Establishment of a New Arrhythmia Model in SD Rats Induced by the Isoproterenol "6+1" Mode and Its Mechanisms

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Abstract

Background

The main purpose is to establish an ideal arrhythmia model with isoproterenol and explore its mechanism.

Methods

Fifty healthy male SD rats were randomly divided into the control (CON) group, subcutaneous injection (SC) group(ISO 5 mg/kg for 2 consecutive days), intraperitoneal injection (IP) group (ISO 5 mg/kg for 2 consecutive days), 2+1 group (ISO 5mg/kg by SC for 2 consecutive days, then ISO 3mg /kg by IP for 1 day), 6+1 group (ISO 5mg/kg by SC for 6 consecutive days, then ISO 3mg /kg by IP for 1 day).The ECG were recorded by BL-420F system. The pathological changes in myocardial tissue were observed by HE and Masson staining. The serum cTnI, TNF-α, IL-6, and IL-1β levels were detected by ELISA. Serum CK, LDH and oxidative stress-related indicators were detected by an automatic biochemical analyser.

Results

The cardiomyocytes in the CON group rats were normal, while those in the other groups showed signs of disorder, unclear borders, and lysis and necrosis, especially in the 6+1 group. The incidence of arrhythmia, arrhythmia score, and the levels of serum myocardial enzymes, troponin, and some inflammatory factors in the 2+1 group and 6+1 group were higher than those in the single injection way ($P<0.01$ or $P<0.05$). The indicators in the 6+1 group were mostly higher than those in the 2+1 group ($P<0.01$), and the SOD level in the 6+1 group was lower than that in the CON group; the MDA and NO higher($P<0.01$ or $P<0.05$).

Conclusion

The combined mode of ISO injection (SC with IP) is more likely to induce arrhythmia than single ISO injection. The "6+1" method of ISO injection can establish a more stable arrhythmia model. Cardiomyocyte damage induced by oxidative stress and inflammation is an important mechanism.

1. Introduction

Arrhythmia is a clinical syndrome of abnormal cardiac pulse frequency or rhythm induced by the origin or conduction disorder of cardiac electrical activity, which is an important cause of sudden cardiac death. According to statistics, approximatelyt 600,000 people die from sudden cardiac death in China each year, of which approximately 90% are induced by malignant arrhythmia[1].Myocardial ischaemia is a common cause of arrhythmia[2]. In animal experiments, the method of ligating the anterior descending coronary artery is often used to establish an acute ischaemia arrhythmia model, but there are problems such as trauma and poor reproducibility. Isoproterenol (ISO) is a β-adrenergic receptor agonist, that is commonly used as an inducer of heart remodelling in experimental animals[3]. The commonly used doses are 3 mg/kg or 5 mg/kg, and the administration methods are different between subcutaneous and

intraperitoneal injections[4, 5]. In this experiment, four different ISO administration dosages and methods were applied to SD rats to establish a simple, reproducible and stable arrhythmia model, laying a foundation for further research on arrhythmia mechanisms.

2. Materials

2.1. Animal

SPF 50 male Sprague Dawley rats weighing 200±20 g were housed on a 12-hour light–dark circadian cycle at 21°C to 23°C and a humidity of 55%. All rats were provided food and water ad libitum. The rats were provided by Liaoning Changsheng Biotechnology Co.,Ltd. The laboratory animal quality certificate number: No. 210726200100192861, and the licence number: SCXK (Liao) 2020-0001. The Ethical Review Committee of Hebei College of Traditional Chinese Medicine approved this experimental study.

2.2. Reagents and drugs

Isoproterenol hydrochloride (Shanghai Hefeng Pharmaceutical Co., Ltd., batch number: 5E20012); creatine kinase (CK) (Changchun Huili Biotechnology Co., Ltd., batch number: A026); cardiac troponin I (cTnI) kit (Abcam, ab246529); lactate dehydrogenase (LDH), malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD) kit (Nanjing Jiancheng Institute of Biological Engineering, batch number: A020-2, A003-1, A012-1, A001-3); and tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β) kit (Xinbosheng Biotechnology Co., Ltd., batch numbers ERC102a, ERC003, ERC007) were used.

2.3. Main instruments

The biological function experiment system BL-420F (Chengdu Taimeng Software Co., Ltd.); an electronic balance (Hunan Instrument and Meter General Factory Balance Factory); a constant temperature water bath (Changzhou Hua Electric Co., Ltd.); an AU-2700 automatic biochemical analyser, and an IX51 Type inverted microscope (Olympus, Japan).

3. Method

3.1. ISO preparation

ISO is dissolved in 0.9% NaCl at 5 mg/kg or 3 mg/kg to make the solvent. Store away from light at -20°C for later use.

3.2. Animal model and drug administration

Before the intervention of experimental animals, we established the inclusion and exclusion criteria of animals. Specifically, according to the published literature, rats with normal basic ECG can be included in this experiment[6]. Arrhythmia is due to the abnormal frequency, rhythm, origin, conduction velocity and excitation sequence of cardiac electrical impulse. Rats with abnormal P wave, QRS wave, T wave, prolonged PR interval, ST segment changes or various types of arrhythmias in ECG will be excluded from

the experiment. All rats were anesthetized with 2% Pentobarbital Sodium (40mg•kg^{-1}, IP), fixed in the supine position, connected to the BL-420F biological function experimental system, and continuously tested for 30 min. After data preservation and analysis, we found that 3 rats had occasional ventricular premature beats, 1 rat had ST segment abnormalities, and 1 rat suspected right bundle branch block in the ECG of 55 rats, which was excluded. The remaining 50 normal rats were included in further experiments.

After adaptively feeding for 7 days, 50 rats were divided into the CON group and 4 experimental groups according to the random number table: the SC group, IP group, 2+1 group, and 6+1 group. Ten rats were in each group. The experimental group was treated as follows: SC group: ISO 5 mg/kg, subcutaneous injection for 2 consecutive days; IP group: ISO 5 mg/kg, intraperitoneal injection for 2 consecutive days; 2+1 group: ISO 5 mg/kg, subcutaneous injection for 2 consecutive days, ISO 3 mg/kg, intraperitoneal injection for 1 day; and 6+1 group: ISO 5 mg/kg, subcutaneous injection for 6 consecutive days, ISO 3 mg/kg, intraperitoneal injection for 1 day. On the last day of ISO intervention in each group, the rats in each experimental group were anesthetized with 2% sodium pentobarbital (40 mg·kg^{-1},IP), fixed in the supine position, connected to the BL-420F biological function experiment system, injected with ISO, and observed and recorded with standard II lead ECG within 60 minutes. After the electrocardiogram recording, the experimenter determined that the rats were still under anesthesia by the disappearance of skin pinching reaction, the flabby muscles of head, neck and limbs, the deep slow and steady breathing, and the disappearance of corneal reflex. If not, the rats were injected with sodium pentobarbital (40mg/kg IP). Then, the blood was taken from the femoral artery of rats, and the blood volume was about 5ml. Before the termination of the study protocol, all rats were euthanized with 2% sodium pentobarbital (150 mg•kg^{-1},IP) according to *the AVMA Guidelines on the Euthanasia of Animals*[7].After the animals were sacrificed, the heart specimens were quickly and meticulously harvested for histopathological and biochemical analysis.

For each animal, four different investigators were involved as follows: a first investigator (ZJG) administered the treatment based on the randomization table. This investigator was the only person aware of the treatment group allocation. A second investigator (ZCW) was responsible for the anaesthetic procedure, whereas a third investigator (KXM, BZD, GPM, YZ) performed electrocardiogram recording and surgical procedure. Finally, a fourth investigator (WJL, WFG) (also unaware of treatment) assessed basic situation of arrhythmia in rats and arrhythmia score.

3.3. Main observation indicators

3.3.1. Observation and scoring of arrhythmia in rats

The electrocardiogram (ECG) of rats before and after induction was continuously recorded, and ventricular premature beat (VP), ventricular tachycardia (VT) and ventricular fibrillation (VF) were determined according to the Lambeth Conventions standard. The early appearance of QRS complexes is used to identify VP; nonsustained VT (NSVT) means that the number of consecutive occurrences of VP is

less than 15 times; sustained ventricular tachycardia (SVT) means that the number of consecutive occurrences of VP is ≥15 times; atrioventricular block (AVB) is between the atria and ventricles, and the impulse is slowed or blocked. The arrhythmia score (severity) is divided into 5 under the basic rating system, as follows: 0=no arrhythmia, 1=single VP, 2=double VP, 3=three VP or NSVT, 4=SVT or AVB, and 5=death[6, 8–9].

3.3.2. Detection of serum index content in rats

Blood was collected from the femoral artery of the anesthetized rat, the blood sample was centrifuged at 4000 r/min and -4°C for 10 min, and the serum was separated for storage. Enzyme-linked immunosorbent assay was used to detect the content of cTnI, TNF-α, IL-6, and IL-1β; the content of CK, LDH, SOD, MDA, and NO was detected by an automatic biochemical analyser.

3.3.3. Calculation of rat heart weight index

After 60 min of ECG detection, the rats were sacrificed to collect their hearts, washed repeatedly with ice normal saline, dried with filter paper, and weighed on an electronic balance. Heart weight index (HWI) = heart mass/body mass.

3.3.4. Histological analysis

The rat myocardial tissue was routinely dehydrated, fixed, embedded in paraffin, sectioned, and stained with haematoxylin-eosin (HE) and Masson staining. Morphological changes in myocardial tissue were observed under a light microscope.

3.4. Statistical analysis.

SPSS statistical software was used for statistical analyses. Counting data are expressed as a percentage (%), and the comparison between the two groups was performed by the chi-square test. The measurement data are expressed as the mean ± standard deviation ($\bar{x}\pm s$). If normality was satisfied, Levene's test was used to test the homogeneity of variance between the two groups. Tukey's test was used to compare the two groups if the variance was homogeneous, and univariate analysis of variance was used for comparisons between multiple groups. Non-normally distributed measurement data are represented by median and quartile [M ($P_{25,}$ P_{75})], and Kruskal-Wallis H test is used.If $P < 0.05$, the difference was considered statistically significant.

4. Results

4.1. Comparison of arrhythmia occurrence of rats in each group

4.1.1. Description of the basic situation of arrhythmia in rats

Compared with the control group, rats in all models showed faster breathing, stronger heartbeat, fatigue, loss of appetite, and increased thirst at different degree. No rats died in the experiment, and the survival rate of the model was 100% (n=50).

4.1.2. Changes in the ECG waveform of rats in each group

Compared with the control group, the ECG of the four experimental groups showed different degrees of abnormalities. In the SC group and IP group, there were only occasional VP and ST segment depression, while in the 2+1 group and 6+1 group, VP and NSVT occurred frequently. SVT was common in the 6+1 group, and the degree of arrhythmia was significantly aggravated. (Figure 1).

4.1.3. The occurrence of arrhythmia in rats in each group

Compared with the control group, the incidence of VP and/or VT was significantly increased in the SC group (P<0.01), and the incidence of VP was significantly different (P<0.01); there was no significant difference in IP group (P>0.05); the incidences of VP and/or VT in the 2+1 group and 6+1 group were significantly increased (P<0.01). Compared with the SC group, there was no significant difference in the incidence of VP and/or VT in the 2+1 group (P>0.05), but it was significantly increased in the 6+1 group (P<0.01). Compared with the IP group, the incidence of VP and/or VT was significantly higher in the 2+1 group and 6+1 group (P<0.01). Compared with that in the 2+1 group, the incidence of VP and/or VT was significantly increased in the 6+1 group (P<0.01). (Table 1)

Table The occurrence of arrhythmia in rats in each group [n (incidence rate%), n=10]

Group	VP	VT	VP and/or VT
CON	2(20)	0(0)	2(20)
SC	6(60)**	0(0)	6(60)**
IP	1(10)##	1(10)**##	1(10)##
2+1	7(70)**△△	3(30)**##	7(70)**△△
6+1	9(90)**##△△□□	6(60)**##△△□□	10(100)**##△△□□
P	0.001	0.003	0.000

Note

** P<0.01 compared to the control group, ## P<0.01 compared to the SC group, △△ P<0.01 compared to the IP group, □□ P<0.01 compared to the 2+1 group. The chi-square test was used.

4.2. Comparison of the severity of arrhythmia in rats in each group

4.2.1. Arrhythmia score

Compared with the control group, the arrhythmia score had no significant change in SC group, IP group, 2+1 group (P>0.05), but increased significantly in the 6+1 group (P<0.01). Compared with that of the SC group, the arrhythmia score of the IP group and 2+1 group had no significant change (P>0.05) but increased significantly in the 6+1 group (P<0.01). Compared with that of the IP group, the arrhythmia score of the 2+1 group had no significant change (P>0.05) but increased significantly in the 6+1 group (P<0.01). Compared with that of the 2+1 group, the arrhythmia score of the 6+1 group was no significantly increased (P>0.05). (Figure 2).

4.2.2. Proportion of SVT and NSVT

Rats in the control group and SC group did not develop VT. Compared with that of rats in the control group, the VT degree of rats in the IP group, 2+1 group, and 6+1 group was significantly increased. Compared with that of the SC group, the VT degree of rats in the IP group, 2+1 group, and 6+1 group gradually increased. Compared with those in the IP group, the proportions of SVT and NSVT in the 2+1 group and 6+1 group increased significantly. Compared with that in the 2+1 group, the proportion of SVT and NSVT in the 6+1 group increased significantly (Figure 3).

4.3. HWI comparison of rats in each group

Compared with that of the control group, the HWI of rats in the 4 experimental groups increased significantly (P<0.01). Compared with that of the SC group, the HWI of rats in the IP group and the 2+1 group did not change significantly (P>0.05); the HWI of rats in the 6+1 group increased significantly (P<0.01). Compared with that of the IP group, the HWI of rats in the 2+1 group did not change significantly (P>0.05); the HWI of rats in the 6+1 group increased significantly (P<0.01). Compared with the 2+1 group, the HWI of the 6+1 group increased significantly (P<0.01) (Figure 4).

4.4. Myocardial histomorphology

The myocardial fibres of the control group had clear borders, regular arrangement, clear contours, uniform staining, and no exudate or inflammatory cell infiltration. The myocardium of the 4 experimental groups showed varying degrees of fibre arrangement disorder, unclear borders with breaks, uneven staining depth, and local lytic necrosis. Among them, the 6+1 group of rats showed significant disturbance of myocardial fibre arrangement and increased lytic necrosis (Figure 5).

4.5. Comparison of myocardial injury in each group

4.5.1. Changes in myocardial enzymes

Compared with those in the control group, serum CK and LDH levels in the 4 experimental groups increased in varying degrees(P<0.01 or P<0.05); compared with the SC group, there was no significant difference between IP group, the 2+1 group, and the 6+1 group (P>0.05). Compared with those in the IP group, serum CK and LDH levels in the 2+1 group and 6+1 group increased (P<0.01 or P<0.05); compared with the 2+1 group, there was no significant difference in 6+1 group (P>0.05) (Figure 6).

4.5.2. Changes in cTnI

Compared with those in the control group, serum cTnI levels in the 4 experimental groups increased (P<0.01), and there was no significant difference between the 4 experimental groups (P>0.05). (Figure 6).

4.6. Comparison of oxidative stress in the rats in each group

Compared with that in the control group, the serum SOD level in the 6+1 group was significantly reduced (P<0.01), while MDA and NO were significantly increased (P<0.05). The other groups had a trend of change (P>0.05); the SOD level in the 6+1 group decreased, and MDA and NO levels increased compared with 2+1 group, but there was no significant difference (P>0.05) (Figure 7).

4.7. Comparison of serum inflammatory factor levels

Compared with those in the control group, serum TNF-α and IL-6 levels in SC group, IP group, 2+1 group had no significant changes (P>0.05), but both of them were significantly increased in the 6+1 group (P<0.05); compared with those in the SC group, TNF-α and IL-6 levels in IP group and the 2+1 group had no significant changes (P>0.05), but the TNF-α in 6+1 group was significantly increased (P<0.05). Compared with those in the IP group, the TNF-α and IL-6 levels in the 2+1 group did not change significantly (P>0.05), and the TNF-α in the 6+1 group was significantly increased (P<0.05); compared with that in the 2+1 group, the IL-6 level in the 6+1 group was significantly increased (P<0.05), and the TNF-α level was increased, but there was no significant difference (P>0.05). Compared with that in the control group, the IL-1β level of rats in each group increased, but there was no significant difference (P>0.05) (Figure 8).

5. Discussion

Arrhythmia has been recognized as one of the common cardiovascular diseases, affecting the lives and health of millions of people worldwide. Researching the mechanism of arrhythmia and research and development of effective drugs is essential to improve clinical efficacy. At present, animal models for arrhythmia research are constructed by injecting barium chloride through the jugular vein to induce arrhythmia in rats[10]; ligation of the left coronary artery to induce arrhythmia[11]; Burst rapid electrical stimulation in vitro under Langendorff perfusion conditions to induce arrhythmia[12] and other approaches. However, most of these methods have high scientific research cost, high technical difficulty, complicated operation, low survival rate of experimental animals, and poor reproducibility, making them difficult to promote and apply. Therefore, it is particularly necessary to construct a simple, reliable, and highly repeatable arrhythmia model. ISO is a catecholamine drug that mainly acts on cardiac beta receptors and can cause relatively strong cardiovascular reactions. In recent years, ISO has successfully been used to construct a variety of rat models of cardiovascular diseases, such as myocardial hypertrophy[13], myocardial injury[14], myocardial infarction[15], and heart failure[16]. However, there are few reports on the direct construction of arrhythmia models by using ISO. In the experimental process, the author established a relatively stable arrhythmia model by using ISO and further explored its mechanism.

ISO subcutaneous injection for 6 days combined with intraperitoneal injection for 1 day ("6+1" mode) can construct an ideal SD rat arrhythmia model. ISO is a β-receptor agonist, that can stimulate the β1

receptors of cardiomyocytes, leading to a faster heart rate, accelerated conduction, increased oxygen consumption, and chronic ischaemi and hypoxia. ISO induces different degrees of arrhythmia with different doses and administration methods. SCs are absorbed through capillaries, while IPs are absorbed through the peritoneum for faster absorption. Repeated SC of ISO causes chronic damage to myocardial cells, and one IP constitutes an acute stimulus, which easily induces arrhythmia quickly. Electrocardiogram testing is the preferred method for evaluating cardiac electrophysiology. The arrhythmia score is an important indicator to evaluate the severity of arrhythmia and cardiac function. From the ECG and arrhythmia incidence, it can be seen that the effect of combination SC with IP is better than that of single administration, and the "6+1" mode is better than the "2+1" mode. From the arrhythmia score and proportion of VT, there was no significant change between the single administration mode and the CON group, but the effect of combined administration was obvious; in particular, the arrhythmia score of "6+1" mode increased significantly, and the proportion of SVT and NSVT increased significantly. In the "6+1" mode, the significantly increased arrhythmia score, the increased proportion of SVT and NSVT, the 100% incidence of arrhythmia, and the obvious VT and AVB phenomenon indicate that the "6+1" mode is superior to the "2+1" mode, forming a relatively stable method for the construction of an arrhythmia model in SD rats.

Cardiomyocyte damage is the cytological basis for the successful induction of arrhythmia by the "6+1" approach. ISO can raise blood pressure, increase the pressure load of the heart, and cause compensatory hypertrophy of myocardial cells, which in turn changes the cell structure, increases the volume and mass of myocardium, and increases HWI. Weiyue Jin et al. subcutaneously injected 5 mg/kg ISO for 14 days to induce myocardial fibrosis in mice and found that the HWI of mice was significantly increased[17]. Similar to reports in the literature, the HWI of each group of rats in this experiment was significantly higher than that of the CON group, especially in the 6+1 group, indicating that the ISO "6+1" mode caused more serious hypertrophic damage to cardiomyocytes. The results of HE and Masson staining confirmed that myocardial cells in the 2+1 and 6+1 groups increased in volume, showed a disorderly arrangement, were vacuolated, and showed interstitial oedema and neutrophil infiltration, which was more obvious in the 6+1 group. Myocardial cell injury causes myocardial enzymes to overflow into the blood, leading to increased serum CK and LDH, and the degree of increase is positively correlated with the degree of injury.

In this experiment, the serum CK and LDH levels in each group were significantly higher than those in the control group, especially in the 2+1 group and 6+1 group, suggesting that repeated ISO stimulation caused more serious damage to cardiomyocytes. cTnI is the most sensitive and specific gold indicator reflecting cardiomyocyte damage. The increase in cTnI in each group of rats in this experiment further proves that the cardiomyocyte damage in the 6+1 group is more serious. Morphological indicators, enzymatic indicators, and myocardial protein indicators all confirm that the "6+1" method causes more serious cardiomyocyte damage, which is more likely to induce arrhythmia.

Oxidative stress (OS) and inflammation are important mechanisms that cause cardiomyocyte damage in the "6+1" approach. ISO stimulates myocardial cells to cause ischaemia and hypoxia. OS and inflammatory reactions run through the entire process of myocardial ischaemia. Hypoxia breaks the

balance between the production and scavenging system of free radicals in cells. The number and activity of antioxidant enzymes such as SOD, CAT and GPX decrease, and peroxide gradually accumulates. Excessive production of free radicals leads to OS and myocardial cell injury. Hypoxia can induce neutrophil infiltration and release a large number of inflammatory factors. IL-1β mediates cardiomyocyte apoptosis. IL-6 causes chronic inflammatory response. TNF-α promotes the activation of vascular endothelial cells and leukocytes and produces an inflammatory response, leading to inflammatory injury of cardiomyocytes. During myocardial ischaemia reperfusion injury, the IL-6 concentration reflects the degree of myocardial injury[18]. The results of this experiment showed that the SOD level in the 6+1 group was significantly reduced, while MDA and NO levels were significantly increased; TNF-α and IL-6 levels were also significantly increased, but the increase in IL-1β was not significant, which may be related to the sample size and sampling site. SOD reflects the body's ability to scavenge free radicals, while MDA and NO reflect the degree of free radical attack on cells[19], TNF-α and IL-6 are markers of inflammation in the body. This shows that the rat cardiomyocytes in the "6+1" group were in a more severe state of OS and inflammation. OS stimulates the body's defensive inflammatory response and produces more inflammatory factors; inflammatory factors promote the production of reactive oxygen species[20]. Therefore, OS interacts with inflammation to form a positive feedback loop, and the two interact with cell damage to form a positive feedback loop, which gradually aggravates myocardial cell damage.

In summary, combined subcutaneous plus intraperitoneal injection of ISO is more likely to induce arrhythmia than single injection; the ISO "6+1" mode can establish an ideal rat arrhythmia model. The "6+1" mode induces cardiomyocyte OS and inflammation, leading to cardiomyocyte oxidative damage and inflammatory damage, causing pathological changes in myocardial tissue, increasing myocardial enzymes and troponin, and inducing more serious arrhythmia. In the future, we will further increase the sample size, optimize the grouping, and use "multi factor analysis of variance" and other methods to deepen the mechanism research, so as to provide a simple, stable and repeatable animal model for the study of late arrhythmia mechanism.

Abbreviations

SC: subcutaneous injection; IP: intraperitoneal injection; CK: creatine kinase; cTnI: cardiac troponin I; LDH: lactate dehydrogenase; MDA: malondialdehyde, NO: nitric oxide; SOD: superoxide dismutase; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; IL-1β: interleukin-1β; HWI: heart weight index; VP: ventricular premature beat; VT: ventricular tachycardia; VF: ventricular fibrillation; SVT: sustained ventricular tachycardia; NSVT: nonsustained ventricular tachycardia AVB :atrioventricular block.

Declarations

Data access

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

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Authors' contributions

ZJ G and ZC W: The main contribution is to participate in experimental research, complete the data acquisition, analysis and writing of the article, participate in the revision of the article, finalize the final version, and agree to be responsible for all aspects of the article. KX M: The main contribution is to participates in experimental research, complete the data acquisition, analysis and writing of the article. BZ D and Y Z: The prominent assist is to improve the idea of the article,take part in the revision of the article, confirm the final version. GP M: The outstanding contribution is to analyze the data, draft the important content of the article. WJ L: The substantial contribution is to improve the concept and design of the article, make strict revisions, determine the final version, and consent to be accountable for all aspects of the work. WF G: The substantial contribution is to improve the concept and design of the article, make strict revisions and consent to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The Ethical Review Committee of Hebei College of Traditional Chinese Medicine approved this experimental study

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests', and all authors should confirm its accuracy.

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Figures

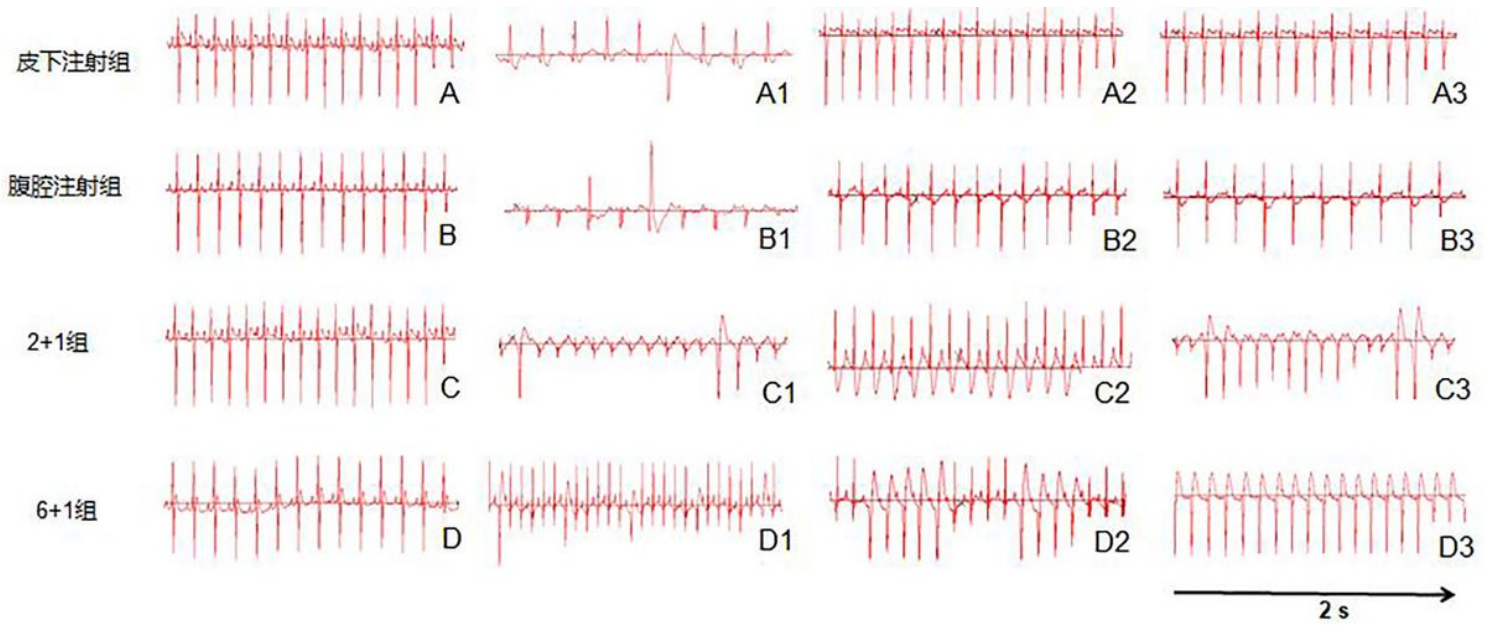

Figure 1

Comparison of ECG of rats in each group. A, B, C, D: the basic ECG of rats in the SC group, IP group, 2+1 group and 6+1 group; A1, B1: the occasional VP in SC group and IP group; C1, D1: frequent ECG of VP in the 2+1 group and 6+1 group; A2, B2: ST-segment slight depression in the SC group and IP group; C2: ST-segment significant depression in the 2+1 group; D2: NSVT in the 6+1 group; A3, B3: ST-segment depression in SC group and the IP group without VT; C3: NSVT in the 2+1 group; D3: SVT in the 6+1 group.

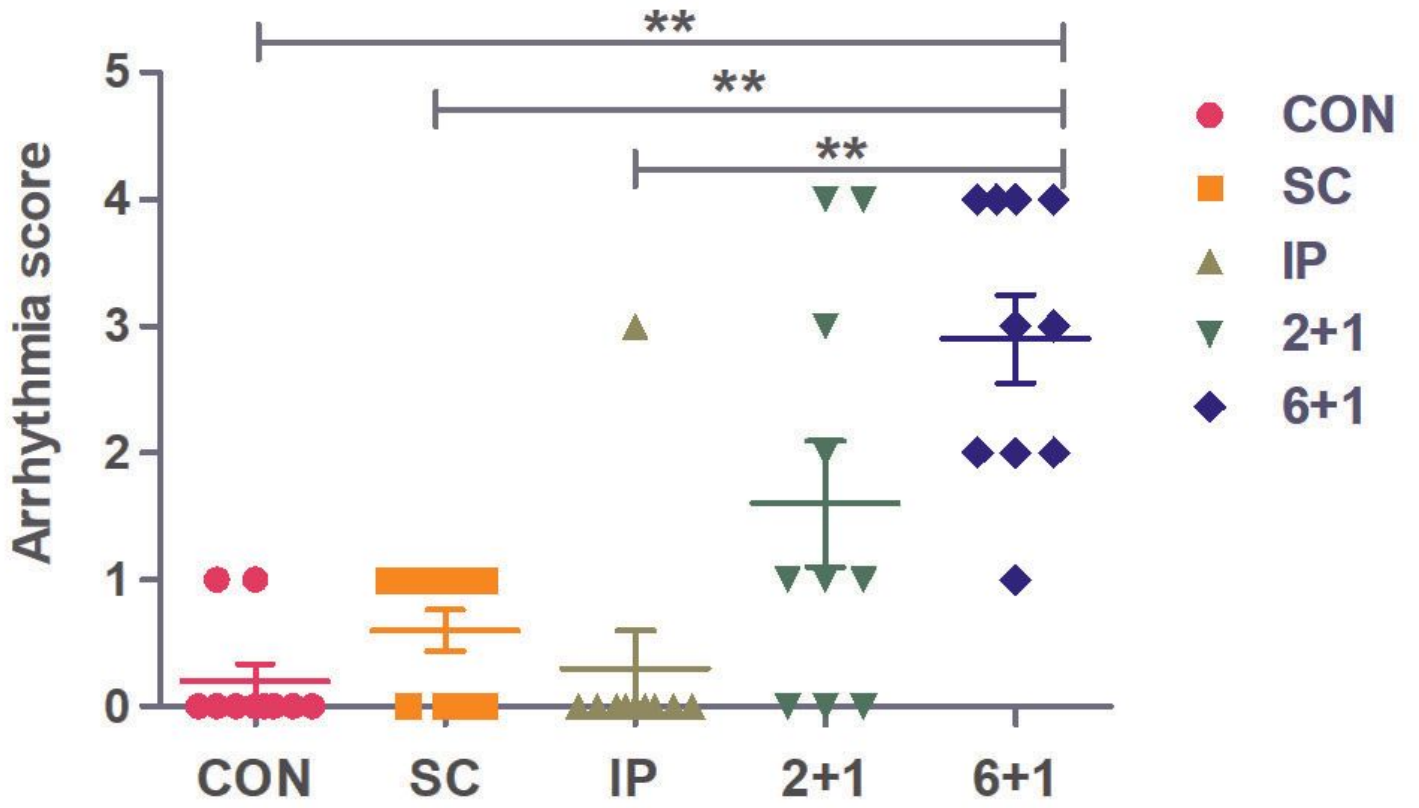

Figure 2

Arrhythmia scores of rats in each group. The data are expressed as the median and interquartile range (n=10). The Kruskal-Wallis H rank test was used, **P□0.01 compared to the CON group.

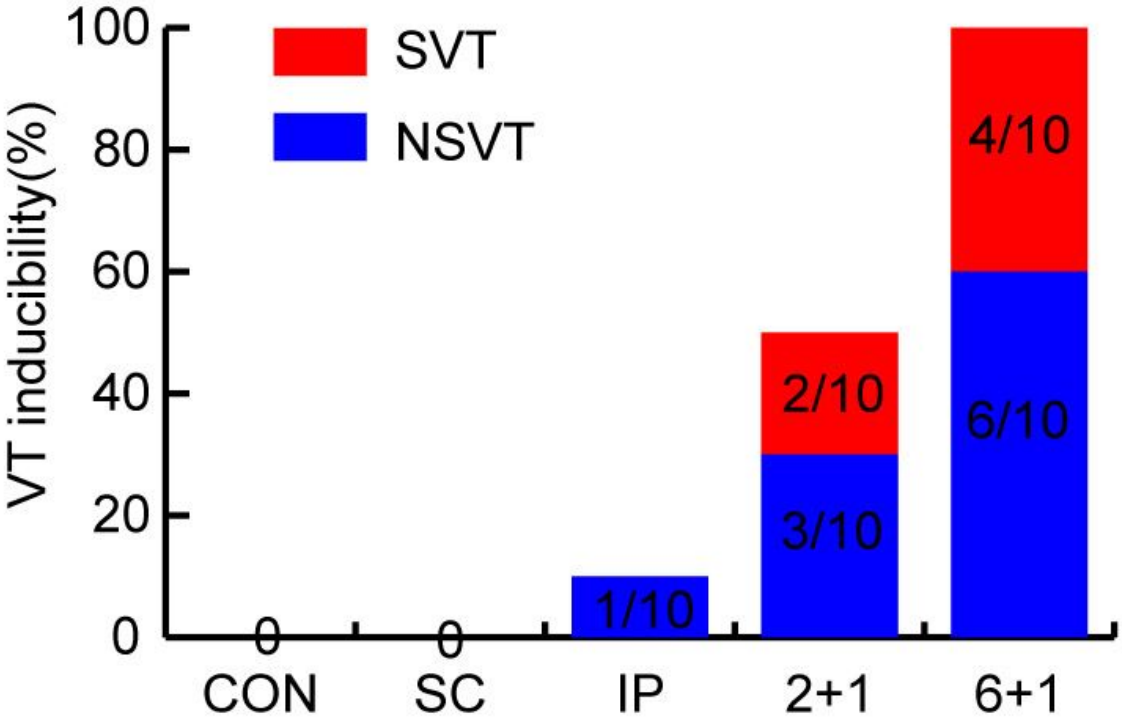

Figure 3

The proportion of SVT and NSVT in rats in each group.

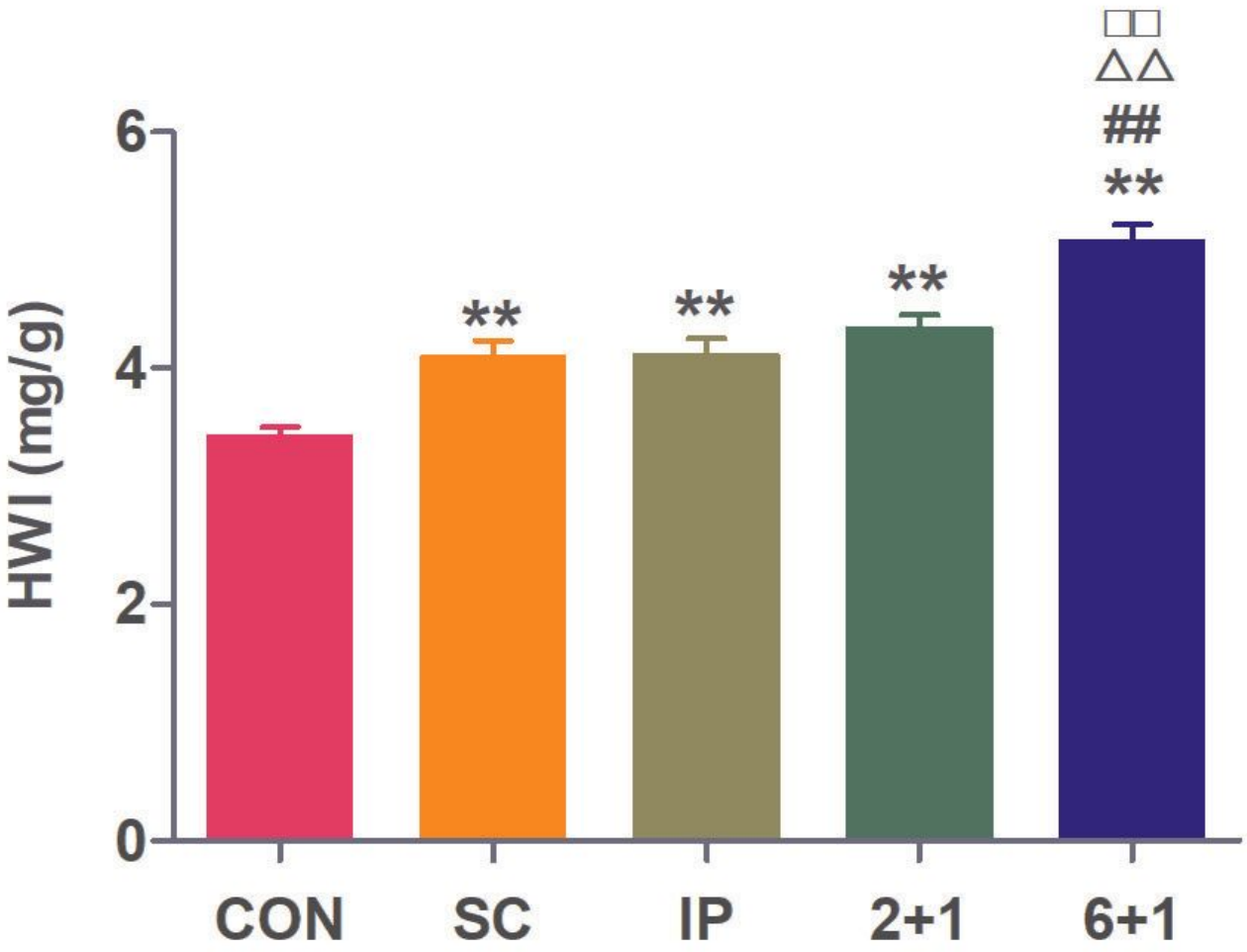

Figure 4

Comparison of HWI of the rats in each group. Data are presented as the mean±SEM (n=10). One-way ANOVA was used, **P□0.01 compared to the CON group, ##P□0.01 compared to the SC group, △△P□0.01 compared to the IP group, □□P□0.01 compared to the 2+1 group.

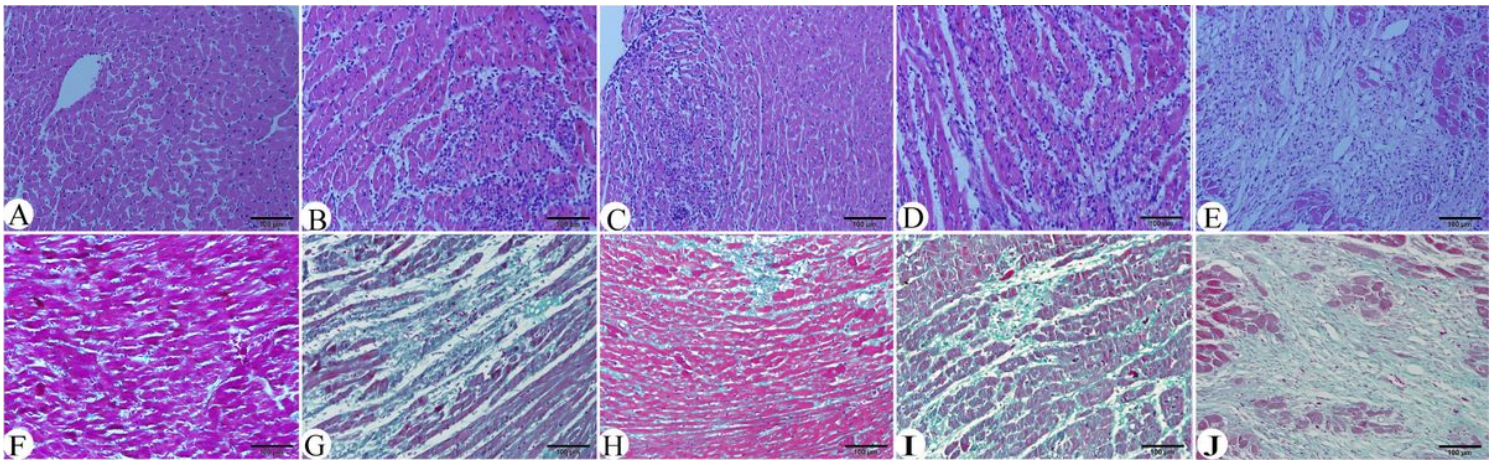

Figure 5

The pathological changes of HE and Masson's trichrome in rat heart tissue (×200). A-E: CON group, SC group, IP group, 2+1 group, 6+1 group, (HE staining). F-J: CON group, SC group, IP group, 2+1 group, 6+1 group (Masson staining).

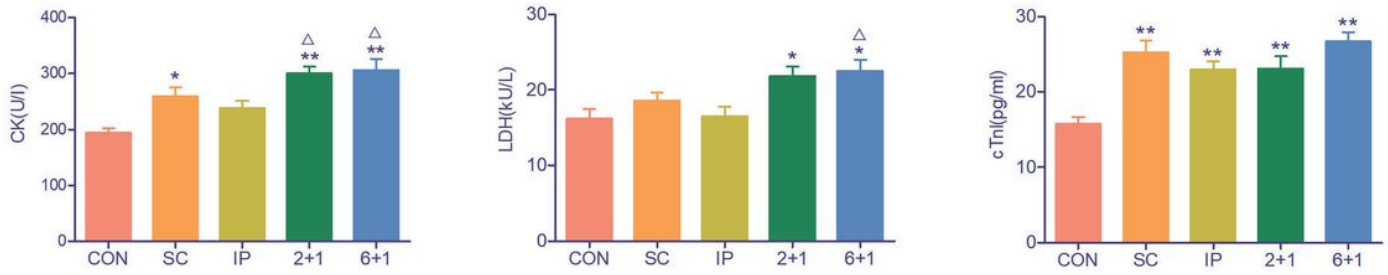

Figure 6

Myocardial injury index levels in each group. Data are presented as the mean±SEM (n=10). One-way ANOVA was used, *P<0.05, **P<0.01 compared to the CON group; △P<0.05, △△P<0.01 compared to the IP group.

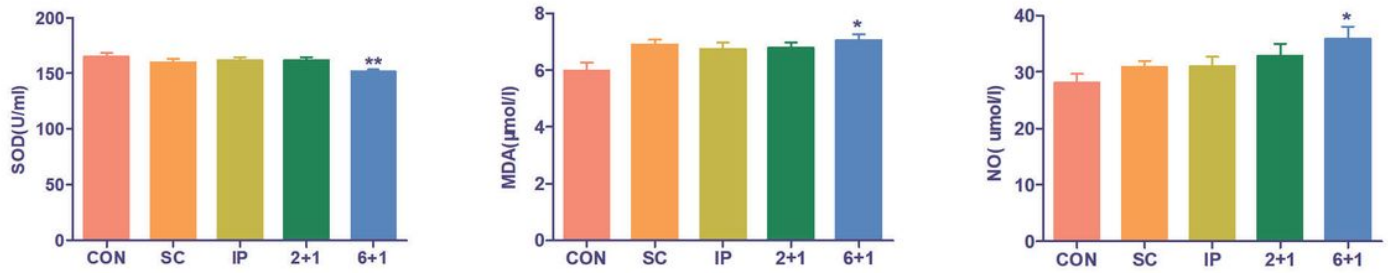

Figure 7

Oxidative stress index levels in each group. Data are presented as the mean±SEM (n=10). One-way ANOVA was used, *P<0.05, **P<0.01 compared to the CON group.

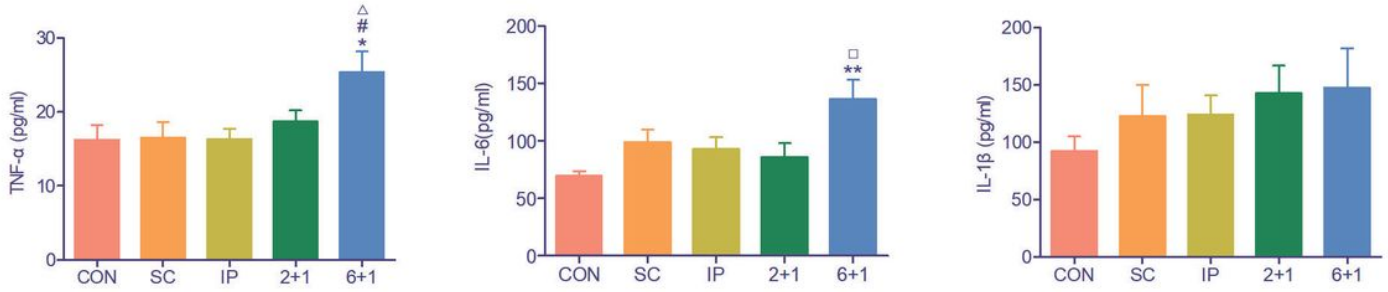

Figure 8

Inflammatory factor levels in each group. Data are presented as the mean±SEM (n=10). One-way ANOVA was used, *P<0.05, **P<0.01 compared to the CON group, # P<0.05 compared to the SC group, △ P<0.05

compared to the IP group, □ P□0.05 compared to the 2+1 group.

Supplementary Files

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