by inflammation through the activation of the β1-adrenergic receptors in the heart [22]. In this study, the rats in the ISO group experienced significant cardiac inflammation as shown by the increases in the inflammatory factors, including TNFα, IL-1β, and IL-6 compared to the control group. However, the dapa treatment significantly reduced the myocardial TNFα, IL-1β, and IL-6 protein levels (P<0.05, **Figure 6A-C**). The S/V treatment markedly decreased the myocardial TNFα and IL-1β protein levels, but not the IL-6 levels (**Figure 6C**). Dapa and S/V both significantly up-regulated the protein levels of anti-inflammatory cytokine IL-10 (P<0.05, **Figure 6D**). AT1R serves as a major mediator of Ang II effects, including the fibrogenic effect and ROS production [23]. In the ISO-treated rats, the cardiac AT1R protein level was higher than it was in the control rats. However, both dapa and S/V downregulated the cardiac protein expressions of AT1R (P<0.05, **Figure 6E**), and this may attenuate the pathogenic effects of Ang II via AT1R on the heart.

Discussion

Dapa, a selective inhibitor of SGLT2, is widely used to treat type 2 diabetes and works by promoting an increase in the glucose excretions in the urine. In addition to its special glycemic effect, there are many other benefits of dapa such as weight loss, a slowdown in cardiovascular disease progression, and so on [1-4]. In this study, we evaluated the influence of the SGLT2 inhibitor dapa on the cardiac remodeling function, and on VAs and oxidative stress in rats with cardiomyopathy induced using ISO. We observed that dapa has obvious cardiovascular protective roles like S/V in the ISO-treated rats: 1) Dapa and S/V both effectively improved

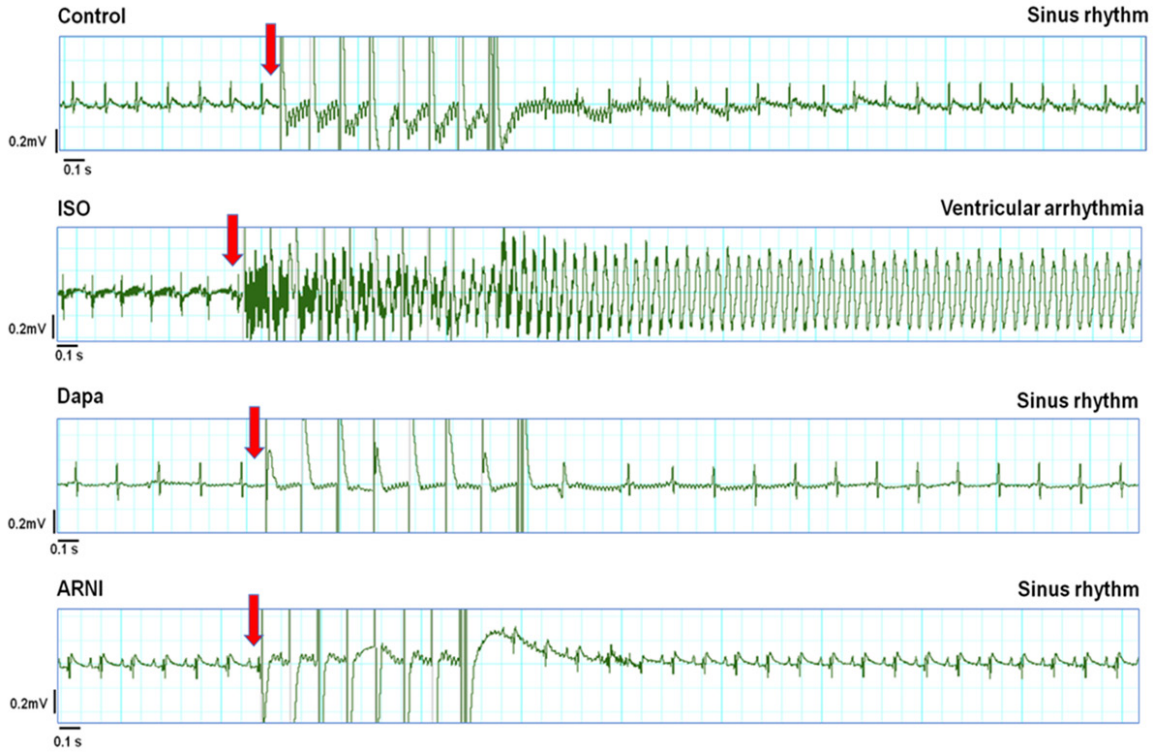


Figure 3. Representative recordings of the electrical stimulations (red arrow) in the control, ISO, dapa, and S/V groups. n=7~10 rats. ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan.

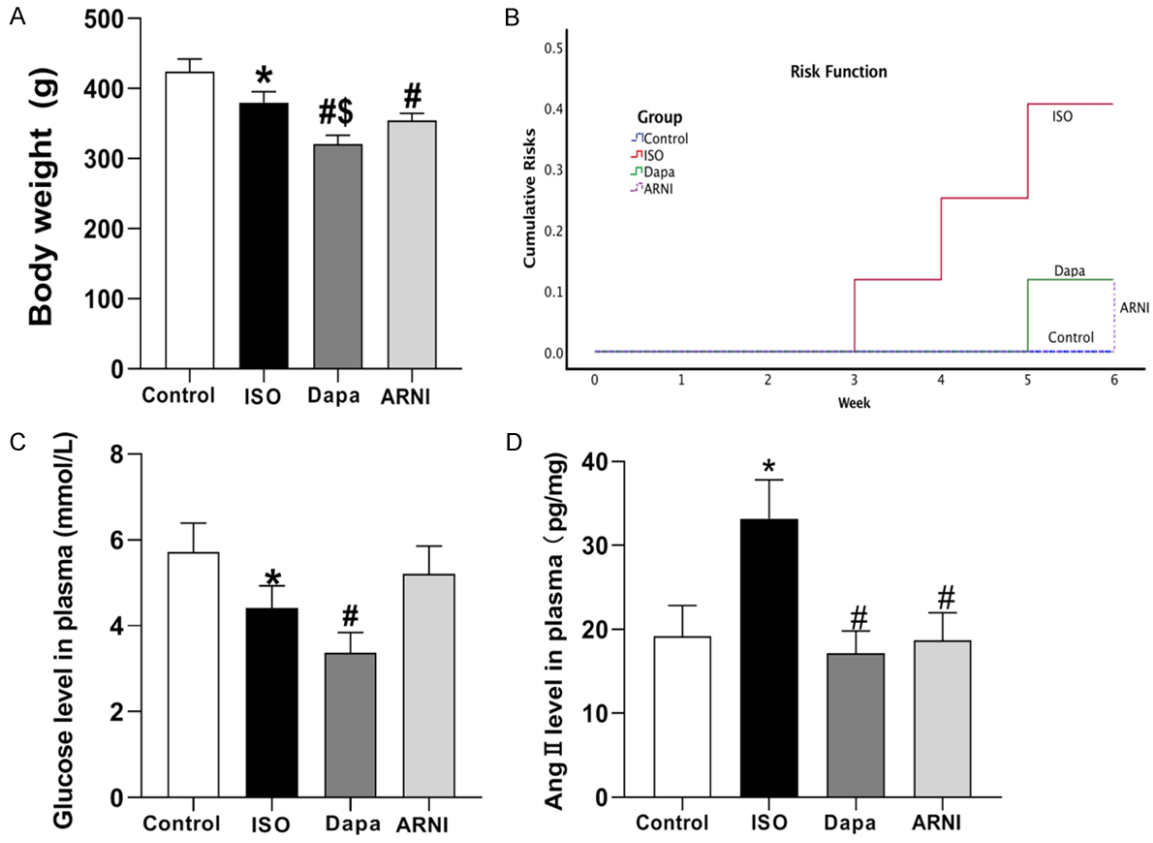


Figure 4. Body weight (A), cumulative risk analysis (B) and the glucose (C) and Ang II levels (D) in rats' plasma at the end of the sixth week in the control, ISO, dapa, and S/V groups. n=7~10 rats. The values represent the means ± SEM. *P<0.05 versus the control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan.

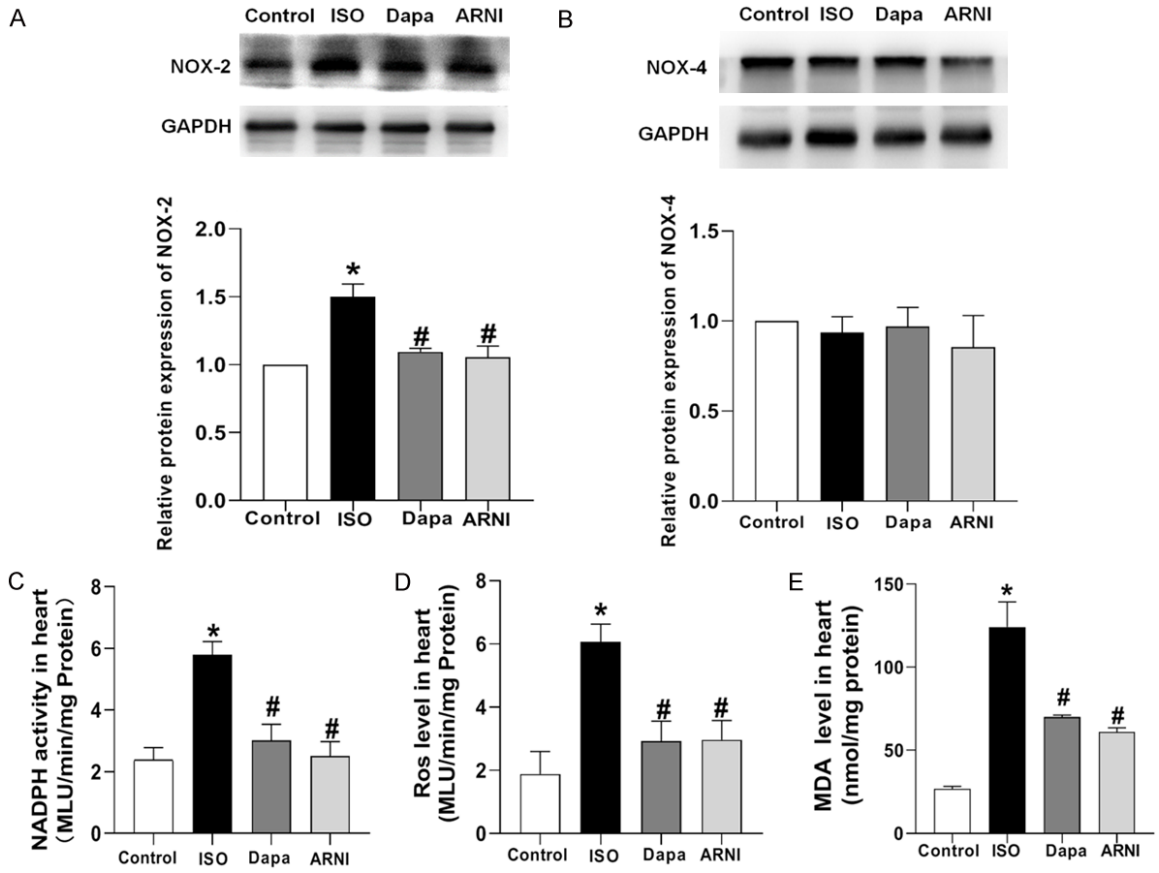


Figure 5. The protein expression levels of NOX2 and NOX4 of the NADPH oxidase isoforms (A, B), NADPH oxidase activity (C), the ROS levels (D) and the MDA content (E) in the rats' cardiac tissues at the end of the sixth week in the control, ISO, dapa, and S/V groups. The values are presented as the means ± SE; n=4~5 for NOX2 and NOX4 (GAPDH was used as an internal control for the Western blotting analysis); n=7~10 for the NADPH oxidase activity, the ROS levels, and the MDA content in each group. *P<0.05 versus the control group; #P<0.05 versus the ISO group. ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan.

the cardiac fibrosis and dysfunction, but the increase in the left ventricular end-diastolic volume induced by the ISO was more markedly improved by dapa than S/V; 2) Dapa reduced the incidence of pacing-induced VAs that had an effective tendency when compared to S/V, and more effectively decreased the heart rates and body weights than S/V; 3) Dapa and S/V both decreased the cumulative risk of death and ameliorated the cardiac oxidative stress levels such as the decreases in the ROS levels and the MDA content in the heart. Dapa may have strong cardiac protective effects like S/V on the ISO-induced cardiomyopathy.

In recent years, many previous studies have focused on the effects of SGLT2i on 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 the β1-adrenergic receptors that are 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 models for chronic cardiac injury. Hung-Yi Chen

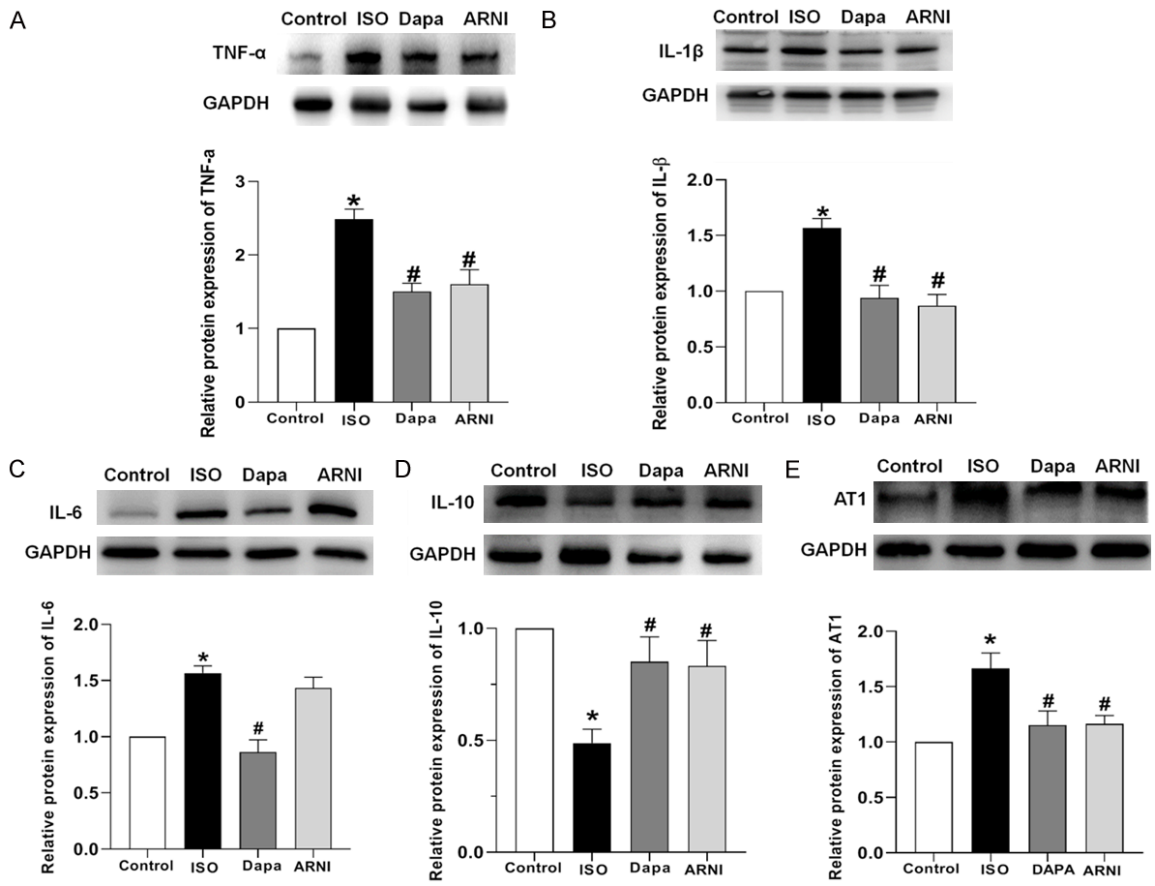


Figure 6. The protein expression levels of the inflammatory mediators TNFα (A), IL-1β (B), IL-6 (C) and IL-10 (D), and AT1R (E) in the rats' cardiac tissue at the end of the sixth week in the control, ISO, dapa, and S/V groups. GAPDH was used as internal control for the Western blotting analysis. The values were presented as the means ± SEM. n=4~5 (A-E). *P<0.05 versus the control; #P<0.05 versus ISO. ISO: isoproterenol; Dapa: dapagliflozin; S/V: sacubitril/valsartan.

et al. [9] reported that patients prescribed with SGLT2i are 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 roles of dapa in VAs in ISO-treated rats. As is well known, S/V is commonly used in clinical treatment of heart failure. For instance, the effect of S/V on VA prevention has been widely reported [17, 26, 27], but not SGLT2i. Therefore, we also used S/V as a reference to compare the effects of dapa in our present study. ISO-induced alterations, including cardiac dysfunction, cardiac fibrosis and an increase in VAs were found in this study, and they were effectively improved by the application of dapa or S/V. Moreover, dapa was more effective at reducing LVEDV, VAs, the heart rate, and body weight than S/V. In this study, the reductions in LVEDV and body weight from our results indicated reductions in the cardiac preload and afterload, which suggested that dapa may play an important role in improving the diastolic heart function and mitigating ventricular loading. The reason for the weight loss may be related to its hypoglycemic action. Moreover, the decreases in the VAs and the heart rates indicated that the use of dapa may have a greater potential to reduce the risk of ventricular arrhythmias. Therefore, the pharmacological intervention of dapa to ameliorate ISO induced cardiac abnormalities may have a potential therapeutic value in preventing the initiation and progression of cardiomyopathy in obese patients characterized by heart failure and high blood glucose levels.

ISO-induced cardiotoxicity is highly associated with cytotoxic free radicals in myocytes, which causes oxidative stress leading to inflammation, structural and functional myocardial dam-

age, and so on [28]. Oxidative stress is due to massive reactive oxygen species (ROS) and imbalanced antioxidant defense mechanisms. ROS can be generated using an activated NADPH oxidase, increased Ang II, proinflammatory cytokines, and so on [29, 30]. These major factors for promoting ROS generation were investigated in this study. Indeed, the application of ISO produced significant increases in the ROS levels, the NADPH oxidase activity, and the proinflammatory cytokine production. Moreover, the Ang II levels in the plasma and the functional receptor AT1R protein expression in the heart, and the oxidative stress-caused LPO production of MDA content in the cardiac tissue were evidently higher than they were in the control group. However, these adverse alterations were effectively reduced by the administration of dapa or S/V. These results indicate that treatment with dapa or S/V significantly attenuates oxidative stress in cardiac tissue as shown by the decreases in the ROS levels and the MDA content, which may occur through a reduction of the NADPH oxidase activity, the AT1R protein levels, and the production of proinflammatory cytokines in the heart. These results also reveal that dapa has antioxidant properties through its protecting cardiac muscle from ISO-mediated oxidative damage.

Obesity is recognized as a cardiovascular risk factor, and the worldwide epidemic of obesity parallels the one observed for heart failure [31, 32]. In addition, obese patients have higher glucose levels, and this may result in oxidative stress and inflammation. Reciprocally, higher glucose levels have been implicated in the development and maintenance of obesity. In this study, we found that dapa not only effectively improved heart failure induced using ISO, but it also reduced the glucose level and body weight. Therefore, dapa may have a more promising clinical application in obese patients.

In conclusion, the protective effects of dapa observed in this study may be due to its potent antioxidant properties, which protect cardiac tissue from oxidative damage and help to maintain 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 in obese patients. However, the potent protective mechanisms of dapa in ISO-induced cardiotoxicity need to be further explored.

Acknowledgements

We gratefully acknowledge the generous support of the Collaborative Innovation Center for Cardiovascular Disease Translational Medicine. This work was supported by the National Natural Science Foundation of China (81970356) and the Science and Education Development Fund of Nanjing Medical University (NMUB-2018186).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhen-Zhen Li, Department of Cardiology, Nanjing BenQ Medical Center, The Affiliated BenQ Hospital of Nanjing Medical University, Nanjing 210021, Jiangsu, China. Tel: +86-17705145276; Fax: +86-25-66862279; E-mail: lizhen.8901@163.com; Dr. Ye-Bo Zhou, Department of Physiology, Nanjing Medical University, 101 Longmian Road, Nanjing 211166, Jinagsu, China. Tel: +86-25-86862885; Fax: +86-25-8686-2885; E-mail: zhouyebo666@njmu.edu.cn

References

[1] Spigoni V, Fantuzzi F, Carubbi C, Pozzi G, Masselli E, Gobbi G, Solini A, Bonadonna RC and 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: 46.

[2] Hodrea J, Balogh DB, Hosszu A, Lenart L, Besztercei B, Koszegi S, Sparding N, Genovese F, Wagner LJ, Szabo AJ and 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: F1017-F1029.

[3] McMurray JJV, DeMets DL, Inzucchi SE, Kober L, Kosiborod MN, Langkilde AM, Martinez FA, Bengtsson O, Ponikowski P, Sabatine MS, Sjöstrand M and Solomon SD. A trial to evaluate the effect of the sodium-glucose co-transporter 2 inhibitor dapagliflozin on morbidity and mortality in patients with heart failure and reduced left ventricular ejection fraction (DAPA-HF). Eur J Heart Fail 2019; 21: 665-675.

[4] Maranghi M, Carnovale A, Durante C, Tarquini G, Tiseo G and Filetti S. Pharmacokinetics, pharmacodynamics and clinical efficacy of dapagliflozin for the treatment of type 2 diabetes. Expert Opin Drug Metab Toxicol 2015; 11: 125-137.

[5] Clegg LE, Penland RC, Bachina S, Boulton DW, Thuresson M, Heerspink HJL, Gustavson S, Sjostrom CD, Ruggles JA, Hernandez AF, Buse JB, Mentz RJ and 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: 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 and 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: 471-482.

[7] Khan MS and Vaduganathan M. What makes sodium-glucose Co-transporter-2 inhibitors stand out in heart failure? Curr Diab Rep 2020; 20: 63.

[8] Rosano G, Quek D and Martinez 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 and Jong GP. The association between SGLT2 inhibitors and new-onset arrhythmias: a nationwide population-based longitudinal cohort study. Cardiovasc Diabetol 2020; 19: 73.

[10] Govindasami S, Uddandrao VVS, Raveendran N and Sasikumar V. Therapeutic potential of Biochanin-A against isoproterenol-induced myocardial infarction in rats. Cardiovasc Hematol Agents Med Chem 2020; 18: 31-36.

[11] Jannesar K, Abbaszadeh S, Malekinejad H and Soraya H. Cardioprotective effects of memantine in myocardial ischemia: ex vivo and in vivo studies. Eur J Pharmacol 2020; 882: 173277.

[12] Zelniker TA and Braunwald E. Mechanisms of cardiorenal effects of sodium-glucose cotransporter 2 inhibitors: JACC state-of-the-art review. J Am Coll Cardiol 2020; 75: 422-434.

[13] Zheng A, Yi H, Li F, Han L, Yu J, Cheng X, Su H, Hong K and Li J. Changes in gut microbiome structure and function of rats with isoproterenol-induced heart failure. Int Heart J 2019; 60: 1176-1183.

[14] Simko F, Bednarova KR, Krajcirovicova K, Hrenak J, Celec P, Kamodyova N, Gajdosechova L, Zorad S and Adamcova M. Melatonin reduces cardiac remodeling and improves survival in rats with isoproterenol-induced heart failure. J Pineal Res 2014; 57: 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 and Dole WP. Pharmacokinetics and pharmacodynamics of LCZ696, a novel dual-acting angiotensin receptor-neprilysin inhibitor (ARNi). J Clin Pharmacol 2010; 50: 401-414.

[16] Thomson SC, Rieg T, Miracle C, Mansoury H, Whaley J, Vallon V and 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: R75-83.

[17] Huo JY, Jiang WY, Chen C, Chen R, Ge TT, Chang Q, Zhu L, Geng J, Jiang ZX and Shan QJ. Effects of angiotensin receptor neprilysin inhibitors on inducibility of ventricular arrhythmias in rats with ischemic cardiomyopathy. Int Heart J 2019; 60: 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 and Zhou YB. Adipose afferent reflex is enhanced by TNFalpha in paraventricular nucleus through NADPH oxidase-dependent ROS generation in obesity-related hypertensive rats. J Transl Med 2019; 17: 256.

[19] Sun HJ, Zhou H, Feng XM, Gao Q, Ding L, Tang CS, Zhu GQ and Zhou YB. Superoxide anions in the paraventricular nucleus mediate cardiac sympathetic afferent reflex in insulin resistance rats. Acta Physiol (Oxf) 2014; 212: 267-282.

[20] Kang Y, Ding L, Dai H, Wang F, Zhou H, Gao Q, Xiong X, Zhang F, Song T, Yuan Y, Zhu GQ and Zhou YB. 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: 4217

[21] Boarescu PM, Chirila I, Bulboaca AE, Bocsan IC, Pop RM, Gheban D and Bolboaca SD. Effects of curcumin nanoparticles in isoproterenol-induced myocardial infarction. Oxid Med Cell Longev 2019; 2019: 7847142.

[22] Attalla DM, Ahmed LA, Zaki HF and 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] Meyers TA, Heitzman JA, Krebsbach AM, Aufdembrink LM, Hughes R, Bartolomucci A and Townsend D. Acute AT1R 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 and Birnbaum Y. SGLT-2 inhibition with dapa-

gliflozin 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: 119-132.

[25] Nichtova Z, Novotova M, Kralova E and Stankovicova T. Morphological and functional characteristics of models of experimental myocardial injury induced by isoproterenol. Gen Physiol Biophys 2012; 31: 141-151.

[26] de Diego C, Gonzalez-Torres L, Nunez JM, Centurion Inda R, Martin-Langerwerf DA, Sangio AD, Chochowski P, Casasnovas P, Blazquez JC and 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: 395-402.

[27] Grabowski M, Ozieranski K, Balsam P, Dabrowski R, Farkowski MM, Gackowski A, Jedrzejczyk-Patej E, Kalarus Z, Leszek P, Nessler J, Sterliński M, Opolski G and 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: 987-993.

[28] Singh PK, Gari M, Choudhury S, Shukla A, Gangwar N and Garg SK. Oleic acid prevents isoprenaline-induced cardiac injury: effects on cellular oxidative stress, inflammation and histopathological alterations. Cardiovasc Toxicol 2020; 20: 28-48.

[29] Tanriverdi LH, Parlakpinar H, Ozhan O, Ermis N, Polat A, Vardi N, Tanbek K, Yildiz A and 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: 772-786.

[30] Liu Q, Zhang Q, Wang K, Wang S, Lu D, Li Z, Geng J, Fang P, Wang Y and Shan Q. Renal denervation findings on cardiac and renal fibrosis in rats with isoproterenol induced cardiomyopathy. Sci Rep 2015; 5: 18582.

[31] Hayes H, Patz J, Corbett J, Afzal MZ, Strande J and Kindel TL. Sleeve gastrectomy in obese Wistar rats improves diastolic function and promotes cardiac recovery independent of weight loss. Surg Obes Relat Dis 2019; 15: 837-842.

[32] Olivier A, Pitt B, Girerd N, Lamiral Z, Machu JL, McMurray JJV, Swedberg K, van Veldhuisen DJ, Collier TJ, Pocock SJ, Rossignol P, Zannad F and Pizard A. Effect of eplerenone in patients with heart failure and reduced ejection fraction: potential effect modification by abdominal obesity. Insight from the EMPHASIS-HF trial. Eur J Heart Fail 2017; 19: 1186-1197.

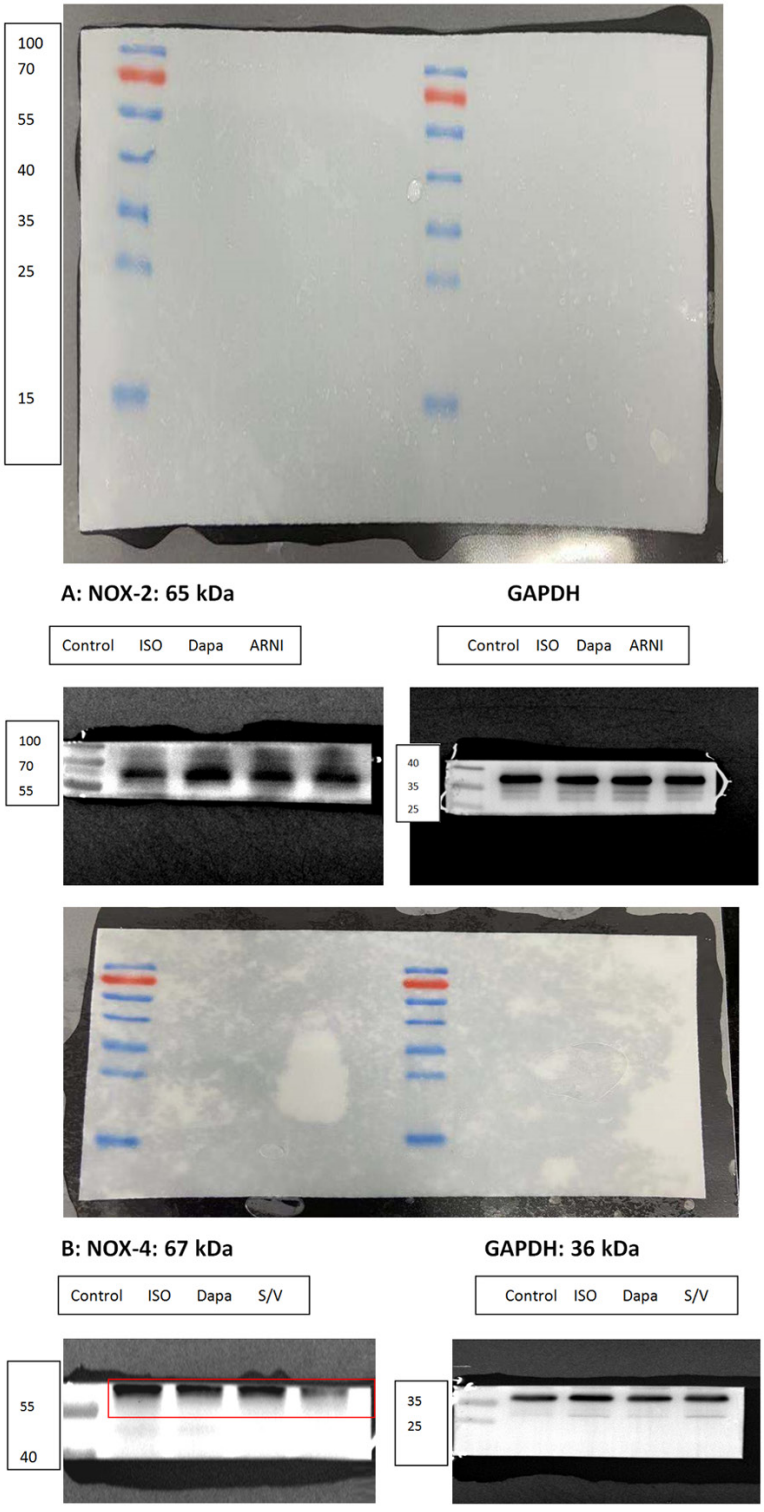
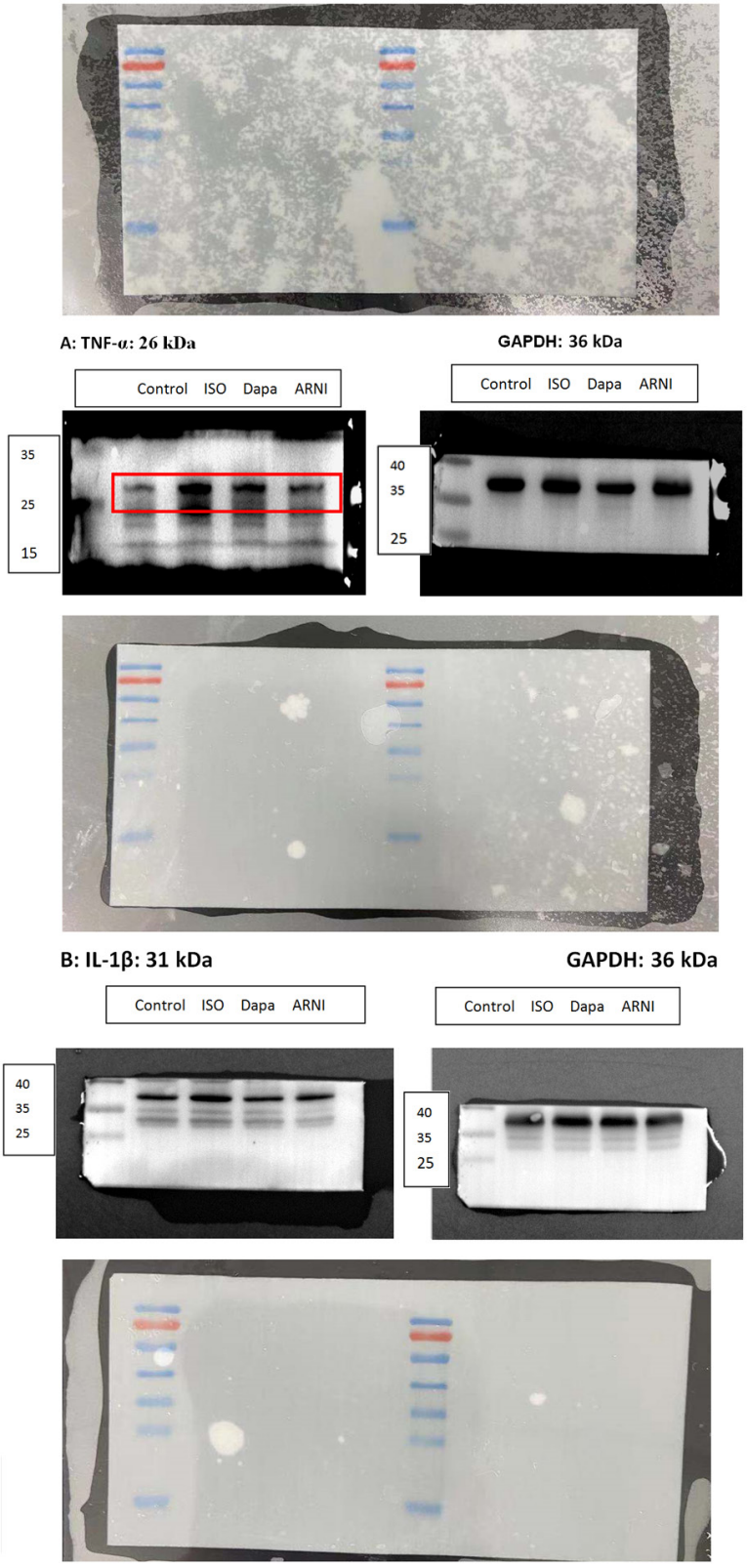
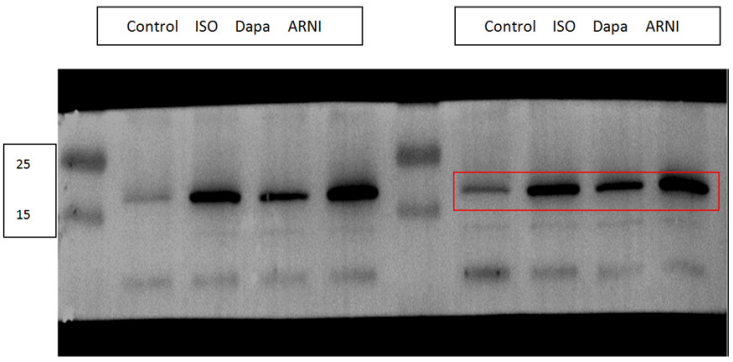


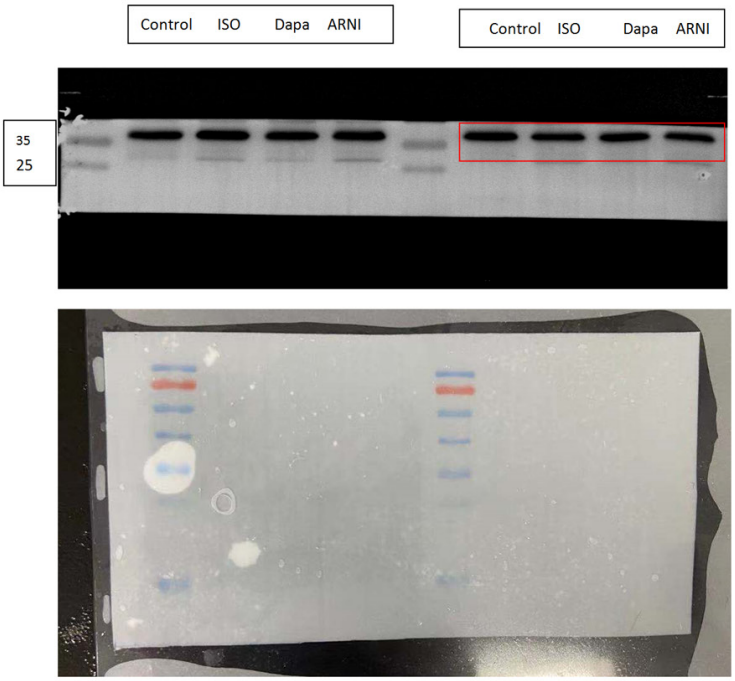
Figure S1. The uncropped western blot results of the NAD(P)H oxidase subunits (NOX-2 and NOX-4) and GAPDH in the rats' cardiac tissues at the end of the 6th week in the control, ISO, Dapa, and S/V groups. We distinguished the different target proteins according to the molecular weight of the target protein as indicated by the markers, and then we cut them off the whole membrane. Every target protein and the corresponding GAPDH blots were from the same whole membrane.


A: TNF-α: 26 kDa
GAPDH: 36 kDa
Control ISO Dapa ARNI
35
25
15
Control ISO Dapa ARNI
40
35
25
B: IL-1β: 31 kDa
GAPDH: 36 kDa
Control ISO Dapa ARNI
40
35
25
Control ISO Dapa ARNI
40
35
25

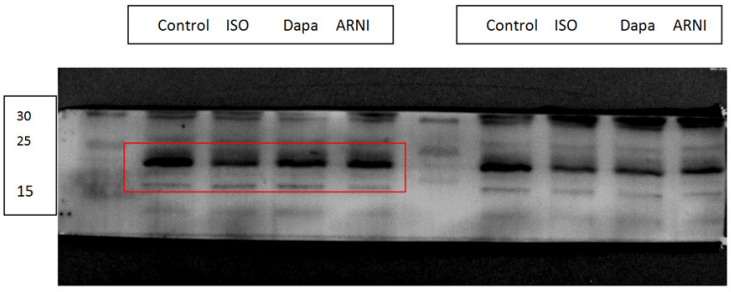
C: IL-6

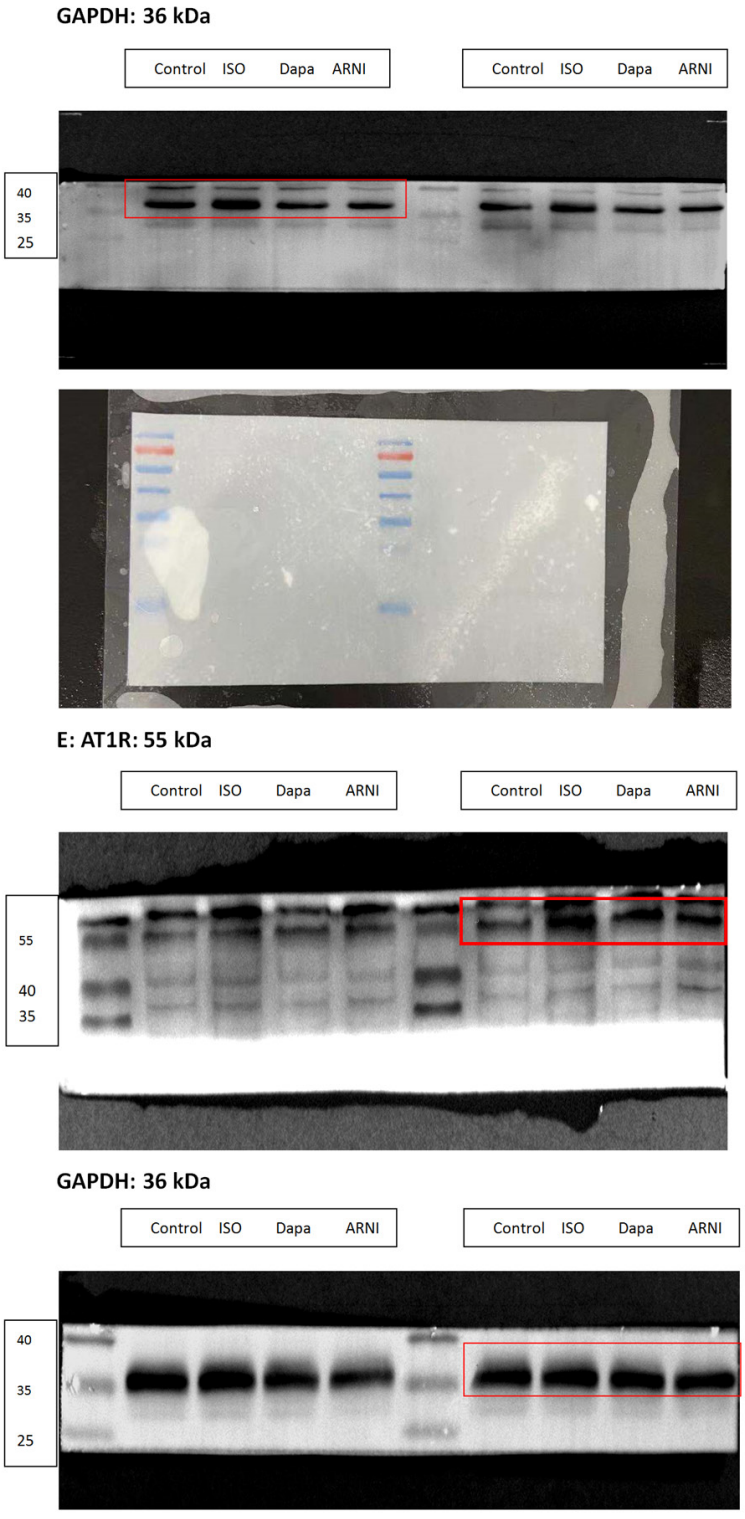


GAPDH: 36 kDa

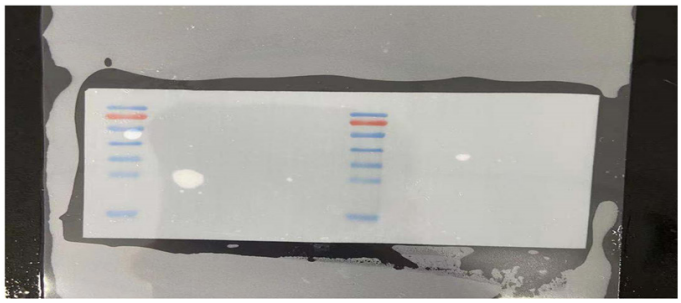


D: IL-10: 19 kDa




GAPDH: 36 kDa
Control ISO Dapa ARNI
Control ISO Dapa ARNI
40
35
25
E: AT1R: 55 kDa
Control ISO Dapa ARNI
Control ISO Dapa ARNI
55
40
35
GAPDH: 36 kDa
Control ISO Dapa ARNI
Control ISO Dapa ARNI
40
35
25

F:



G:

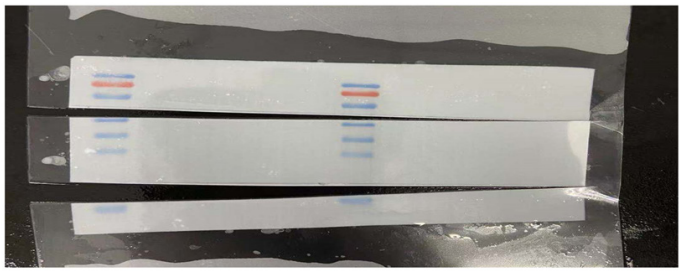


Figure S2. The uncropped western blot results of the inflammatory mediators TNFα, IL-1β, IL-6, and IL-10, the angiotensin II type-1 receptor (AT1R), and GAPDH in the rats' cardiac tissue at the end of the 6th week in the control, ISO, Dapa, and S/V groups. We distinguished the different target proteins according to the molecular weight of the target protein as indicated by the markers, and then we cut them off the whole membrane. Every target protein and the corresponding GAPDH blots were from the same whole membrane as shown in figures (F & G).