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Original Article

A risk analysis of clarithromycin on sudden cardiac death in rats with chronic heart failure

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Abstract: Administration of macrolide antibiotics, including clarithromycin, may induce dose-dependent cardiac toxicity in patients with heart disease. This study intended to observe the cardiac death risk of clarithromycin on chronic heart failure (CHF) in a rat model and to analyze its impact on cardiac structure, function, and hemodynamics. A CHF model was established using the abdominal aortic constriction method. After 4 weeks, the surviving rats were further randomly divided into the heart failure model group (C, n = 17), the heart failure + clarithromycin high dose group (D, n = 17), and the heart failure + clarithromycin low dose group (E, n = 16). The heart rate variability parameters were recorded. Cardiac structure and function was determined by ultrasonic electrocardiogram. The rats' mortality within 4 weeks was observed. The mortality in groups C, D, and E was 17.65%, 58.85%, and 37.50%, respectively ($P < 0.05$). Groups D and E showed clearly lower LVEF, FS, IVS, and LVPW, but they had higher LVS and LVD compared with group C ($P < 0.05$). Mhp extended in groups D and E compared with group C. APU, APH, and RUN in groups D and E were markedly higher than they were in group C ($P < 0.05$). The APU, RUN, and APH peaks in group D appeared at 5, 5, and 10 minutes, but their peaks in group E occurred at 10, 10, and 5 minutes. Clarithromycin can increase the sudden death risk in CHF rats, which is related to left ventricular dysfunction and a reduced stability in the cardiac autonomic nervous system.

Keywords: Clarithromycin, chronic heart failure, left ventricular reconstruction

Introduction

Clarithromycin, which belongs to the group of macrolide antibiotics, is mainly used for respiratory diseases, skin, soft tissue, and wound infections, such as pneumonia, tonsillitis, bronchitis, and esophagitis, etc. It also can be applied for *Ureaplasma urealyticum* or *Chlamydia trachomatis* induced genital urinary system infection [1, 2]. In recent years, some studies have suggested that macrolide antibiotics may cause cardiac toxicity [3, 4]. Some patients suffered sudden cardiac death or torsade de pointes after erythromycin oral administration or intravenous drip. Clarithromycin oral may lead to QT interval prolongation and torsade de pointes, resulting in fatal arrhythmia, cardiac insufficiency, electrolyte disorders, or cardiac toxicity in patients with idiopathic QT interval prolongation. Thus, the potential for QT interval prolongation risk in patients with cardiovascular risk should be noted when prescribing clarithromycin [5, 6]. Sudden cardiac death is the major adverse cardiovascular event in patients with chronic heart failure (CHF). Sudden death risk is associated with cardiac function classification and left ventricular ejection fraction. The risk of sudden cardiac death elevates in CHF patients with cardiac function deterioration [7, 8]. Numerous studies showed that CF patients with significantly reduced left ventricular ejection fraction (LVEF) (≤ 30%~40%) are at high risk for sudden cardiac death [9, 10]. For coronary heart disease (CHD)/myocardial infarction patients with LVEF ≤ 30%~40%, their sudden cardiac death risk increased 2.2~9.6 times. For non-ischemic dilated cardiomyopathy patients with LVEF ≤ 45%, the risk of sustained ventricular tachycardia/ventricular fibrillation increased 2.28 times per 10% LVEF reduction. There is a close relationship between sudden cardiac death and cardiac function. Worsening heart failure often indicates an increased probability of sudden cardiac death. Heart rate variability (HRV) can quantitatively analyze cardiac autonomic nerve balance and tension [11, 12]. This research study established a CHF rat model,

observed the cardiac death risk of clarithromycin on the CHF rat model, and analyzed its impact on cardiac structure, function, and hemodynamics.

Materials and methods

Materials

Experimental animals and grouping: Healthy male SD rats, 7-weeks old and weighing 200~220 g were provided by the Yan' an University (Shaanxi, China) Animal Experiment Center (license SYXK-2013-0025). Rats were fed in an SPF laboratory according to the established standard for experimental animals and were randomly divided into the control group (A, n = 8), the sham group (B, n = 8), and the model group (n = 60). A CHF model was established using the abdominal aortic constriction method. After 4 weeks, the surviving rats were further randomly divided into the heart failure model group (C, n = 17), the heart failure + clarithromycin high dose group (D, n = 17), and the heart failure + clarithromycin low dose group (E, n = 16).

The rats were used for all the experiments, and all procedures were approved by the Animal Ethics Committee of Yan' an University Affiliated Hospital (Shaanxi, China).

Experimental drugs and reagents: Clarithromycin was provided by Shanghai Xinhua Pharmaceutical Co., LTD. The water for the injections was obtained from Kelun Pharmaceutical Co., LTD. Pentobarbital was bought from the Shanghai Chemical Reagent Co., LTD. Aldosterone and angiotensin II were purchased from the Beijing North Institute of Biological Technology. A MPA-2000 multichannel biological signal analysis system was provided by Second Military Medical University. A Sonos 7500 ultrasonic diagnostic instrument was obtained from Philips Ultrasound (Bothell, Wash., USA).

Experimental methods

Animal model: The CHF model was established using the abdominal aortic constriction method, according to the reference [13]. The rats were anesthetized with a 1% pentobarbital intraperitoneal injection. Their abdominal peritonea were opened from the middle, and the abdominal aortas and left renal arteries were isolated. A bent type 9 needle was put into the long axis of the abdominal aorta of each rat and ligated with the aorta using a 3-0 nonabsorbent silk thread. After removing the needle, the abdominal aorta left a residual cavity of about 1 mm. After being observed for 3 minutes to confirm there was no bleeding, the abdominal cavity on each rat was closed. The vena cavae were only isolated without ligation in the sham group, and the control group received no treatment. Gentamicin was dripped into abdominal cavities after the surgeries to prevent infection. The rats were free to eat and drink after their surgeries. After 4 weeks, no rats in the sham or control groups died, but 10 rats died in the model group (16.67%). Eight rats were randomly selected to have an ultrasonic cardiogram examination performed to determine their left ventricular ejection fraction (LVEF), their interventricular septum thickness (IVS), their left ventricular short axis reduced rate (FS), their left ventricular end-diastolic diameter (LVD), their left ventricular wall thickness (LVPW), and their left ventricular end systolic diameter (LVS). The surviving rats were further randomly divided into the heart failure model group (C, n = 17), the heart failure + clarithromycin high dose group (D, n = 17), and the heart failure + clarithromycin low dose group (E, n = 16).

Medication

Clarithromycin was intravenously injected to the rats in groups D and E at 50 and 100 mg/kg, respectively. The rats in groups A, B, and C received equal amounts of normal saline. After fasting for 8 hours, the rats received abdominal anesthesia and endotracheal intubation. The drug was medicated through the rats' jugular veins within 10 min. A II-lead electrocardiosignal was collected and analyzed by HRV software after Vc-10 oscilloscope amplification. The HRV parameters were recorded at 0, 5, 10, 20, and 30 minutes, respectively. An ultrasonic cardiogram was applied to determine the cardiac structure and function. The rats were observed for 4 weeks and their mortality was recorded.

Statistical analysis

SPSS 19.0 was used for the data analysis. The measurement data conforming to a normal distribution are presented as the mean ± standard deviation ($\bar{X}\pm SD$). The data was compared by a one-way ANOVA or an LSD test. $P < 0.05$ was considered statistical significant.

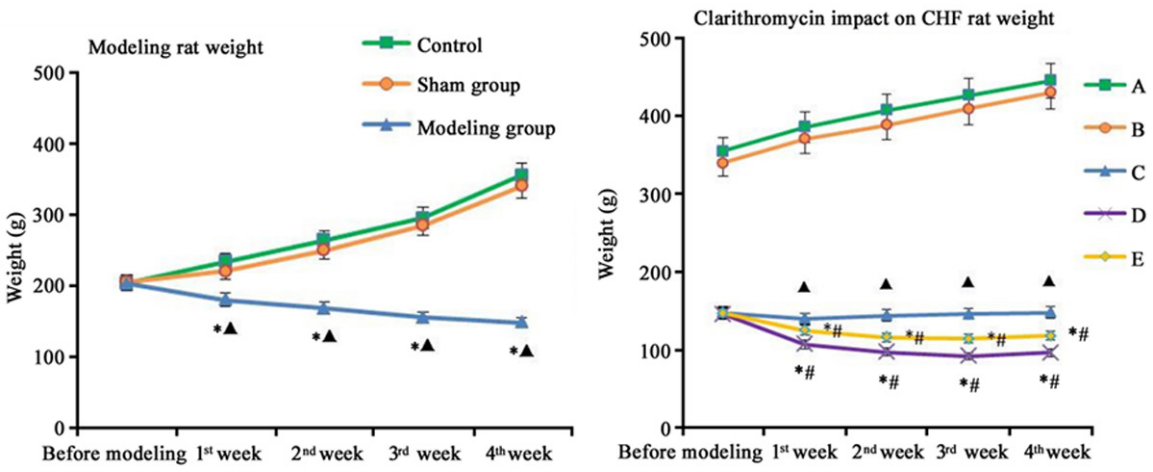

Figure 1. Clarithromycin's impact on CHF rat weight. A. Control; B. Sham group; C. CHF group; D. CHF+ clarithromycin high dose group; E. CHF+ clarithromycin low dose group. *$P < 0.05$, compared with before modeling or medication; ▲$P < 0.05$, compared with the control or sham group; #$P < 0.05$, compared with the CHF group.

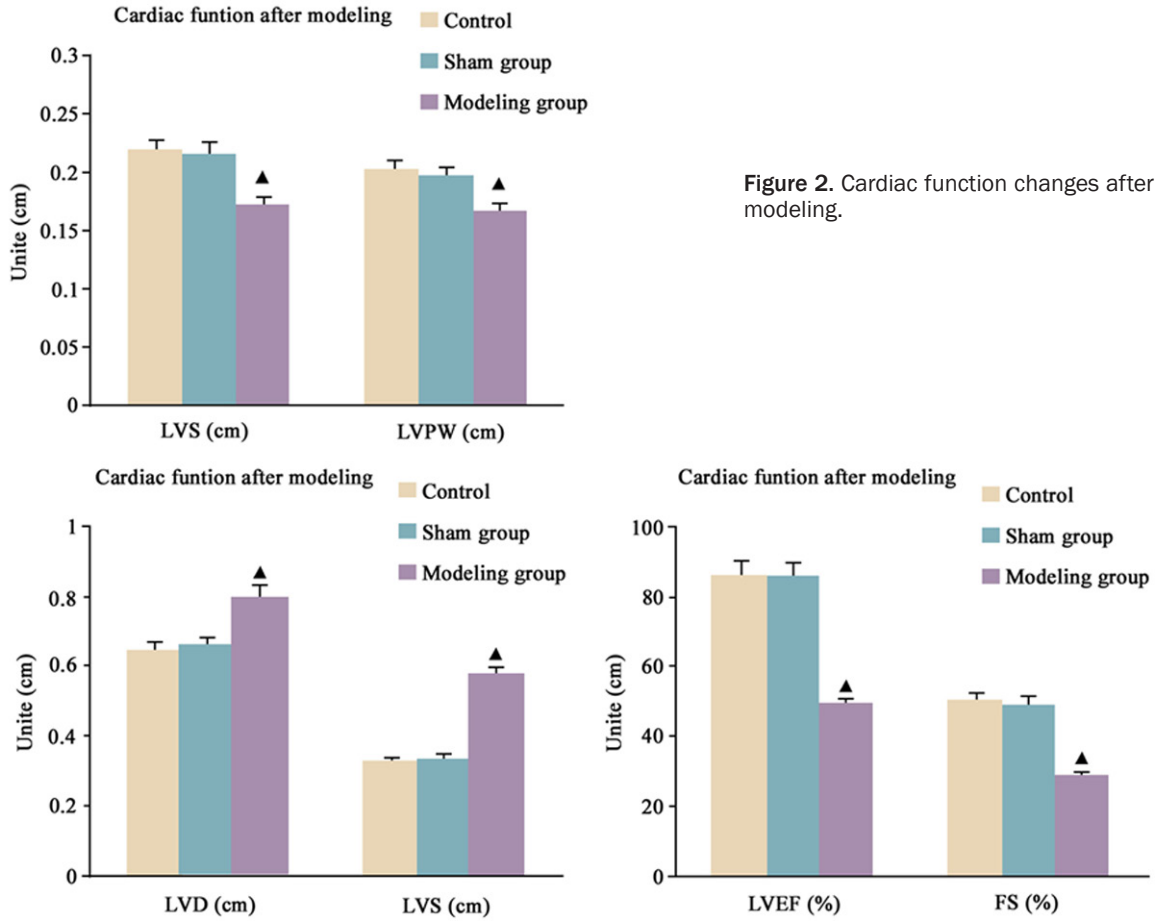

Figure 2. Cardiac function changes after modeling.

Results

The abnormal characteristics and higher mortality rate of the CHF rats

The rats in the control and sham groups showed normal eating and drinking. Compared with the control, the CHF group ate and drank less, had worse nutrition, had loose hair, and showed decreased activity. Ten rats died during the observation as the mortality rate reached 16.67%. The rats were furthered observed for 4 weeks after receiving the medication. Three

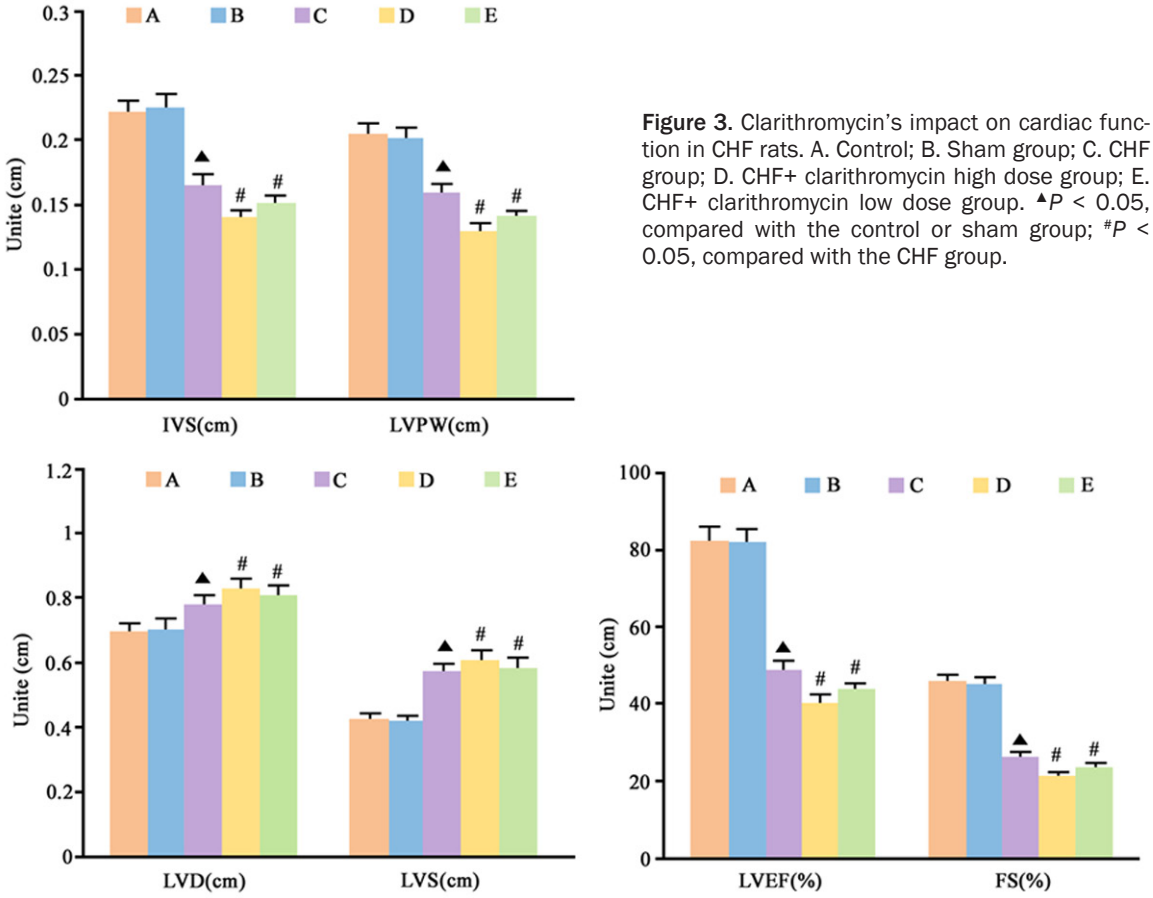

Figure 3. Clarithromycin's impact on cardiac function in CHF rats. A. Control; B. Sham group; C. CHF group; D. CHF+ clarithromycin high dose group; E. CHF+ clarithromycin low dose group. ▲$P < 0.05$, compared with the control or sham group; #$P < 0.05$, compared with the CHF group.

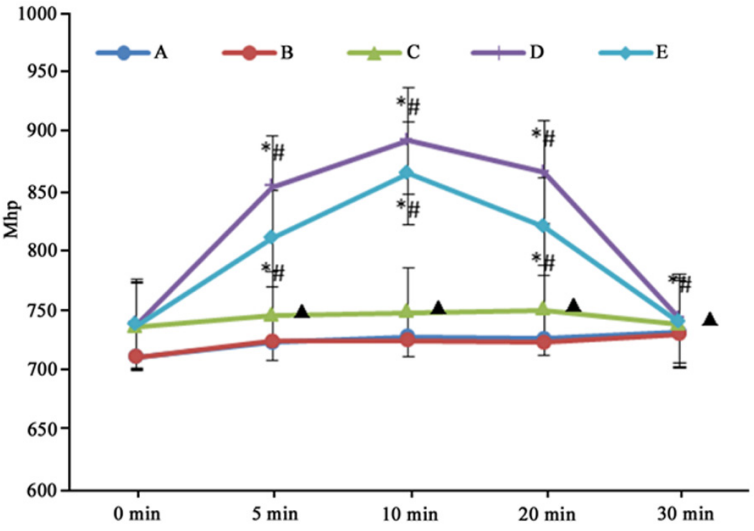

Figure 4. Clarithromycin's impact on Mhp in CHF rats. A. control; B. sham group; C. CHF group; D. CHF+ clarithromycin high dose group; E. CHF+ clarithromycin low dose group. ▲$P < 0.05$, compared with the control or sham group; #$P < 0.05$, compared with the CHF group.

rats died in group C (17.65%), 10 in group D (58.85%), and 6 in group E (37.50%) (χ^2 = 6.1195, P < 0.05). The weight change in each group is shown in **Figure 1**.

Clarithromycin induces abnormal cardiac function

Cardiac function was evaluated at 4 weeks after the modeling. Compared with the sham group, the model group showed clearly lower LVEF, FS, IVS, and LVPW levels and higher LVS and LVD levels (P < 0.05) (**Figure 2**). After receiving the clarithromycin medication, groups D and E presented markedly lower LVEF, FS, IVS, and LVPW levels, and higher LVS and LVD levels than group C (P < 0.05) (**Figure 3**).

Increased Mhp and decreased Mhr in CHF rats after clarithromycin treatment

Compared with the control and sham groups, the rats in group C showed extended Mhp (mean heart period). Mhp was extended in groups D and E compared with group C (P <

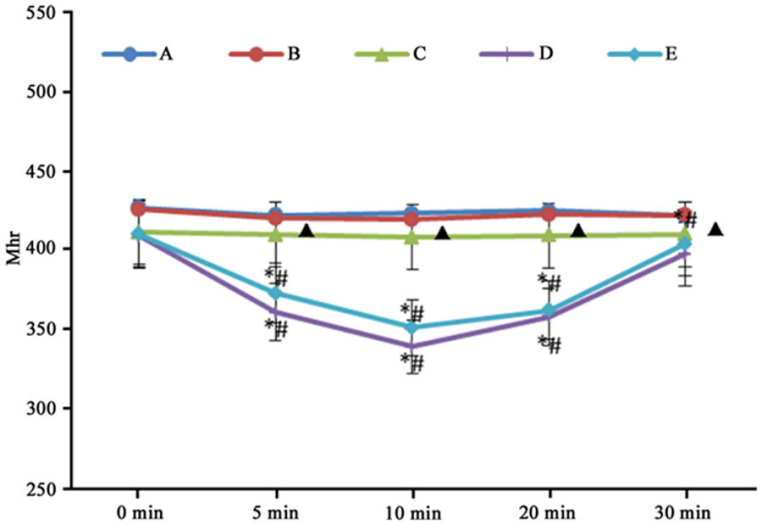

Figure 5. Clarithromycin's impact on Mhr in CHF rats. A. control; B. sham group; C. CHF group; D. CHF+ clarithromycin high dose group; E. CHF+ clarithromycin low dose group. $^{\blacktriangle}P < 0.05$, compared with the control or sham group; $^{\#}P < 0.05$, compared with the CHF group.

0.05). The variation amplitude of Mhp increased following the dose elevation ($P < 0.05$). This suggested that clarithromycin's impact on Mhp is dose dependent. It reached a peak at 10 minutes and recovered after 30 minutes. The mean heart rate (Mhr) went in the opposite direction with the heart period, as it decreased at first and then elevated (**Figures 4** and **5**).

Sympathetic activity enhancement after clarithromycin treatment

APU (absolute power of ultra-low frequency band) is a power in the ultra-low frequency band that reflects sympathetic activity. APH (absolute power of high-frequency band) is a power in the high frequency band that reflects parasympathetic activity. RUN equals APU/APH, which represents the balance between sympathia and parasympathia. RUN (ratio of power in ultra-low to high frequency band) elevation means sympathetic activity enhancement, while its reduction means parasympathetic activity enhancement. APU, APH, and RUN in groups D and E were markedly higher than they were in group C ($P < 0.05$). The APU, RUN, and APH peaks in group D appeared at 5, 5, and 10 minutes, while their peaks in group E occurred at 10, 10, and 5 minutes. APU and APH are presented as natural logarithms (**Figure 6**).

Discussion

Clarithromycin is a type of macrolide antibiotic. Two recent studies reported that macrolide antibiotics can cause cardiac toxicity [14, 15]. The oral administration of clarithromycin may lead to abnormal changes in cardiac electrical activity, resulting in fatal arrhythmias. The risk of sudden cardiac death is associated with cardiac function classification and left ventricular ejection fraction. The risk of sudden cardiac death increases in heart failure patients following cardiac function deterioration [16, 17]. To determine whether sudden cardiac death is related to cardiac function, this research observed the cardiac death risk of clarithromycin in a CHF rat model and analyzed its impact on cardiac structure, function, and hemodynamics. The results showed that 3 rats died in group C (17.65%), 10 in group D (58.85%), and 6 in group E (37.50%). The results indicated that clarithromycin may increase the CHF rat sudden death risk. Furthermore, clarithromycin's impact on CHF rat cardiac function was observed through an ultrasonic electrocardiogram. Cardiac function was evaluated at 4 weeks after modeling. Compared with the sham group, the model group showed observably lower LVEF, FS, IVS, and LVPW, and higher LVS and LVD. Clarithromycin medication significantly lowered LVEF, FS, IVS, and LVPW, and elevated LVS and LVD, suggesting that clarithromycin increases CHF rats' sudden death risk and is associated with left ventricular dysfunction.

The sinoatrial node is mainly controlled by the right sympathetic and vagus nerves. The vagus nerve is distributed more widely than the sympathetic nerve. Vagus nerve excitement may slow the heart rate, but sympathetic nerve excitement may increase it. The impact of sinoatrial node pacemaker cells on vagal excitation is relatively faster than it is on sympathetic nerve action. Cardiac rhythm is basically controlled by the vagus nerve, and the sympathetic

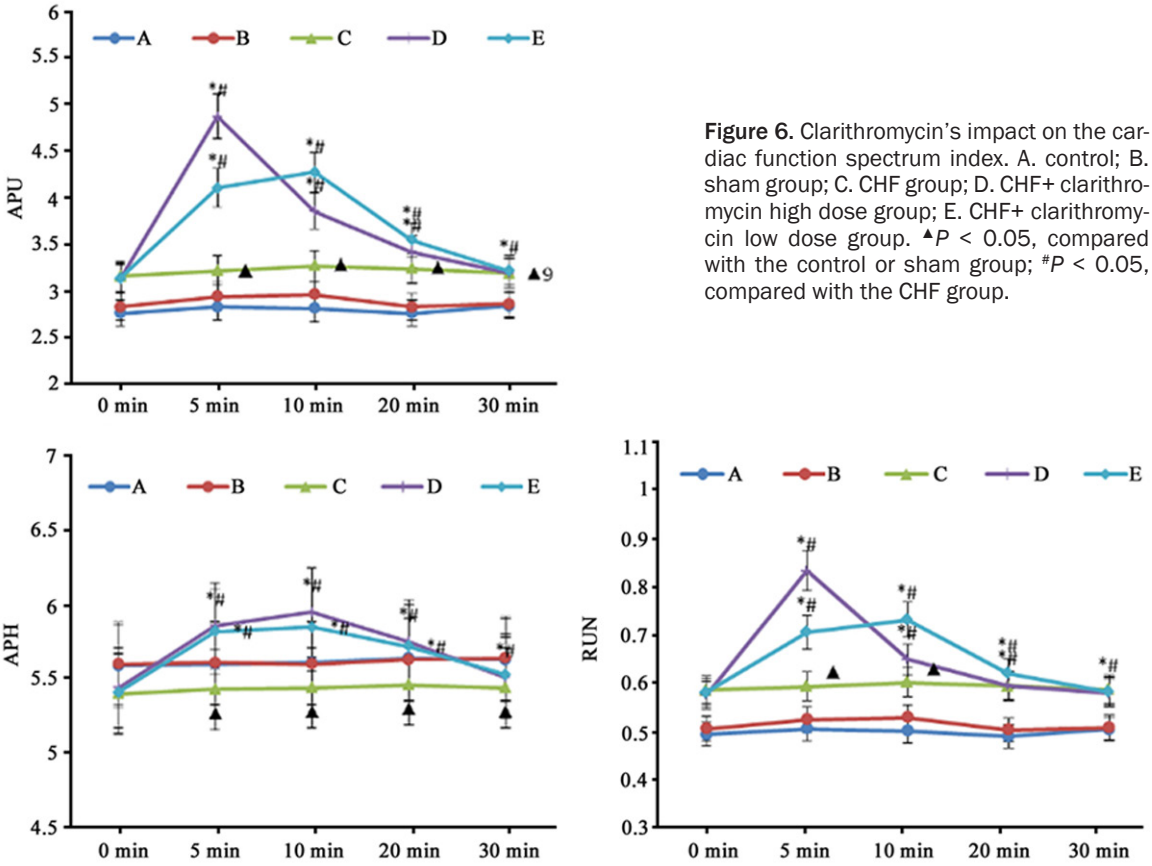

Figure 6. Clarithromycin's impact on the cardiac function spectrum index. A. control; B. sham group; C. CHF group; D. CHF+ clarithromycin high dose group; E. CHF+ clarithromycin low dose group. ▲$P < 0.05$, compared with the control or sham group; #$P < 0.05$, compared with the CHF group.

nerve plays an auxiliary role. Normal heart activity and normal cardiac rhythm are completed by the coordination of the right vagus and sympathetic nerves [18-20]. This study applied the heart rate variation method to analyze clarithromycin's impact on CHF rats. APU is a kind of power in the ultra-low frequency band that reflects sympathetic activity. APH is a kind of power in the high frequency band that reflects parasympathetic activity. RUN equals APU/APH, which represents the balance between sympathia and parasympathia. A RUN elevation means sympathetic activity enhancement, and its reduction means parasympathetic activity enhancement. In the present study, clarithromycin treatment significantly increased Mhp, reduced Mhr, and enhanced APU, APH, and RUN in the CHF model in a dose dependent manner, indicating that both vagus and sympathetic nerve activity were increased in CHF rats after clarithromycin treatment. Since sinoatrial node pacemaker cells have a faster effect on vagal excitation than on the sympathetic nerve, the heart rate change trend is consistent with a high frequency power. However, the exact mechanism by which clarithromycin affects the vagus and sympathetic nerve activities in CHF rats remains poorly understood. Clarithromycin may act on the potassium channels on the myocardial cell membrane, resulting in a repolarization time extension, heart rate reduction, and cardiac output declination, which then enhances the sympathetic nerve and weakens the parasympathetic nerve excitability through the baroreceptor reflex, leading to heart rate recovery. However, further study is required to verify this hypothesis. Based on the present study, our results indicated that clarithromycin may elevate the sudden death risk in CHF rats, which is associated with the reduced stability of the rat cardiac autonomic nervous system.

In summary, clarithromycin can increase the sudden death risk of CHF rats, a risk which is related to left ventricular dysfunction and the reduced stability of the cardiac autonomic nervous system.

Disclosure of conflict of interest

None.

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