Untargeted metabolomics analysis reveals the efficacy of Xinsuning capsules in ameliorating arrhythmias in rats

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Author contributions
Du RJ, Wang RR and Jiang MM designed this project, processed the samples, performed the experiments. Du RJ wrote the article. Lei P, Mwangi CN, Zhou ZR revised the article. Liu CJ, Huang SJ analyzed the data. Jiang MM and Ren M guided the experiment and provided funding for the research.

Competing interests
The authors declare no conflicts of interest.

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Abbreviations
ECG, electrocardiogram; TCM, traditional Chinese medicine; FC, fold change; AA, arachidonic acid; COX, cyclooxygenase; PGF2, prostaglandin F2α; ROC, receiver operating characteristic; CYP450, cytochrome P450.

Citation

Abstract
Xinsuning capsules are a safe and effective drug against phlegm-heat-disturbed arrhythmia (a type of rapid arrhythmia in traditional Chinese medicine. The clinical manifestations include chest tightness, irritability, vomiting, insomnia, and dreaminess) with no noticeable adverse effects. However, the molecular biological basis of Xinsuning capsules in the treatment of arrhythmia is relatively unexplored. Methods: In order to assess the antiarrhythmic effects of Xinsuning capsules, a rat arrhythmia model was constructed by injecting barium chloride into the tail vein. Metabolomic analysis was performed by UHPLC/Q-Exactive-Orbitrap mass spectrometry. The selection of differential variables in the metabolic data were necessitated fold change ≥ 1.5 or fold change ≤ 0.67, together with P < 0.05. Results: Barium chloride-induced arrhythmias in rats were significantly delayed in beginning and cut short in duration, according to electrocardiogram monitoring. Left ventricles of rats exhibited significantly higher Na⁺-K⁺-ATPase activity after taking Xinsuning capsules compared to the model group. Metabolic analysis showed that Xinsuning capsules could regulate arachidonic acid, prostaglandin F2α, 15-HETE, 15-HPETE, 12-HETE, 12(13)-DHOMA, 17B4, inosine, and hypoxanthine. These metabolomics mainly involved arachidonic acid metabolism, unsaturated fat acid biosynthesis, and purine metabolism. Conclusion: The therapeutic effects of Xinsuning capsules against arrhythmia had been investigated with metabolomics, providing the basis for further comprehensive research.

Keywords: arrhythmias; Xinsuning capsules; metabolomics; arachidonic acid metabolism
Medical history of objective
Xinsuning capsule was derived from an ancient formula Huanglian Wendan Tang included in “Liu Yin Tiao bian”. Huanglian Wendan Tang was used to treat insomnia, bile reflux gastritis, non-alcoholic fatty liver disease, H-type hypertension, and other diseases in ancient times. Professor Ding Shuwen developed Xinsuning capsules based on Huanglian Wendan Tang to address the phlegm heat disturbance heart type arrhythmia (premature pulsation).

Introduction
Arrhythmia is a common and serious type of cardiovascular illness, which is the main cause of death worldwide [1–4]. Arrhythmia is an irregularity in the frequency or rhythm of the heartbeat brought on by a blockage at the site of impulse production or impulse conduction. In addition to exacerbating pre-existing heart conditions, it frequently results in unexpected death. Arrhythmias present with a variety of clinical signs and symptoms, depending on the exact conditions. Arrhythmias can manifest clinically as a variety of symptoms, ranging from moderate discomfort in less serious circumstances to abrupt cardiac death and life-threatening cardiac arrest [5]. Unfortunately, arrhythmias are still not adequately treated [6]. A significant part of cardiovascular disease research concentrates on the prevention and treatment of arrhythmias. Arrhythmias can be caused on by a variety of factors, such as taking specific medications, having particular biological cardiac illnesses, or having issues with the nervous or endocrine systems [7]. The pathophysiology of arrhythmias is complex. Currently, anti-arrhythmic medications can be classified into four groups based on their electrophysiological and pharmacological mechanisms, such as sodium channel blockers (I class), beta-blockers (II class), potassium channel blockers (III class), and calcium channel blockers (IV class). The III class of medications is the most popular group, but it is also associated with serious side effects [8].

Xinsuning capsule is an effective formula for treating arrhythmia caused by phlegm heat disturbance, which is derived from an ancient formula Huanglian Wendan Tang included in “Liu Yin Tiao bian”. In ancient times, Huanglian Wendan Tang is used to treat insomnia, bile reflux gastritis, non-alcoholic fatty liver disease, H-type hypertension, and other diseases. Professor Ding Shuwen developed Xinsuning capsules based on Huanglian Wendan Tang to address the phlegm heat disturbance heart type arrhythmia (premature pulsation). In the clinic, Xinsuning capsules are often used in patients with mild to moderate premature ventricular beats caused by coronary heart disease and viral myocarditis. In recent years, with the continuous research and progress on clinical practice and theory, the benefits of traditional Chinese medicine (TCM) in treating cardiac arrhythmias have gradually been apparent [9]. Xinsuning capsules have been approved by the State Food and Drug Administration, which are currently often used to treat cardiac arrhythmias [10]. Eleven botanical drugs that make up Xinsuning capsules are Coptidis Rhizoma (Coptis chinensis Franch.), Pinelliae Rhizoma (Pinellia ternata (Thunb.) Breit.), Poria (Poria cocos (Schw.) Wolf), Aurantii Fructus Immaturus (Citrus aurantium L.), Dichroae Radix (Dichroa febrifuga Lour.), Nelumbinis Pluma (Nelumbo nucifera Gaertn.), Sophorae Flavescentis Radix (Sophora flavescens Ait.), Armeniaceae Annuae Herba (Artemisia annua L.), Ginseng Radix et Rhizoma (Panax ginseng C. A. Mey.), Ophiopogonis Radix (Ophiopogon japonicus (L.) Ker-Gawl.) and Glycyrrhizae Radix et Rhizoma (Glycyrrhiza uralensis Fisch.), mainly used for clearing heat, resolving phlegm, and tranquilizing the heart to calm palpitations [11]. The clinical anti-arrhythmic efficacy of Xinsuning is based on its class I and Class III anti-arrhythmic properties without obvious adverse reactions being reported [12–14]. Furthermore, numerous studies have demonstrated that Xinsuning capsules have great anti-arrhythmic therapeutic benefits [15–18].

Metabolomics is a new historical method used to investigate the comprehensive metabolic profile in biological systems [19]. It examines the dynamics and profile of metabolites in complex samples, which is consistent with TCM’s overarching perspective [20]. It has been extensively applied in a variety of medically related domains, including illness diagnostics, pharmacodynamics, and drug toxicology [4, 21, 22]. Non-targeted metabolomics methods are used to investigate the metabolic profile and biomarkers of cardiovascular disease as well as to find new biomarkers and pathways [23, 24]. There are limited investigations on the biomarkers and therapeutic mechanisms in treating ventricular tachycardia with Xinsuning capsules, despite the existence of a quality control system that correlated efficacy and combined qualitative and quantitative elements of Xinsuning capsules [25].

In earlier researches conducted by our lab, gas chromatography-mass spectrometry and liquid chromatograph mass spectrometer techniques were used to qualitatively analyze the chemical components of Xinsuning capsules. The principal components of Xinsuning capsules were identified using the rapid ultra-performance liquid chromatography combined with electrospray ionization triple quadrupole mass spectrometry (UHPLC-QQQ-MS/MS) method [26]. In this investigation, we verified the efficacy of Xinsuning capsules on treating the rats with arrhythmia caused by barium chloride. The metabolomic analysis based on UHPLC/Q-Exactive-Orbitrap MS was applied to discover prospective biomarkers and metabolic pathways that affected arrhythmia.

Materials and methods
Reagents and materials
Amiodarone was purchased from Sanofi (Hanzhou, China) Pharmaceutical Co., Ltd. (Zhejiang, China). Barium chloride dihydrate was obtained from Tianjin Jingdong Tianzheng Precision Chemical Reagent Factory (Tianjin, China). Carboxymethyl cellulose was purchased from Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Acetonitrile and methanol (Optima® liquid chromatograph mass spectrometer grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Formic acid (MS grade) was purchased from Anaqua™ Chemicals Supply (Wilmington, DE, USA). Ultra-micro Na+–K+–ATPase test kit (No. A0070-2-2), total superoxide dismutase assay kit (A001-3-2), malondialdehyde assay kit (No. A005-1-2) were purchased from Nanjing Jiancheng Biological Engineering Co., Ltd. Xinsuning capsules were supplied by Shaanxi Momentum Qinxeue Pharmaceutical Co., Ltd. (Shaanxi, China, batch No. 20190501).

Animals
Sixty male 8-week-old Wistar rats were obtained from SPF Biotechnology Co., Ltd (Beijing, China) and housed in a temperature controlled (24 ± 2 °C) room at 50 ± 10% relative humidity under a consistent lighting cycle (12 h light/dark). This study was conducted by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Laboratory Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (ethics approval number TCM-LAE20200067).

Diets and treatment
Wistar rats were equally divided into five groups (n = 12) after a week of acclimatization: including control group (C), model group (M), positive drug group (A, I.g. 54 mg/kg of amiodarone), low dosage group (L, I.g. 0.52 g/kg of Xinsuning capsules) and high dosage group (H, I.g. 1.04 g/kg of Xinsuning capsules) for seven days. Xinsuning capsules and amiodarone were made into a suspension with carboxymethyl cellulose sodium salt. C and M groups were also...
gavaged with carboxymethyl cellulose sodium salt suspension. All rats fed a regular diet. On the seventh day, after being fasted overnight, the rats were anesthetized by intraperitoneal instillation of tribromoethanol (0.75 g of tribromoethanol dissolved in 2 mL of tert-amyl alcohol and 48 mL of water, i.p. 0.8 mL/100 g in rat). Electrocardiogram (EGG) was recorded for one hour following medication administration. After the ECG became stabilized, C group was administered physiological saline injections through the caudal vein, while the other four groups received injections of 0.4% barium chloride solutions. ECG was recorded for 30 minutes before rats being sacrificed. Finally, all the rats were euthanized by taking blood from the abdominal aorta. The biological samples were immersed immediately in liquid nitrogen and stored at –80 °C for further analysis.

Biochemical analysis
Weighted left ventricular tissue was added with normal saline in a weight-to-volume ratio of 1:9 and the homogenized on ice. The homogenate was centrifuged for 10 minutes at 4 °C and 3,585 g. After a 10 times dilution, The activity of Na+-K+-ATPase in the supernatant was assayed according to kit instruction.

Serum metabolomic analysis
One hundred microlitre of serum sample was added with 400 μL ice-cold acetonitrile, vortexed for 5 minutes, and centrifuged at 17,209 g for 15 minutes at 4 °C. The solvent was removed under nitrogen blowing and then redissolved in 100 μL of methanol aqueous solution (4:1, v/v) that had been pre-cooled. Samples were centrifuged at 17,209 g for 10 minutes at 4 °C after being vortexed for 2 minutes. The supernatants were collected for analysis. In order to ensure reproducibility and stability throughout the whole analysis procedure, quality control samples were mixed by taking 10 μL from each sample, and placed into every ten samples for testing.

Serum metabolomic analysis was conducted on a Thermo Scientific UltiMate 3,000 ultra-high-performance liquid chromatography system (Thermo Scientific, Waltham, MA, USA) coupled to a Q-Exactive-Orbitrap MS system (Thermo Scientific, Waltham, MA, USA). Separation was performed on a Waters ACQUITY UPLC BEH C18 (2.1 × 100 mm, 1.7 μm) at 30 °C. A binary solvent system was used, in which mobile phase A consisted of 0.1% formic acid water and mobile phase B of acetonitrile. A 9 minutes gradient with a flow rate of 0.4 mL/min was used, 0–9 min, 97%-0% A, 3%-100% B. All the MS spectra were acquired on a Q-Exactive-Orbitrap MS with a heater temperature at 350 °C, sheath gas flow rate of 35 L/h, auxiliary gas flow rate of 10 L/h, sweep gas flow rate of 0 L/h, a capillary temperature at 350 °C, first-level full scan ranges from 100 to 1,500, and data-dependent second-level mass spectrometry (dd-MS2, Top n = 10). The full scan and fragment spectra were collected with resolutions of 70,000 and 17,500, respectively.

Tissue metabolomic analysis
Left ventricular tissue (50 mg) was homogenized with 1.5 mL of ice-cold methanol aqueous solution (2:1, v/v) using an automatic homogenizer (Shanghai Jingxin Industrial Development Co., Ltd, Model TissueLyser-32). Samples were vortexed and centrifuged at 1016 g for 10 minutes at 4 °C. The solvent was removed under nitrogen blowing and then redissolved in 100 μL of pre-cooled methanol aqueous solution (4:1, v/v). Samples were centrifuged at 17,209 g for 10 minutes at 4 °C after being vortexed for 2 minutes. The supernatant was collected for analysis. In addition, quality controls were injected with every ten samples.

Tissue metabolomic analysis was conducted on a Thermo Scientific UltiMate 3000 ultra-high-performance liquid chromatography system (Thermo Scientific, Waltham, MA, USA) coupled to a Q-Exactive-Orbitrap MS system (Thermo Scientific, Waltham, MA, USA). Separation was performed on a Waters ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm) at 50 °C. A binary solvent system was used, in which mobile phase A consisted of 0.1% formic acid water, and mobile phase B of 0.1% formic acid-methanol. A 21 minutes gradient with a flow rate of 0.4 mL/min was used, including 0–2 min: 99.9%–99.9% A, 0.1%–0% B; 2–6 min: 99.9%–75% A, 0.1%–25% B; 6–10 min: 75%–20% A, 25%–80% B; 10–12 min: 20%–10% A, 80%–90% B; 12–21 min: 10%–0.1% A, 90%–99.9% B. All the MS spectra were acquired on a Q-Exactive-Orbitrap MS with a heater temperature at 350 °C, sheath gas flow rate of 35 L/h, auxiliary gas flow rate of 10 L/h, sweep gas flow rate of 0 L/h, a capillary temperature at 350 °C, first-level full scan ranges from 100 to 1,500, and data-dependent second-level mass spectrometry (dd-MS2, Top n = 10). The full scan and fragment spectra were collected with resolutions of 70,000 and 17,500, respectively.

Statistical analysis
The original data was converted into “ab” format using ABF Converter software, and then imported into MS-DIAL software for peak extraction, peak recognition, peak alignment, normalization and other pre-processing to obtain a three-dimensional data matrix containing compound name, retention time, peak area, etc. The database of MS-DIAL software (MSMS-Public-VS15), MS FINDER, Xcalibur and HMDB database were used for metabolite identification. The multivariate statistical analysis was completed using SIMCA-P (Umetrics, Vasterbottens Lan, Sweden). Fold change (FC) values and P values of t-tests for variables in different groups were computed using MetaboAnalyst (www.metaboanalyst.ca). The selection of differential variables in the metabolic data required that FC ≥ 1.5 or FC ≤ 0.75, and P < 0.05. Graphpad prism software was used to analysis biochemical indicators.

Results
Histopathology and biochemical parameters
The results of ECG were shown in Figure 1A and Figure 1B. In comparison to model group, I and H groups experienced greatly delayed arrhythmia beginning (P < 0.05), while L group experienced no appreciable changes. In comparison to model group, I and H groups significantly reduced arrhythmia duration (P < 0.05), while A group experienced no appreciable changes. The effects of Xinsuning capsules on Na+-K+-ATPase activities in left ventricular tissue were shown in Figure 1C. The Na+-K+-ATPase activities in M group were significantly lower than those of C group (P < 0.05). Compared to those of M group, the Na+-K+-ATPase activities in A, H and L groups were significantly higher (P < 0.01, P < 0.001). These findings revealed that H group showed a better effect than that of L group, hence H group was chosen for metabolic analysis.

Metabolomics analysis
Quality control during analysis. In this experiment, the instrument’s stability was evaluated by measuring quality control samples. The principal component analysis scores were shown in Supplementary Figure S1. The fluctuations of quality control samples in the positive and negative ion modes were within the range of 3 times of the standard deviation, indicating that the instrument and method were relatively stable during the experiment.

Multivariate statistical analysis. For the purpose of multivariate statistical analysis, the raw data were processed by MS-DIAL and imported into SIMCA-P14.1 software. Parameters of orthogonal partial least squares discriminant analysis (Figure 2A, 2B) among serum samples in 3 groups were R² X = 0.568, R² Y = 0.811, Q² = 0.687 in positive ion detection mode and R² X = 0.558, R² Y = 0.919, Q² = 0.740 in negative ion detection mode, respectively. Parameters of orthogonal partial least squares discriminant analysis (Figure 2C, 2D) among tissue samples in 3 groups were R² X = 0.722, R² Y = 0.963, Q² = 0.713 in positive ion detection mode and R² X = 0.635, R² Y = 0.977, Q² = 0.791 in negative ion detection mode, respectively. The score plots revealed a substantial clustering differentiation effect between M and C groups, demonstrating how the metabolic profiles of rats were impacted by barium chloride-induced arrhythmia. H group could be easily separated from M group, demonstrating an effectiveness of Xinsuning capsules in treating
Figure 1 Effect of Xinsuning capsules on ECG and Na⁺-K⁺-ATPase in arrhythmic rats between control group C, model group M, positive drug group A, low dosage group L and high dosage group H. (A) The occurrence time of arrhythmia in each group. (B) The duration of arrhythmia in each group. (C) Statistics of Na⁺-K⁺-ATPase contents in each group. (n = 6) *P < 0.05, **P < 0.01 and ***P < 0.001 compared with model group. *P < 0.05 and **P < 0.01 compared with control group. ECG, Electrocardiogram.

Figure 2 The score plots of OPLS-DA among samples in control C, model M, high dosage H groups. (A) Serum samples in positive ion detection mode. (B) Serum samples in negative ion detection mode. (C) Left ventricular tissue samples in positive ion detection mode. (D) Left ventricular tissue samples in negative ion detection mode. OPLS-DA, orthogonal partial least squares discriminant analysis.

arrhythmias.

Screening for differential metabolites. Metaboanalyst platform was used to analysis the metabolomics data matrix after MS-DIAL processing. Non-parametric tests (P < 0.05) and fold change (FC ≥ 1.5 or FC ≤ -0.67) calculations were used to select for screening differential metabolites and plotting volcanoes (Supplementary Figure S2). Compared to the serum samples of C group, 35 metabolites were up-regulated and 34 metabolites were down-regulated in those of M group. Compared to the serum samples of M group, 54 metabolites were up-regulated and 51 metabolites were down-regulated in those of H group (Figure 3A). Compared to the tissue samples of C group, 70 metabolites were up-regulated and 160 metabolites were down-regulated in those of M group. Compared to the tissue samples of M group, 69 metabolites were up-regulated and 34 metabolites were down-regulated in those of H group (Figure 3B). In total, 26 metabolites in serum samples (Figure 4A, Table S1) and 34 ones in tissue samples (Figure 4B, Table S2) were regulated back to normal levels after Xinsuning capsule administration.

Receiver operating characteristic (ROC) curve of differential metabolites were displayed in Supplementary Figure S3, S4. The area under ROC curve between M and C groups was significantly higher than 0.916, indicating great accuracy. Since these metabolites would likewise alter when an arrhythmia occurred, the prediction accuracy was 83%. The area under ROC curve between H and M groups was
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Figure 3 Venn plots for screening differential metabolites. (A) In serum samples. (B) In left ventricular tissue samples.

Figure 4 Heatmaps of differential metabolites that regulated back to normal levels after Xinsuning capsule administration. (A) in serum samples. (B) in left ventricular tissue samples.

significantly greater than 0.807 and the prediction accuracy was 64.7%. It was proposed that Xinsuning capsules might have an anti-arrhythmic effect by altering the expression level of these various metabolites.

Metabolic pathway analysis. Metabolic pathway analysis of 26 different metabolites in serum revealed that they were primarily enriched in the pathways for arachidonic acid and unsaturated fatty acids metabolism. Thirty-four differential metabolites in tissues were largely enriched in purine metabolism (Figure 5A). Xinsuning capsules could significantly back-regulate the flagged metabolites of arachidonic acid, 15-HETE, 15(S)HPETE, leukotriene B4, 15-HETE, prostaglandin F2α, 12,13-DHOME, inosine, hypoxanthine (Figure 5B). Regulated metabolic pathways of Xinsuning capsules against arrhythmias were summarized in Figure 6.

Discussion

Xinsuning capsule, a Chinese patent drug against premature ventricular contractions, had been successful without causing noticeably harmful side effects. In the present work, the anti-arrhythmic effect of Xinsuning capsules was evaluated by a comparison of the period of arrhythmia development and duration across groups in the barium chloride-induced arrhythmia rats. Rat ventricular arrhythmia models were frequently created via coronary artery ligation and drug-induced stimulation [27]. Arrhythmia induction with barium chloride was simple, time-saving, and with stable results [28–31]. Artificial intelligence technology have created a strong foundation for identifying cardiac arrhythmias [32]. It revealed that Xinsuning capsules considerably postponed the start of arrhythmias and shortened their length.
Figure 5 Analysis of relevant pathways of differential metabolites and histograms of relevant metabolites. (A) Metabolic pathway enrichment of different metabolites in serum and tissues. (B) Changes in relative amounts of the flagged metabolites that regulated by Xinsuning capsules in serum and tissues. *P < 0.05, **P < 0.01 and ***P < 0.001 compared with model group. *P<0.05 and **P < 0.01 compared with control group.

Figure 6 Arachidonic acid metabolism and purine metabolism. (The red font represented the differential metabolites which regulated by Xinsuning capsules in the treatment of arrhythmia).
Na\textsuperscript{+}-K\textsuperscript{+} pump is a biofilm enzyme system in cell membrane. Na\textsuperscript{+} -K\textsuperscript{+}-ATPase hydrolyzes adenosine triphosphate to provide energy for sodium and potassium ion transport across the membrane and to maintain a balance between the concentration of ions inside and outside the cell membrane to support typical physiological functions [33, 34]. Inhibiting Na\textsuperscript{+} -K\textsuperscript{+}-ATPase activity results in an inward flow of extracellular Na\textsuperscript{+} and an outward flow of intracellular K\textsuperscript{+} in cardiac myocytes. In addition, the autoregulation of cardiomyocytes increases, which lowers their conductivity and makes them more susceptible to arrhythmias [35]. At the same time, the intracellular potassium ion concentration decreases. It suggested that Xinsuning capsules had a therapeutic impact on arrhythmias due to their effect on the activity of Na\textsuperscript{+} -K\textsuperscript{+}-ATPase.

The developed method known as metabolomics has been included in systems biology [36]. It has been extensively employed in many different fields, including studies on plant composition, microbial changes, the diagnosis and treatment of disease [37–41]. Chinese drugs possess multi-component and multi-target features. Metabolomics is also well-known as a popular method to examine the overall pharmacodynamic effects of Chinese drugs. Metabolomics was employed to investigate the mechanism of the anti-arrhythmic effect of Xinsuning capsules. Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} channels are the most significant channels in cardiac myocytes, and abnormalities in these channels can result in arrhythmias. Na\textsuperscript{+} -Ca\textsuperscript{2+} exchange proteins are active with the increase of intracellular sodium ions, which allow Ca\textsuperscript{2+} to transfer from one cell to another and one result an overload of Ca\textsuperscript{2+}. In mitochondria, Ca\textsuperscript{2+} accumulation interferes with the typical physiological function and preventing the production of adenosine triphosphate. Additionally, Ca\textsuperscript{2+} overload activates proteases which change xantine dehydrogenase into xantine oxidase, increases oxygen radical production and worsens damage to cell membranes [42]. Similarly, phospholipase A is also triggered to degrade phospholipids in biomembranes and release arachidonic acid for damaging cell membranes. Arachidonic acid (AA) is a type of eicosanoid unsaturated fatty acid present in all mammals, and be metabolized by three separate enzymes, including cyclooxygenase (COX), lipoxigenase and cytochrome P450 (CYP450) [43]. Prostaglandins and thromboxanes are the two products of AA catalyzed by COX, which exhibit a wide range of biological functions, and are crucial for inflammatory reactions. Initiating the inflammatory response is mediated by prostaglandin F2a (PGF2), 15-keto-dihydro-PGF2 is a primary metabolite of COX-mediated inflammatory response [44, 45]. We assessed the expression of PGF2 in rat serum, which was elevated in arrhythmic rats, and down-regulated by Xinsuning capsules administration. The anti-arrhythmic activity of Xinsuning capsules was assumed to be a result of an reduction of pro-inflammatory induced by PGF2. When lipoxigenase enzymes are used, AA often derives into some substances with anti-inflammatory properties, such as hydroxyeicosatetraenoic acid series, leukotriene series, and LXs. The 5/12-LOX enzyme convert arachidonic acid into 5/12-15-HPETE and then quickly degrade into 5/12-15-HETE, which are the signals of lipid peroxidation damage [46]. One of these derivates, 5-HPETE is dehydrated to create LTA4, and then break down to produce LTB4. Several different blood cell types produce more pro-inflammatory substances when expose to LTB4, 12-HETE and 15-HETE [47, 48]. Vascular endothelial cells gather leukocytes triggered by LTB4, and produce oxygen radicals which contribute to the formation of inflammatory reactions [49]. Oxygen radicals oxidize unsaturated fatty acids on cell membranes, increase their permeability and fluidity, affect typical physiological functions and induce inflammatory responses [50, 51]. Under the catalysis of CYP450 enzymes, AA generates a series of epoxy compounds. These metabolites are generally believed to possess a number of advantages, such as anti-inflammatory, anti-arrhythmic, and vascular protection.

In addition, CYP450 enzymes also catalyze into epoxycadecanoic acids, which are subsequently hydrolyzed by epoxide hydrolases to produce dihydroxyoacetadecanoic acids. Dihydroxyoacetadecanoic acids have been demonstrated to cause mitochondrial dysfunction, inhibit Na\textsuperscript{+} -K\textsuperscript{+}-ATPase activity, and altered ion channels in cardiac myocytes [52–54].

In this study, Xinsuning capsules have been demonstrated to influence the metabolism of arachidonic acid, the production of unsaturated fatty acids, and the metabolism of purines in arrhythmic rats. It provides a possible action mechanism of the treatment of arrhythmia with Xinsuning capsules administration. It still has several limitations. Metabolic pathways had only been discovered from a metabolomics perspective, and biological validation had not yet been conducted.

**Conclusion**

Our study provided a theoretical foundation for the use of Xinsuning capsules to treat arrhythmias. The results of metabolomics analysis revealed that Xinsuning capsules could alleviate arrhythmias by regulating arachidonic acid and purine metabolism.

**References**


25. Zhang XX. Study on the material basis of Xinsunxing capsules. Beijing Univ Chin Med 2017. (Chinese) Available at: https://kns.cnki.net/kcms/article/aspect?v=0VG0WnPAtl4F-_2WEGO3qQw5WshpPXRq2uvNVCfYz2Z3eI,MXZlbvR-NK5R4vP1LP9JblvBsk3lHxrlDq9rPq2BYY1Pf3S3PBYLzhJozZekKLZJEU1QIb5WyGNQpdS30E &uniplatform=NZKPT&langage=CHS


35. Yan Z. Effect of Dingjinfumai Decoction on Electrocardiogram and Myocardial Cx43 in Rats with Ventricular Arrhythmia. Southwest Med Univ 2018. (Chinese) Available at: https://kns.cnki.net/kcms/article/aspect?v=0VG0WnPAtl4D39xeBWzvzMDH3CPWu8CuZfsQk7L7Qqj_1JyBL675USpPj-hzwLZw_09e0CeDI0Ge6V4Yx0xaBx8E65H6eBsn5mXvYDbvSG8P9Wkl4ZV4CvCx4x0uIOiXPS &uniplatform=NZKPT&langage=CHS


40. Ciborowski M, Teul J, Martín-Ventura JL, Egido J, Barbás C. Metabolomics with LC-QTOF-MS Permits the Prediction of Disease Stage in Aortic Abdominal Aneurysm Based on Plasma


