Establishment and effect evaluation of a stress cardiomyopathy mouse model induced by different doses of isoprenaline

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Abstract. The optimum dose of isoprenaline (ISO) required to induce stress cardiomyopathy (SC) in mice is not known. The present study aimed to investigate the dose-response association and determine the optimum dose of ISO to establish a high-morbidity/low-mortality SC mouse model to simulate the clinical symptoms of SC. A total of 72 6-week-old wild-type female mice (C57BL/6) were randomly divided into control mice administered normal saline and mice treated with increasing ISO concentrations (5, 10, 25, 50 and 100 mg/kg ISO intraperitoneal injections daily for 14 consecutive days). All mice were analysed by body weight assessment, open field test (OFT), echocardiography (Echo), electrocardiogram (ECG), assessment of myocardial pathology and quantification of cortisol, brain natriuretic peptide (BNP), cardiac troponin T (cTnT), catecholamine (CA) and C-reactive protein (CRP). Compared with the control group, the 25 and 50 mg/kg ISO groups exhibited the most prominent weight changes and lower mortality. The open-field test showed a significant decrease in autonomous activity behaviour in the 25 and 50 mg/kg ISO groups compared with the control group (P<0.05). Echo revealed that the apex of the heart was balloon-like in the 25 and 50 mg/kg ISO groups, along with prominent left ventricular dyskinesia. ECG showed a significant increase in ST segment amplitude, QT interval and Q amplitude (P<0.05) in the 25 and 50 mg/kg ISO group compared with the control group. Haematoxylin and eosin staining of heart tissue showed a disordered arrangement of myocardial cells, dissolution of myocardial fibres and cytoplasm, notable widening of myocardial cell space, oedema and hyperaemia of the interstitium, whereas heart tissue of the control group was structurally intact. Compared with the control group, the 25 and 50 mg/kg ISO groups exhibited significantly higher levels of cortisol, BNP, cTNT, CA and CRP (P<0.05). A high-incidence low-mortality SC model was successfully and stably developed by administration of 25 and 50 mg/kg ISO. Such models may provide a basis for the development of other animal models of SC.

Introduction

The incidence of stress cardiomyopathy (SC), an acute cardiomyopathy caused by mental stimulation or physical stress also known as Takotsubo syndrome, increased considerably during the COVID-19 pandemic (1-3). It is reported that suffering the pandemic, the proportion of SC in acute coronary syndromes patients increased from 1,8% before to 7,8% now (4). This disease has increased complications in patient, leading to increased adverse outcomes, medical costs and waste of medical resources (5). The clinical symptoms of SC are similar to those of myocardial ischemia (6). Its manifestations include chest pain, dyspnoea, abnormal electrocardiogram (ST-segment elevation), changes in myocardial enzymology and left ventricular (LV) motor dysfunction (7,8). In addition, a body of evidence has shown that the clinical prognosis of SC is worse compared with that of myocardial ischemia (9,10). Apart from the Renin-Angiotensin-Aldosterone System inhibitors, no therapeutic interventions have been effective in decreasing mortality now, improving prognosis or preventing recurrence in the acute or chronic stages of SC (11). SC has become a serious public health problem (12).

It has been hypothesized that SC development can be attributed to the cardiotoxicity caused by stress-induced excessive generation of catecholamines (CAs) (13). When CA levels spike superphysiologically in circulation and combined with β1-AR in the bottom of the heart, the contractility of bottom of the heart increase (14), which accelerates heart rate, inducing insufficient coronary artery perfusion and LV dilation and mild-moderate cardiac biomarker elevation (15,16). Therefore, CAs (including isoprenaline, epinephrine, norepinephrine, dopamine and phenylephrine) are commonly used in current studies to induce SC models (17,18). However, isoprenaline

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is the most commonly used drug to induce SC models with apical heart dysfunction (19).

In general, there have been some achievements in the research on SC but the criteria and evaluation indicators for establishing the mouse animal model of this disease have been unclear and vary widely (20-22). So it is crucial to establish successful animal SC models to elucidate the pathological mechanism underlying the disease and for the development of effective drug therapy.

In the present study, SC mouse models were generated via daily intraperitoneal injection of isoprenaline (ISO) at varying doses for 14 consecutive days. The present study aimed to determine the optimal modelling dose and establish a stable SC mouse model and evaluation criteria consistent with human pathological characteristics.

Materials and methods

Experimental animals. A total of 72 female C57BL/6 mice (age, 6 weeks; body weight, 20±2 g) were purchased from Zhejiang Ziyuan Laboratory Animal Technology Co., Ltd. [animal license no. SCXK (Zhe) 2019-0004]. Animals were bred in the Experimental Animal Center of Anhui University of Traditional Chinese Medicine. The protocol was approved by the Experimental Animal Ethics Committee of Anhui University of Traditional Chinese Medicine [approval no. AHUCM-mouse-2022045].

Animal grouping and modelling. Mice were housed in individual cages (six mice/cage) under standard laboratory conditions and were given food and water *ad libitum*. Mice were kept at 20-22˚C, with 50% humidity and a fixed 12/12-h light/dark cycle. Mice were randomly divided into two parts (six groups each part, six mice each group) according to a random number table, including a control group of untreated mice and five groups treated with varying doses of ISO (5, 10, 25, 50 and 100 mg/kg; APExBIO Technology LLC). ISO was administered intraperitoneally once daily for 14 consecutive days (Fig. 1A). The mice in the first part were observed to make a survival curve, and other thirty-six mice in the second part were used for open field test, echocardiography, ECG, haematoxylin and eosin staining, and ELISA. All mice were weighed daily until the end of the experiment. The whole experiment lasted 14 days.

Open field test. At 30 min after ISO injection on day 14, the mice were placed in the central compartment of an open field box (Shanghai Xinsoft Information Technology Co., Ltd.), and their activity was recorded for 5 min using a camera system. At the end of the experiment, computer software (Super Maze V2.0, Shanghai Xinsoft Information Technology Co., Ltd.) was used to analyse the video and indexes of the total distance (track length of free movement of mice in open field), degree across the grid (total number of mesh traversed freely by mice in open field), time in the centre (the amount of time of the mice in the intermediate zone in the open field) and the grid number in the centre (the total number of intermediate mesh traversed by mice in open field) were assessed for each group.

Echocardiography. Alteration in LV function of the mice was measured using echocardiography [Vinno Technology (Suzhou) Co., Ltd.] after the open field test. LV function was assessed by measuring the following parameters: LV internal end-diastolic diameter (LVIDd, mm); LV internal end-systolic diameter (LVIDs, mm); LV end-diastolic volume (LVEDV, ml); LV end-systolic volume (LVESV, ml); ejection fraction (EF, %) and fractional shortening (FS, %). Mice were anaesthetized with 1% isoflurane gas during the electrocardiogram (ECG) and echocardiography detection to minimise effect on the heart rate, autonomic nervous system and blood oxygen saturation (23).

ECG. After the echocardiography, a PowerLab system (ADInstruments, Ltd.) was used to record the lead ECG on all limbs of mice to monitor their cardiac function. The electrode was inserted into the right upper and left lower limbs subcutaneously and right lower limbs intramuscularly. Once the waveform was stabilized, ECG was recorded continuously for 15 min to observe the following parameters: ST segment; QT interval and Q wave amplitude.

Animal euthanasia. The experiments followed the principle of minimizing pain and fear in animals. Euthanasia was performed using 5% isoflurane inhalation for >1 min. After euthanasia the absence of heartbeat and breathing were used to confirm death. The bodies were transported to the designated recycling room at the Laboratory Animal Center of Anhui University of Traditional Chinese Medicine.

Of seven mice that died during ISO administration, two, one and four belonged to the 10, 50 and 100 mg/kg ISO groups, respectively. In the 10 mg/kg ISO group, one mouse died due to adverse reactions following ISO injection, causing a sharp increase in myocardial contractility and oxygen consumption in a short period, resulting in arrhythmia and myocardial ischemic necrosis. The remaining three mice in the 10 mg/kg ISO group were euthanised due to weight loss caused by insufficient food and water intake. A total of one mouse in the 50 mg/kg group and three mice from the 100 mg/kg group were euthanised due to extreme physical discomfort caused by myocardial ischemia as well as tension and stress induced by long-term injection of high ISO doses. The fourth mouse in the 100 mg/kg group suffered extremely slow heartbeat when ECG and echocardiography were performed under isoflurane anaesthesia.

Haematoxylin and eosin (H&E) staining. Histopathological changes in myocardial tissue were observed using H&E staining (cat. nos. BA4097, BA4099, Zhuhai Beso Biotechnology Co. LTD). The myocardial tissue samples were fixed in 4% paraformaldehyde for 24 h (4˚C). Samples were placed in 70, 85 and 95% ethanol for gradient dehydration. The samples were were embedded in melted paraffin wax for 3 h (50-60˚C). Then, the embedded sample is cut into pieces 20 μm thick and placed onto a slide. Thereafter, sections were dewaxed three times in xylene (room temperature) and then washed with anhydrous ethanol for 3 min (room temperature). The sections were immersed in 95, 80, and 70% ethanol for 1-3 min and washed with pure water for 1 min (room temperature). Sections were stained with haematoxylin for 3 min at room temperature. Following washing with water, samples were treated with PBS for 1 min (room temperature), followed by
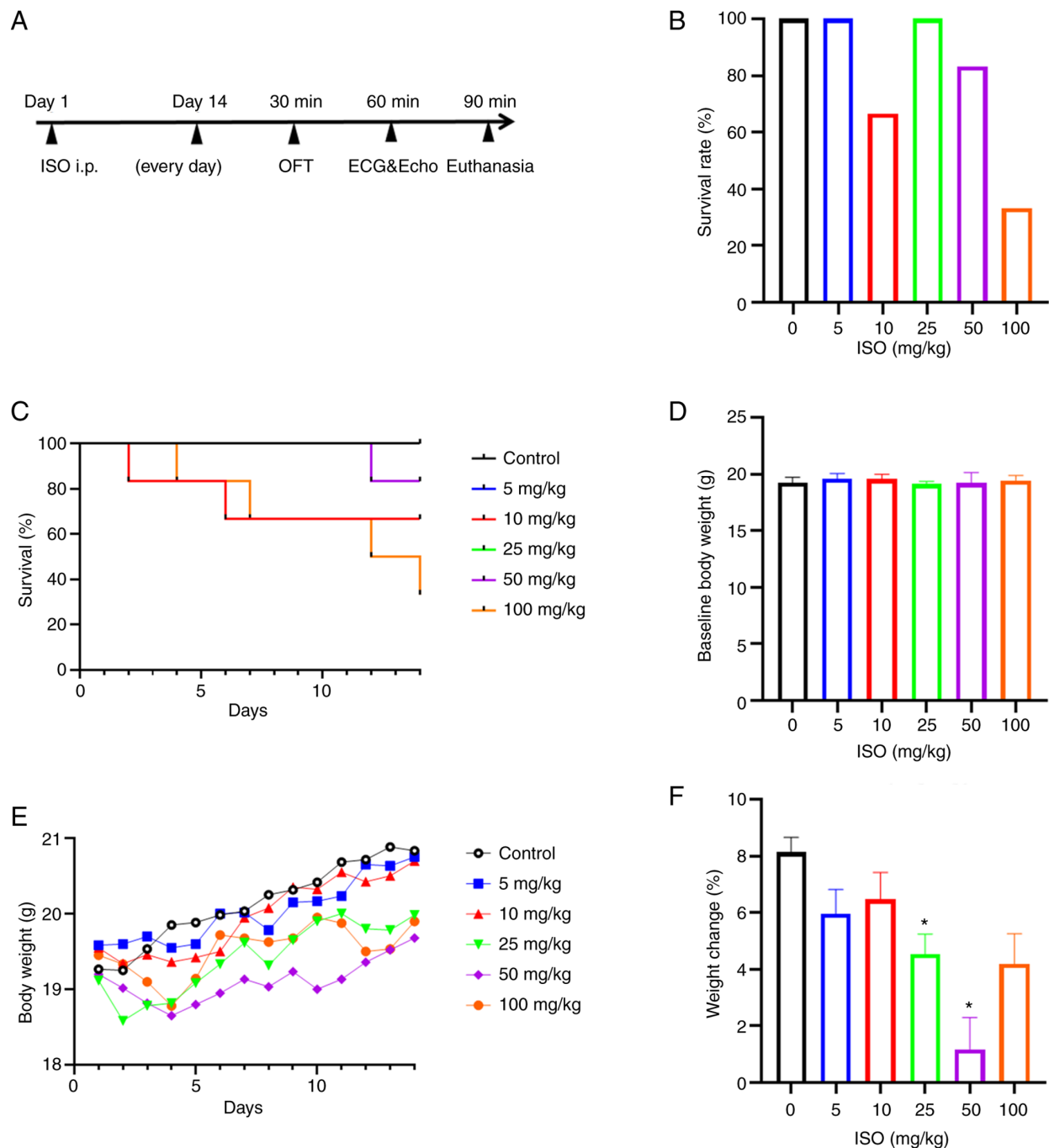
Figure 1. Effect of ISO on body weight and growth of mice. (A) Experimental flow chart. Following daily i.p. injection of ISO for 14 consecutive days, an open field test was performed 30 min after the last injection and echocardiography and electrocardiogram were performed at 60 min. Serum and heart samples were collected within 90 min. (B) Survival rate. (C) Survival curve. (D) Baseline body weight. (E) Body weight change within 14 days. (F) Percent change in body weight. For the control and 5 and 25 mg/kg ISO group, n=6. For the 10 mg/kg ISO group, n=4. For the 50 mg/kg ISO group, n=5. For the 100 mg/kg ISO group, n=2. *P<0.05 vs. control. ISO, isoprenaline; OFT, open field test; ECG, electrocardiogram; Echo, echocardiography; i.p., intraperitoneal.

staining with eosin for 1 min at room temperature. Finally, the samples were immersed in 70, 85, and 95 ethanol for gradient dehydration and sealed using neutral gum. The morphology of myocardial tissue was observed under fluorescent microscope using bright field (NIKON ECLIPSE C1, Nikon Corporation; magnification, x400).

ELISA. Within 90 min of the last ISO injection, mice were anaesthetized with 2% isoflurane and sacrificed via decapitation after blood (1 ml) was extracted through the orbital vein.

The blood samples were centrifuged (1,006.2 x g for 10 min, room temperature) and the supernatant was collected and stored at -80˚C until further analysis. Brain natriuretic peptide (BNP), cardiac calcitonin T (cTnT), cortisol, CA or C-reactive protein (CRP) ELISA reagent (JYM0380Mo, JYM1157Mo, JYM0759Mo, JYM0392Mo, JYM0563Mo, respectively; all Wuhan Jiyinmei Technology Co., Ltd.) was placed at room temperature and equilibrated for 30 min, as per the manufacturer's instructions. Serum samples (50 μl) were placed in a 96-well plate with 10 μl antibody
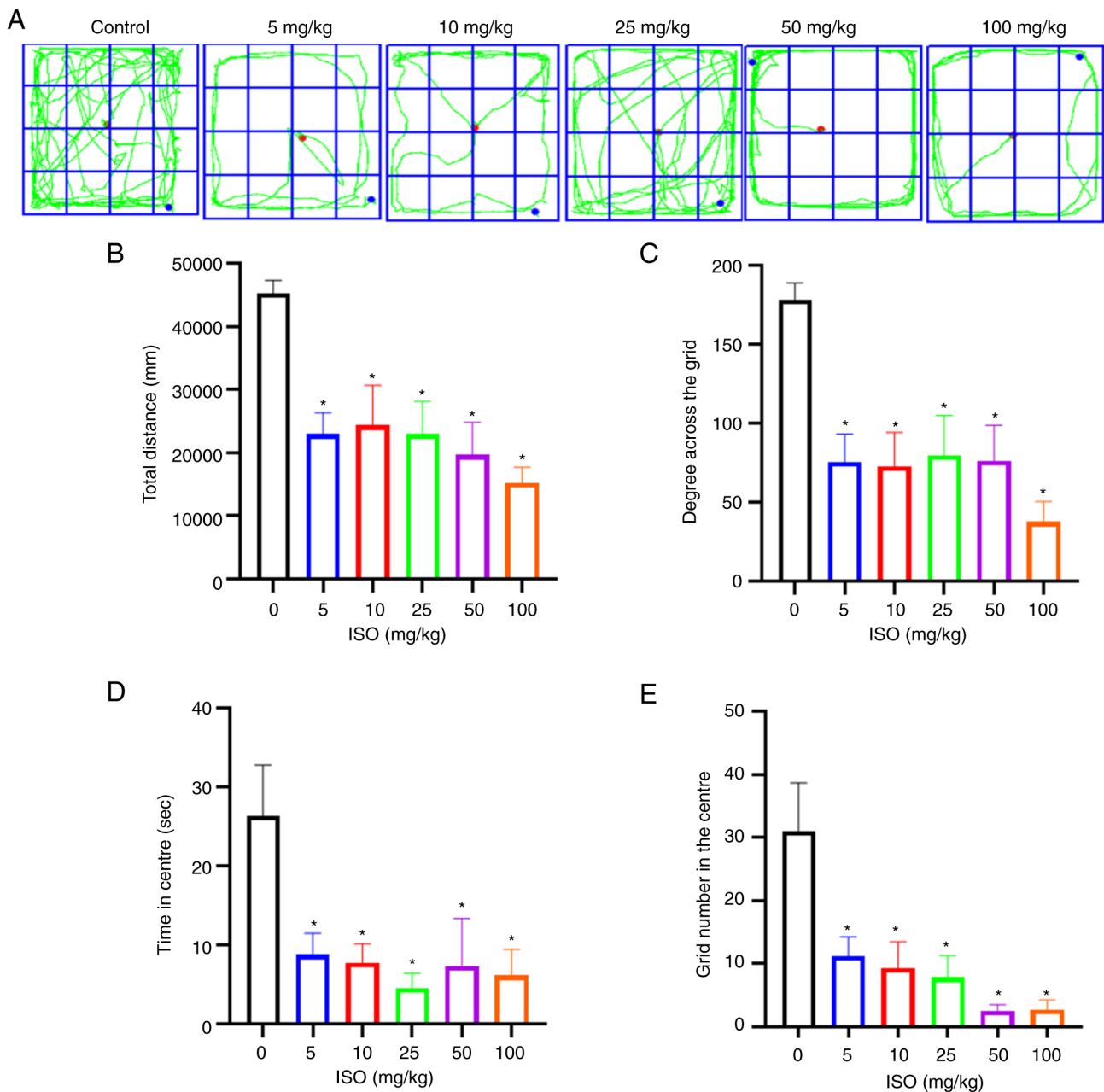
Figure 2. Effect of ISO on stress response in mice. (A) Representative open field test images. (B) Total distance travelled. (C) Degree across the grid. (D) Time in the centre. (E) Grid number in the centre. n=6. *P<0.05 vs. control. ISO, isoprenaline.

(all 1:5) against BNP, cTnT, cortisol, CA or CRP. Following incubation at 37˚C, 30 min), plate washing, adding color developing agent (37˚C, 10 min and dark treatment), and adding termination fluid, the absorbance of the mixture was measured at 450 nm using an automatic microplate reader (RT-600, Shenzhen Redu Life Science Co., Ltd.), and the concentration of the sample was calculated according to a standard calibration curve.

Statistical analysis. SPSS 25.0 (IBM Corp.) statistical software was used for analysis and GraphPad Prism 8.0 (GraphPad Software, Inc.; Dotmatics) was used for data plotting. Data are expressed as the mean ± standard deviation (n=6). The differences between groups were analysed using one-way ANOVA and Tukey's post hoc test. Pearson's

correlation coefficient was used to analyse correlation. P<0.05 was considered to indicate a statistically significant difference.

Results

Inhibitory effect of different doses of ISO on body weight and growth. The survival rate of each group was assessed after daily administration of ISO for 14 consecutive days to draw a survival curve. The survival rates of 5, 10, 25, 50 and 100 mg/kg ISO groups were 100.0, 66.7, 100.0, 83.3 and 33.3%, respectively (Fig. 1B). There was no significant difference in the mean survival time between the 5, 10, 25, 50 and 100 mg/kg groups (14, 10.3±5.8, 14, 13.5±1.2 and 10.2±4.6, respectively; Fig. 1C) with 0 mg/kg group.
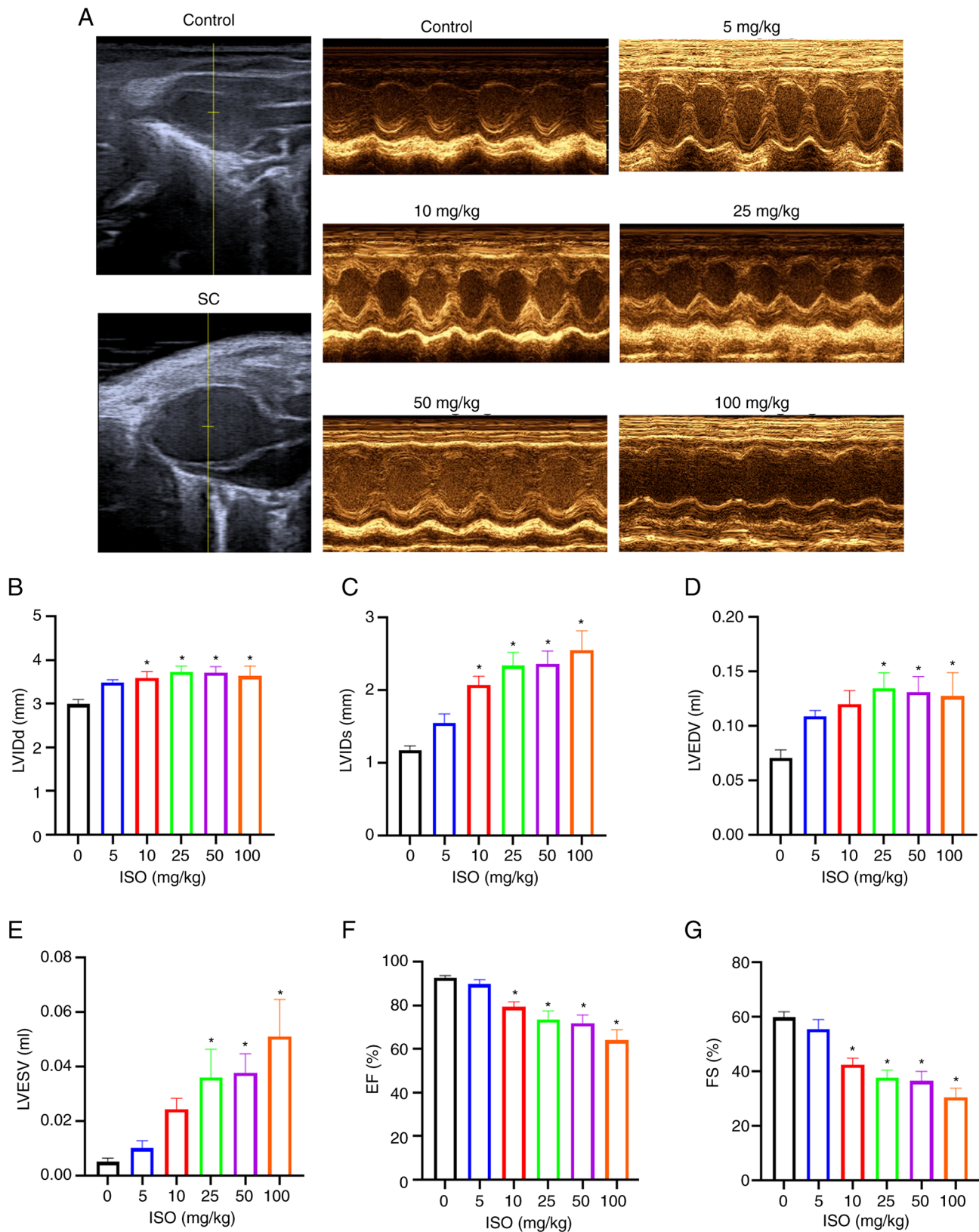
Figure 3. Effect of ISO on left ventricular systolic function in mice. (A) Representative M-mode echocardiogram images. (B) LVIDd. (C) LVIDs. (D) LVEDV. (E) LVESV. (F) EF. (G) FS. n=6. *P<0.05 vs. control. ISO, isoprenaline; LVIDd, left ventricular internal end-diastolic diameter; LVIDs, left ventricular internal end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; FS, fractional shortening.

At baseline, no significant differences in body weight were observed between groups (Fig. 1D). During the experiment, standardized feeding was adopted to ensure that the diet were at the same level as much as possible. However, an increase in body weight was observed in all ISO-treated mice (Fig. 1E); the increase in body weight was significantly lower in the 25
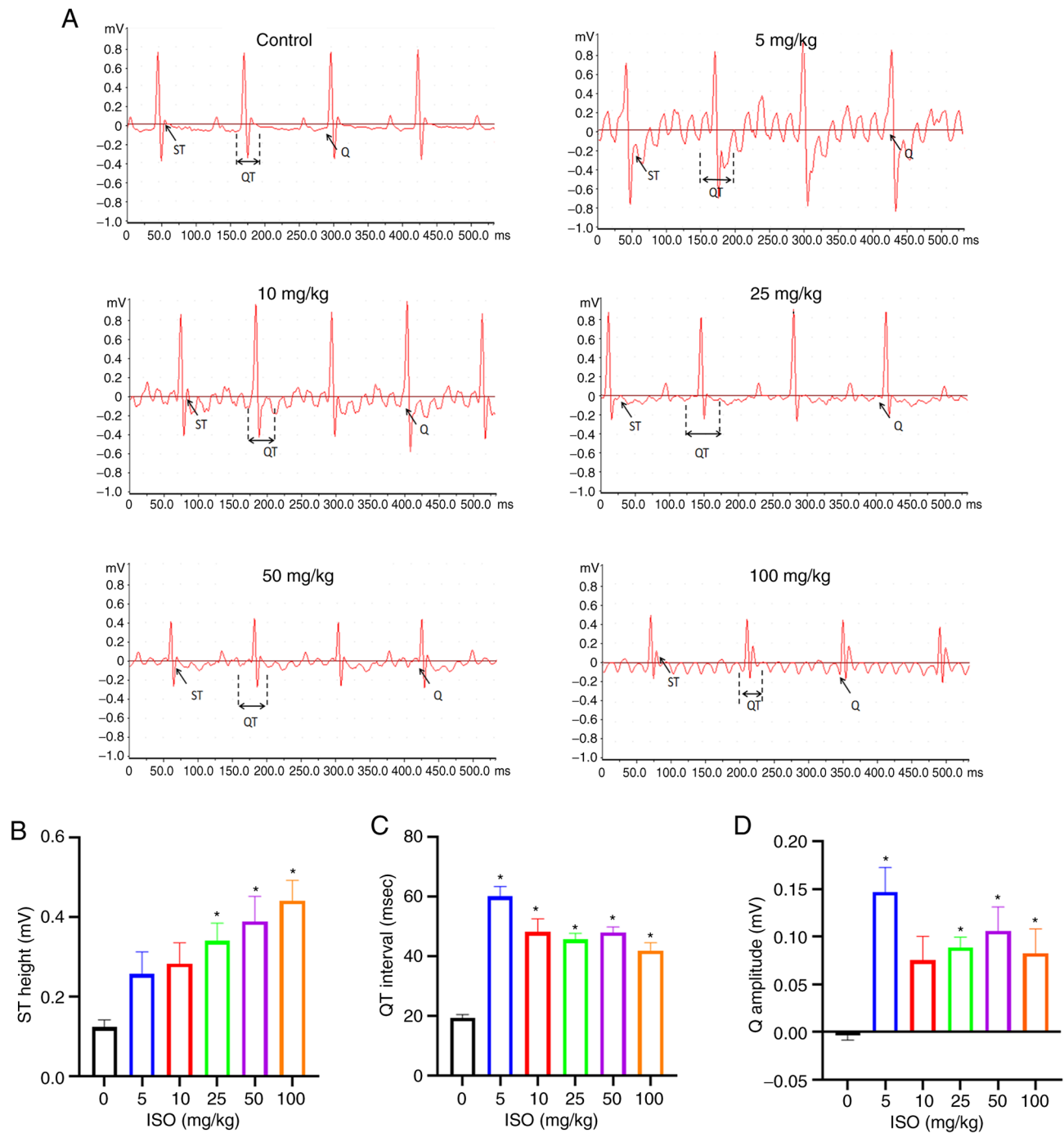
Figure 4. Effect of ISO on the electrocardiogram in mice. (A) Representative electrocardiogram images. (B) ST height. (C) QT interval. (D) Q wave amplitude. n=6. *P<0.05 vs. control. ISO, isoprenaline.

and 50 mg/kg ISO groups compared with that in the control (Fig. 1F). Following intraperitoneal injection of ISO, the mice showed signs of excessive resting on the ground, trembling, urination, cowering in the corner and increased sensitivity to stress. After 5 days of ISO administration, a more prominent stress response in the form of increased aggressiveness was observed in the 10 mg/kg ISO group.

Effect of different doses of ISO on stress response in mice. The open field test revealed changes in behavioural parameters in all ISO groups compared with those in the control group (Fig. 2A). Compared with the control group, mice in all the

ISO groups showed a significant decrease in the total distance travelled, degree across the grid, time spent at the centre of the field and grid number in the centre (Fig. 2B-E). These results showed that the level of spontaneous activity was significantly decreased after ISO administration and activity and exploration of the mice were confined to the peripheral grid at the bottom of the box. ISO injection for 7 days also resulted in decreased voluntary movement (Fig. S1A-D), with the most prominent decrease observed in the 5, 50 and 100 mg/kg ISO groups.

Effect of different doses of ISO on left ventricular systolic function in mice. Echocardiography of ISO-treated mice showed a
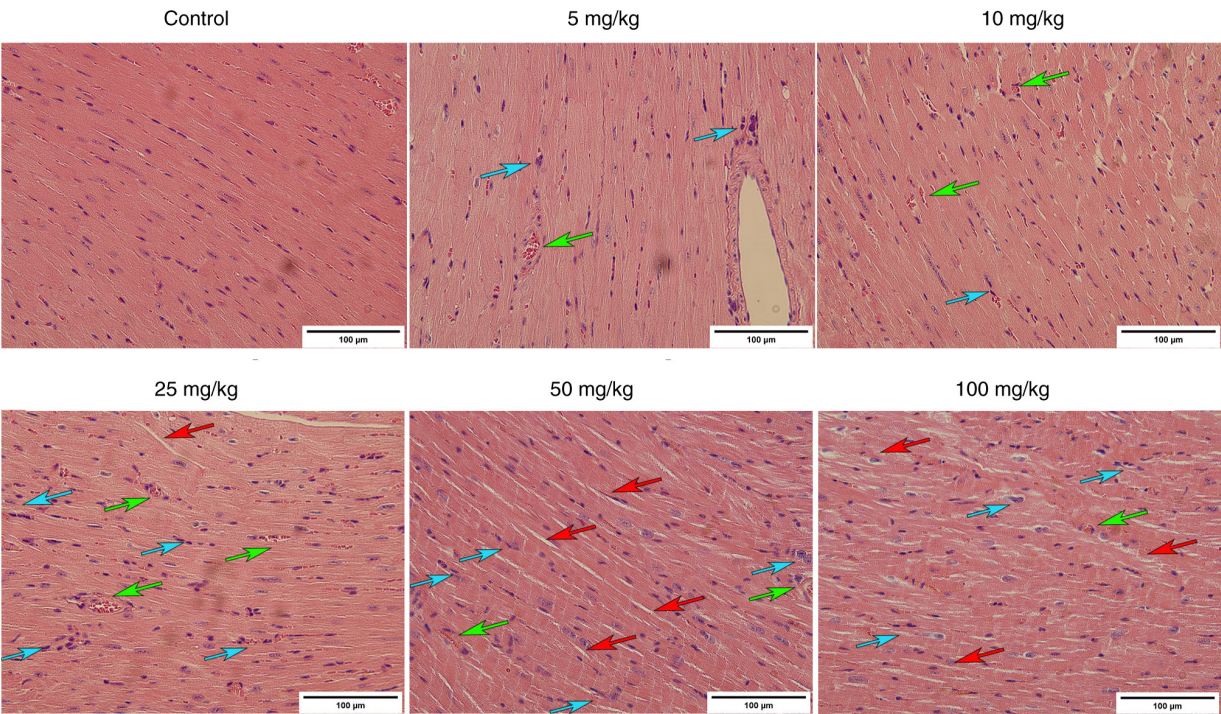
Figure 5. Cardiac pathological changes in mice treated with ISO. Haematoxylin and eosin staining of Con and ISO groups (magnification, x400). Blue arrows indicate cardiomyocyte abnormalities with myocardial fibre and cytosol lysis, red arrows indicate marked expansion of the cardiomyocyte space, and green arrows indicate interstitial oedema and hyperaemia. ISO, isoprenaline.
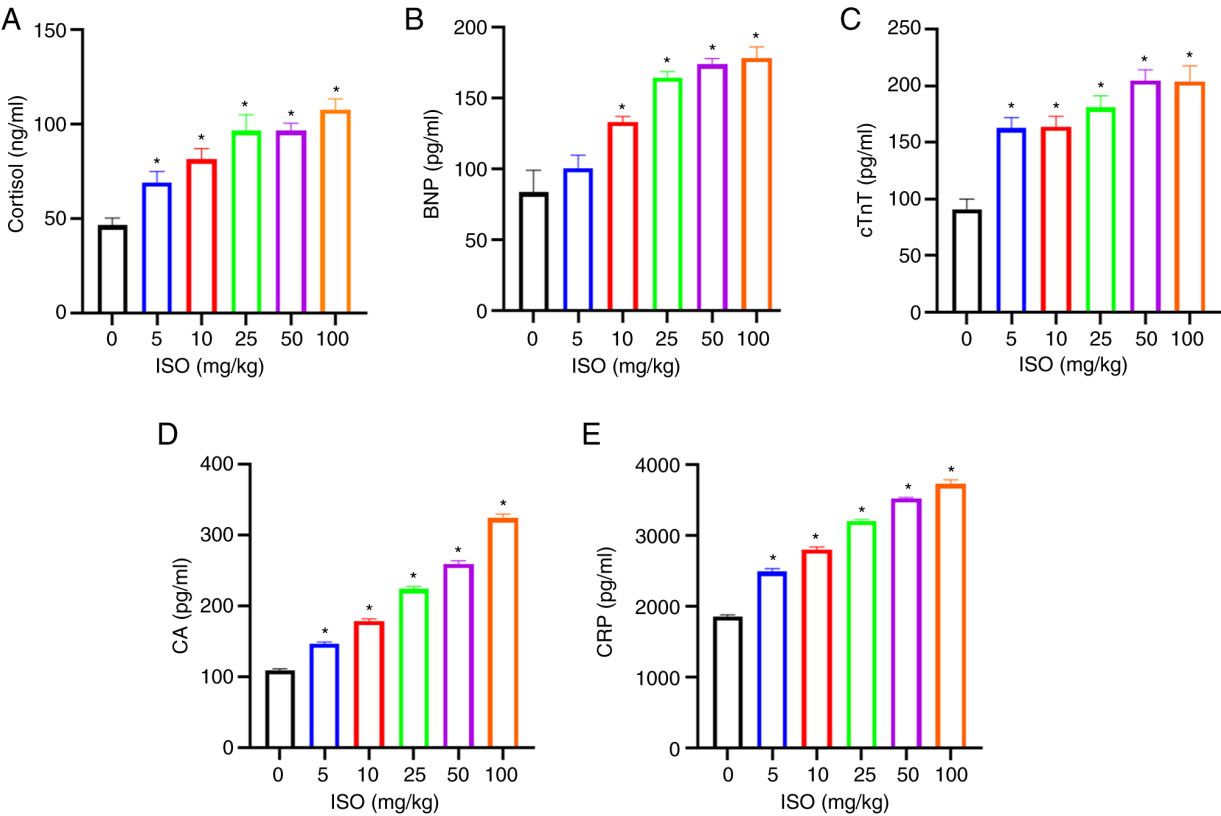

Figure 6. Effect of ISO on serum indexes in mice. (A) Cortisol, (B) BNP, (C) cTNT, (D) CA and (E) CRP levels. n=6. *P<0.05 vs. control. BNP, brain natriuretic peptide; cTnT, cardiac troponin T; CA, catecholamine; CRP, C-reactive protein; ISO, isoprenaline.

balloon-like enlargement of the apex of the heart (Fig. 3A), abnormal contractile movement of the apex or middle part of the heart and normal contractile function of the base of the heart. Compared with the control group, LVIDd and LVIDs

Table I. Previously used methods for administering ISO to induce stress cardiomyopathy.

First author/s, year	Mouse model	Dose, mg/kg	Dosage regimen	(Refs.)
Liao *et al*, 2022	C57BL/6J	200	Single intraperitoneal injection of ISO	(38)
Shao *et al*, 2013	C57BL/6J	400	Single intraperitoneal injection of ISO	(39)
Khurana *et al*, 2021	129/Sv	25	Continuous subcutaneous injection of ISO for 5 days	(40)
Walsh-Wilkinson *et al*, 2021	C57BL/6J	30	Micro-osmotic pump inserted subcutaneously to inject ISO for 21 consecutive days	(41)
Deng *et al*, 2004	Konmin	30	Continuous intraperitoneal injection of ISO for 3 days	(42)

ISO, isoprenaline.

were significantly increased in the 10, 25, 50 and 100 mg/kg ISO groups while LVEDV and LVESV were significantly increased in the 25, 50 and 100 mg/kg ISO groups (Fig. 3B-E). On the other hand, compared with the control group, EF and FS were significantly decreased in the 10, 25, 50 and 100 mg/kg ISO groups (Fig. 3F and G).

These results indicated that the ISO group exhibited enlarged apex and LV dyskinesia and a significant increase in the diameter and volume of the left ventricle, which was consistent with the pathological characteristics of SC (24). After 7 days of ISO administration, diastolic function of mice in all ISO groups remained normal compared with that in the control (Fig. S2A and C). Furthermore, mice in the 50 mg/kg ISO group showed LV systolic dysfunction and significantly increased LV diameter and volume with the control group (Fig. S2B and D-F).

Effect of ISO on the ECG in mice. ECG of the mice in all groups was recorded after 14 days of ISO treatment. ECG of all ISO-treated mice differed from that of the control group (Fig. 4A). Compared with the control, the ST height was significantly increased in the 25, 50 and 100 mg/kg ISO groups, the QT interval was significantly increased in all ISO-treated groups and the Q wave was significantly increased in the 5, 25, 50 and 100 mg/kg ISO groups (Fig. 4B-D).

These results indicated that ISO administration for 14 days induced myocardial ischemia (ST segment elevation), atrioventricular block (prolonged QT interval) and damage to the heart (abnormal Q wave), which is consistent with the ECG characteristics of SC (25). Following ISO administration for only 7 days 25 and 100 mg/kg group exhibited significant increases in ST height, QT interval and Q wave compared with control (Fig. S3A-D).

Following a single ISO injection, ECG revealed an inclined depression in the upper ST segment within 30-60 min, followed by normalization of the ECG. In addition, SC pathology was not maintained post-single injection (Fig. S4A-D).

Effect of ISO on cardiac pathological changes in mice. H&E staining showed that the heart of mice in the control group was normal-sized, with a clear short cylindrical myocardial cell structure, with neat horizontal stripes and oval nuclei in the centre. Compared with the control, myocardial tissue of the mice in all ISO-treated groups exhibited abnormal myocardial cells, dissolution of myocardial fibres and cytoplasm, widening
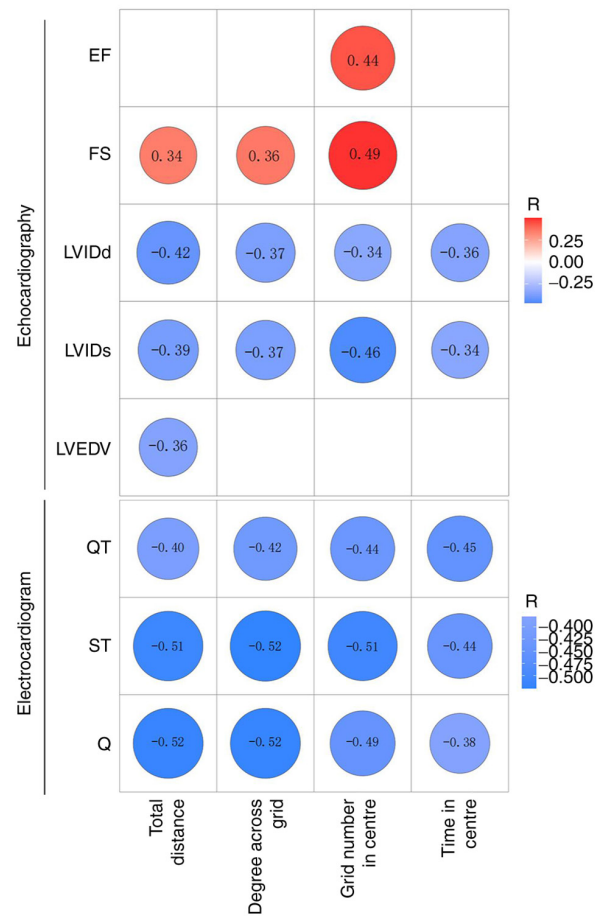

Figure 7. Stress is associated with heart injury in mice with stress cardiomyopathy. Heat map of correlation analysis between parameters assessed in the open field test with echocardiography and electrocardiogram. Red represents a positive correlation and blue represents a negative correlation. A stronger correlation is indicated by colour intensity. The numbers in the circle are the R-values. LVIDd, left ventricular internal end-diastolic diameter; LVIDs, left ventricular internal end-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; EF, ejection fraction; FS, fractional shortening.

of myocardial cell space and oedema and hyperaemia of the interstitium (Fig. 5).

Effect of different doses of ISO on serum indexes in mice. Compared with those in the control, cortisol, cTnT, CA and

CRP levels were significantly increased in the 5, 10, 25, 50 and 100 mg/kg ISO groups while BNP levels were increased in the 10, 25, 50 and 100 mg/kg ISO groups (Fig. 6A-E).

Stress is associated with heart injury in mice with SC. Correlation analysis was performed to assess the correlation between total distance, the number of degrees across the grid, the time in the centre and number of grids in the centre of the open field with LVIDd, LVIDs, LVEDV, EF and FS. The total distance correlated positively with FS (R=0.34) and negatively with LVIDd, LVIDs and LVEDV (R=-0.42, R=-0.39 and R=-0.36, respectively; Fig. 7). The number of degrees across the grid correlated positively with FS (R=0.36) and negatively with LVIDd and LVIDs (R=-0.37 and R=-0.37, respectively). Furthermore, grid number in the centre correlated positively with EF and FS (R=0.44 and R=0.49, respectively) and negatively with LVIDd and LVIDs (R=-0.34 and R=-0.46, respectively); however, the time in the centre was negatively correlated with LVIDd and LVIDs (R=-0.36 and R=-0.34, respectively).

Further correlation analysis was performed to determine if total distance, the number of degrees across the grid, time in the centre and the number of grids in the centre in the open field test correlated with QT interval, ST segment and Q wave amplitude. The total distance was negatively correlated with QT interval, ST segment and Q wave amplitude (R=-0.40, R=-0.51 and R=-0.52, respectively). Moreover, the number of degrees across the grid was negatively correlated with QT interval, ST segment and Q wave amplitude (R=-0.42, R=-0.52 and R=-0.52, respectively). Furthermore, the number of grids in the centre was negatively correlated with QT interval, ST segment and Q wave amplitude (R=-0.44, R=-0.51 and R=-0.49, respectively). Finally, the time in the centre negatively correlated with QT interval, ST segment and Q wave amplitude (R=-0.45, R=-0.44 and R=-0.38, respectively; Fig. 7).

Discussion

The success in establishing an SC model is demonstrated by assessing the consistency of myocardial manifestations with clinicopathological features under stress (26). Clinical diagnosis is based on psychological stress levels, upregulation of CA, cardiac hypertrophy, apical balloon-like change, motor dysfunction, ECG manifestations of myocardial blood deficiency, increase in BNP levels and other manifestations (27,28). Intense emotional or physical stress overstimulates the sympathetic nervous system, leading to the excessive release of CAs, which is hypothesized to trigger SC (29-31). ISO simulates the stress-state levels of CAs. Furthermore, it causes pathological myocardial damage in mice, similar to the stress-state myocardial injury in individuals with hypertrophic cardiomyopathy (32). Thus, isopropyl CA hormone epinephrine is often used as the primary component of SC-induction drugs. On the other hand, a joint scientific statement from the Heart Failure Association Takotsubo Syndrome Study Group and Myocardial Function Working Group of the European Society of Cardiology has reported that 90% of patients with SC are female (33). Compared with male patients, female patients have a higher incidence of angina, depression and other concomitant symptoms (34-37). For this reason, the present study used female mice as research subjects.

Previous studies (38-42) have reported that SC models can be constructed using either continuous intraperitoneal injection of a small dose of ISO (5-100 mg/kg) or a single intraperitoneal injection of a large dose of ISO (200-400 mg/kg; Table I). The present study showed that a single intraperitoneal ISO injection was not enough to induce SC, which was in agreement with a previous study (43). In addition, studies have shown that animal models prepared with high-dose ISO injection exhibit a high mortality rateA recent study found that injection of 400 mg/kg ISO proved lethal and the mice died on account of acute myocardial ischemia within 5 min of 400 mg/kg ISO injection (44). Hence, the present study aimed to determine the optimum dosage regimen of ISO to establish a stable mouse SC model for investigating the pathogenesis of the disease, exploring the dose-effect association and devising more effective treatment approaches.

Open-field testing is a widely used classical method to study rodent exploration behaviour and assess their emotional state (45,46). Total distance travelled is calculated to assess rodent activity based on the assumption that the central area is more threatening to rodents than the peripheral areas (44). The level of movement in the central areas is used to assess anxiety (47). All ISO groups showed a decrease in values of all the open field test parameters, including total distance travelled, the number of degrees across the grid, the time in the centre and number of grids in the centre, which indicated that ISO injection for 14 consecutive days induced a stress response in mice.

ECG and echocardiography are common methods to evaluate cardiac function (48). After onset of SC, ST segment on ECG immediately elevates and the QT interval prolongs for an extended time (49,50), which is accompanied by pathological Q wave (51,52) and atrioventricular block (53,54). SC is characterized by decreased LV systolic function and abnormal ventricular motor function, as well as decreased EF (55) and increased LV diameter and volume at end-diastolic and -systolic stages, which manifests in the form of an abnormal balloon-like shape of the left ventricle (56).

The present study showed a high mortality rate in mice injected with 100 mg/kg ISO and a slow growth rate in mice injected with 25, 50 and 100 mg/kg ISO. The open-field test showed that all ISO-treated mice exhibited a notable stress response. Echocardiography revealed alterations in cardiac function of mice in the 25 and 50 mg/kg ISO groups. The same groups showed a significant increase in inner diameter and volume of the left ventricle and a significant decrease in EF and FS, which are typical manifestations of SC (57). Moreover, ECG showed a significant increase in ST segment in the 25, 50, and 100 mg/kg ISO groups, which indicated myocardial ischemia (58,59). Furthermore, all ISO groups exhibited marked prolongation of QT interval; however, pathological Q wave was observed in the 5, 25, 50, and 100 mg/kg ISO groups. Correlation analysis showed that stress was associated with cardiac function change in the present animal model.

An increase in levels of serum BNP, which is produced by ventricular myocytes and indicates impaired cardiac function (60,61), is a common indicator of SC (62-64). At the onset of

SC, BNP and peak cTnT levels increase significantly and they act as biomarkers that distinguish SC from acute myocardial ischemia (65). The levels of cortisol, another classic stress biomarker, also increase during the development of SC (66,67). CRP is a powerful predictor and risk factor for myocardial injury (68). A key factor in SC progression is the overactivation of the sympathetic nervous system so high levels of CA can effectively reflect the degree of sympathetic nerve activation (69,70).

The present study indicated that ISO administration led to a significant increase in cortisol, cTnT, CRP and CA levels in all ISO groups, indicating that the sympathetic nervous system was activated following ISO treatment in mice, which produced a stress state and caused myocardial damage and inflammation. Moreover, increased BNP levels found in 10, 25, 50 and 100 mg/kg ISO groups indicated damage to heart function caused by ISO injection.

In conclusion, intraperitoneal injection of 25 or 50 mg/kg ISO for 14 consecutive days in mice induced a stable SC model. Compared with the 50 mg/kg ISO group, the 25 mg/kg ISO group exhibited a lower mortality rate with more prominent changes in ECG and levels of serum markers. Nonetheless, a stable mouse SC model can be established via 25 or 50 mg/kg ISO administration. The model in the current study showed several advantages, such as a simple and affordable modelling method, good stability and low mortality. It not only demonstrated the effect of different doses of ISO on stress response and cardiac function but also screened the most appropriate dose to establish a viable model and provide a basis for future SC research.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HW and SC confirm the authenticity of all the raw data. SC, HW and MZ conceived and designed the study. HW and HS collected data. HW, HS, CZ and SW analysed and interpreted the data. HS, CZ and SW revised the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Study protocols were reviewed and approved by the Experimental Animal Ethics Committee of Anhui University of Traditional Chinese Medicine (approval no. AHUCM-mouse-2022045).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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