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Study on the Absorption Components of Xin-Su-Ning Capsule in Rat Plasma and Myocardial Tissue Based on Uhplc-Qqq-Ms/Ms Analysis

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Keywords:

Xin-su-ning Capsules; Plasma Components; Myocardial Tissue; Pathway prediction; Arrhythmia

1. Abstract

1.1. Background: The absorption process of Xin-su-ning Capsules (XSNC) in rats has not been reported, so this study studied the absorption components of XSNC in rat plasma and myocardial tissue based on the chemical composition characterization methods of XSNC. To elucidate the absorption process of XSNC in rats and provide a reference for the clinical use of XSNC.

1.2. Methods: UHPLC-QQQ-MS/MS was used to characterize the chemical components of components of XSNC in rat plasma and myocardial tissue, and network pharmacological analysis was carried out on the identified components.

1.3. Results: The method validation of the six compounds in rat plsam was performed with acceptable linearity (R2, 0.9994-0.9999), stability (RSD≤4.62%), measured concentration (RSD≤13.04%) and extraction recovery and matrix effect (85.97%-110.74%); the method validation of the five compounds in rat myocardial tissue was performed with acceptable linearity (R2, 0.9934-0.9992), stability (RSD≤2.78%), measured concentration (RSD≤14.03%) and extraction recovery and matrix effect (85.05%-108.33%). The results of GO and KEGG pathway enrichment analysis showed that the Calcium signaling pathway can be verified as the main pathway of arrhythmia.

1.4. Conclusion: In rat plasma, matrine has the highest concentration peak, and palmatine has the lowest concentration peak; in rat myocardial tissue, matrine has the highest concentration peak, and nobiletin has the lowest concentration peak.

2. Introduction

Cardiac arrhythmias are the abnormalities or perturbations in the normal activation or beating of heart myocardium [1], is a major cause of morbidity and mortality worldwide, Cardiac arrhythmias affect about 2% of community-dwelling adults, with an incidence of about 0.5% per year, sudden arrhythmias, such as ventricular tachycardia and ventricular fibrillation, account for 10% to 20 % of all deaths in the United States [2]. Arrhythmias and sudden cardiac death are one of the sources of serious cardiovascular complications, sudden cardiac death is the most common cause of ventricular arrhythmias, accounting for about 25% to 50% of all cardiovascular related deaths. Moreover, the high incidence, prevalence and lifetime risk make arrhythmia an important epidemiological and public health problem, which brings a heavy burden to patients and society [3]. People pay more and more attention to reducing this burden. Arrhythmia is a common and extremely dangerous cardiovascular diseases, it can not only aggravate the pre-existing heart disease, but also cause sudden death of patients [4], anti-arrhythmic therapies are based on ion channel-blocking drugs that further downregulate these channels and exhibit proarrhythmic risk [5]. For example, amiodarone and other class I and III antiarrhythmic drugs are commonly used to treat arrhythmias, but there are significant side effects. Long-term use can cause serious side effects on multiple organs and tissue types, including the

heart, such as inducing significant bradycardia. Ocular changes are also the most common, with corneal microdeposition occurring in up to 98% of patients [6]. There is a clear need for effective drugs with better safety in the long-term use of drugs in the treatment of arrhythmias. Due to the limitations of chemical drugs in the treatment of arrhythmia, traditional chinese medicine may have potential advantages in the treatment of arrhythmia, and traditional chinese medicine is increasingly recognized in the treatment of arrhythmia. Related studies confirmed that [7] XSNC double-blind, placebo-controlled, multicenter clinical trial recruited 861 patients (CHICTR-TRC-14004180), and the results showed that XSNC is an effective multi-component antiarrhythmic drug for the treatment of ventricular arrhythmias with no side effects on patients. By blocking hERG potassium channels and hNaV1.5 sodium channels of class I and class III anti-arrhythmic mechanism of strong support, and extend the AP duration of ventricular muscle cells to anti-arrhythmic [8]. UHPLC-QQQ-MS/MS is an important method for quantitative analysis of multi-component Chinese herbal compounds [9]. Since the absorption components of XSNC in rat plasma and myocardial tissue have not been reported, a UHPLC-QQQ-MS/MS method was developed to determine the content of the relevant components of XSNC in rat body. The quantification of XSNC components can provide a reference for its quality evaluation and lay a foundation for further research on its pharmacological substances and clinical medication.

3. Materials and Methods

3.1. Materials and Reagents

Methanol and acetonitrile (chromatographic purity) was purchased from Fisher

company (USA), formic acid (MS grade) was purchased from ACS company (USA), and distilled water was purchased from Guangzhou Watsonsfood and beverage company (Guangzhou, China), Reference standards of sophocarpine, matrine, berberine, palmatine, Tangeratin, nobiletin were purchased from Sichuan Weikeqi Biotechnology Co., Ltd. XSNC were supplied by Shanxi Momentum Pharmaceutical Co., Ltd (Shanxi, China); Sodium carboxymethyl cellulose was purchased from Dalian Meilun Biotechnology Co., LTD. (Dalian, China) .

3.2. Analysis of the Components of plasma of XSNC and Discussion of its Changes During the Time Period

Male SD rats (200±20g, n=6) were given XSNC suspension (12g/kg) [10], the dosage volume was 10ml/kg, blood samples were collected from the orbital vein at 0, 0.083, 0.167, 0.333, 0.667, 1, 1.5, 2, 4, 6, 8, and 10 h after XSNC administration.

3.3. Analysis of the Components of Myocardial Tissue Distribution of XSNC and Discussion of its Changes During the Time Period

After fasting for 12 h, 30 Sprague-Dawley (SD) rats were randomly divided into 5 groups, each with 6 rats (Grouped by time of administration). Single intragastric administration of XSNC suspension (6g/kg) [11], then the rats were decapitated 0.25h, 0.5h, 1h, 2h, and 4h after gavage, and blood was taken immediately, after perfusion and cleaning with 0.9% Nacl, removing a small amount of sedimentation and tissue inclusions, removing the water with filter paper and storing at -80℃. Another 6 male SD rats were selected as blank control group,the blank control group was given the same volume of 0.9% Nacl, and the sampling method was the same.

3.4. Prediction of Effective Targets Based on Network Pharmacology “plasma components - Disease Targets”

The structures of the six plasma components were obtained in PubChem database (https://pubchem.ncbi.nlm.nih.gov). Then, the targets of drug components were detected from the TCMSP database (http://tcmspw.com/tcmsp.php). The targets of arrthymia were obtained in the OMIM database(http://omin.org/), PharmGKB database (http:// pharmgkb.org/home/), Genecard database (https:// www.Genecards.org/), TTD database (http://db.idrblab.net/TTD/). The common targets of drug components and arrhythmia-related targets were detected. The XSNC targets and arrthymia-related gene set was used to construct PPI network by using STRING database (https://string-db.org/). The PPI network from STRING was then imported into Cytoscape 3.9.0 to construct a compounds-target-disease network. The David database (https://david.ncifcrf.gov) was used to performed enrichment analysis, including gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Potential personalized treatment mechanisms are revealed from biological processes (BP), cellular components (CC), molecular functions (MF), and key pathways.

3.5. Sample Solutions Preparation

3.5.1. Plasma Sample

The 50μL plasma sample was put into EP tube, and 150μl acetonitrile, 50μl internal standard working solution and 50μL 50% acetonitrile solution were added successively, then the sample was fully dissolved by vortexing for 5min. After centrifugation at 10000 r/min for 10min at 4℃, the solution was filtered through a 0.22 micron syringe. The filtrate was stored at 4 ℃ in preparation for UHPLC-QQQ-MS /MS quantitative analysis.

3.5.2. Tissue Sample

About 100mg of myocardial tissue was accurately weighed, 0.9% Nacl (W/V=1:3) and 3uL of internal standard solution were added, the tissue was cut and ground. The obtained ground supernatant was centrifuged at 4℃ for 10min, and then the supernatant was carefully absorbed to 200μL, then 600μL methanol was added, and the supernatant was vortexed for 1min. After centrifugation (as above), the supernatant was taken, the precipitate was mashed and then 600μL methanol was added again for 10000r/min. After centrifugation for 10min, the supernatant was combined with the

previous two tissue supernatants, blown dry with nitrogen, and re-dissolved with 200μl methanol. After centrifugation for 10min at 10000r/min, the supernatant was taken again, and the solution was filtered through a 0.22μm syringe filter. The filtrate was stored at 4℃ to prepare for UHPLC-QQQ-MS /MS.

3.6. Standard Solutions Preparation

3.6.1. Plasma Sample

The standards for sophocarpine, matrine, berberine, palmatine, tangeratin, nobiletin were weighed accurately and dissolved in methanol for preparation of single reference substance mother solutiou. The concentrations of reference substance mother solutions were as follows: sophocarpine 750 ng/ml, matrin 6000 ng/ml, berberine 60 ng/ml, palmatine 30 ng/ml, tangeratin 60 ng/ml, nobiletin 60 ng/ml. Preparation of standard solutions: The highest concentration of the mixed reference solution was diluted in methanol 1:1 (v: v) for 2, 4, 8, 16, 32, 64 times, etc., to obtain a series of mixed reference solutions.

Concentration of internal standard solution: etrahydropalmatine 10 ng/ml.

3.6.2. Tissue Sample

The standards for sophocarpine, matrine, berberine, tangeratin, nobiletin were weighed accurately and dissolved in methanol for preparation of single reference substance mother solution. The concentrations of reference substance mother solutions were as follows: sophocarpine 3000 ng/ml, matrin 6000 ng/ml, berberine 1200 ng/ml, tangeratin 1200 ng/ml, nobiletin 600 ng/ml. Preparation of standard solutions: The highest concentration of the mixed reference solution was diluted in methanol 1:1 (v: v) for 2, 4, 8, 16, 32, 64 times, etc., to obtain a series of mixed reference solutions. Concentration of internal standard solution: tetrahydropalmatine 200ng/ml.

3.7. Chromatographic and Mass Spectrographic Conditions

Chromatographic analysis was performed on an ACQUITY UPLC Ultra Performance Liquid Chromatograph (American, waters company); Chromatographic separation was conducted on a Waters UPLC ACQUITY BEH C18 (1.7 μm, 2.1 mm×100 mm) maintained at 35 ℃, the mobile phase consisted of 0.1% formic acid solution (A) and acetonitrile (B) using a gradient elution as following: 0-2 min, 5-10% B; 2-5 min, 10-20% B; 5-8 min, 20-25% B; 8-10 min, 25-30% B; 10-15 min, 30-45% B; 15-20 min, 45-95% B; the flow rate was kept at 0.3mL/min [12]. MS detection was performed on Waters Xevo TQ-S Triple Quadrupole Mass Spectrometer (American, waterscompany). Quantification was performed using multiple reaction monitoring (MRM) mode. The optimized MS conditions for the positive ion mode were as follows: capillary voltage 3.0 KV, cone voltage 30 V, solvent removal temperature 350℃. The optimized MS conditions for the negative ion mode were as follows: capillary voltage 2.0 KV, cone voltage 37 V, desolvation temperature 350℃ [12]. The mass spectrometry analysis conditions of the 16 compounds were optimized and summarized in (Table 1).

Table 1: Condition parameters of mass spectrometry analysis of 6 compounds in Xin-Su-Ning capsules

Compound	Formula	Parent	Daughters	CV	CE	Detection mode
Sophocarpine	C15H22N2O	247.14	136.13	82	28	positive
Matrine	C15H24N2O	249.16	148.16	80	26	positive
Berberine	C20H18NO4	336.16	320.24	20	28	positive
Palmatine	C21H22NO4	352.12	308.13	62	28	positive
Tangeretin	C20H20NO7	373.03	343.14	26	26	positive
Nobiletin	C21H22NO8	403.1	373.15	84	26	positive

3.8. Verification of UHPLC-QQQ-MS /MS Method

The analytical method was validated according to the bioanalyticalmethod guidelines suggested by Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission 2020). The linear relationship, precision, extraction recovery and matrix effect, the method of establishing UHPLC-QQQ-MS/MS in plasma and myocardial tissue was verified. Taking plasma samples as an example, the specific method verification is as follows: the standard curve was drawn with the ratio of the peak volume of each analyte to the peak volume of the internal standard solution as the Y-axis and the mass concentration of each analyte as the X-axis; the intraday accuracy and interday accuracy was assessed by calculating QC samples (low, medium, and high), measured six times a day for three consecutive days; the extraction recoveries and matrix effects were obtained using three different concentrations of high, medium, and low concentrations of QC samples, as well as six times in parallel for each concentration, set A:the blank plasma samples treated with “2.5.1.” plus QC samples with high, medium and low concentrations were analyzed by UHPLC-QQQ-MS/MS; set B: the blank plasma samples plus QC samples with high, medium and low concentrations were processed by “2.5.1.” for UHPLC-QQQ-MS/MS analysis; set C: for water to replace blank plasma samples with high, medium, and low three concentrations of QC samples by UHPLC-QQQ-MS/MS analysis. The peak areas are A, B and C, extraction recovery rate=A/B×100%, matrix effect rate=B/C×100%; the stability of the high-, medium-, and low-concentra-

tion QC samples under four conditions was investigated: the samples were placed in the refrigerator at 4°C for 0h, 2h, 4h, 8h, 16h, and 24h for UHPLC-QQQ-MS/MS analysis. Finally, the RSD% value of QC was calculated according to the sample peak area.

4. Results

4.1. Methodological Validation of Plasma Samples

LC-MS was used for the quantitative analysis of sophocarpine, matrine, berberine, palmatine, Tangeratin, nobiletin. The 6 index components had a good linear relationship within the corresponding concentration range, and their R2 were all greater than 0.999, the results were shown in (Table 2). The relative standard deviation (RSD) values of stability, measured concentration were all less than 4.62 %, 13.04%, indicating that the instrument had good precision, the method had high repeatability, and the sample solution was stable for 24 h at room temperature. The sample extraction recovery and matrix effect RSD were between 85.97%-110.74%, indicating that the recovery rates of the 6 compounds in XSNC were good,the results were shown in (Table 3). The positive ion mode Xin-Su-Ning capsule TIC is shown in (Figure 1). Multiple reaction monitoring (MRM) is a highly specific and sensitive mass spectrometry technique for quantifying predefined compounds of interest. The UHPLC-MS/MS analysis method described above was subsequently used to simultaneously quantify 6 compounds of XSNC in rat plasma, the results were shown in (Table 2).

Table 2: Linearity, concentration of 6 analytes in UHPLC-QQQ-MS/MS

Compound	Regression equation	R^2	Linearity range (ng/ml)	Compounds concentration								
				0.083h (ng/ml)	0.167h (ng/ml)	0.25h (ng/ml)	0.333h (ng/ml)	0.5h (ng/ml)	1h (ng/ml)	2h (ng/ml)	4h (ng/ml)	6h (ng/ml)
Sophocarpine	y = 0.1189x - 0.0255	R^2 = 0.9995	0.50-125	9.05±2.41	16.03±1.21	40.79±10.14	44.56±6.50	73.87±3.74	79.80±3.72	31.34±6.54	19.24±2.95	16.30±4.47
Matrine	y = 0.0362x - 0.4000	R^2 = 0.9998	8-1000	185.20±41.58	226.90±15.67	367.00±46.12	396.90±47.20	872.70±6.83	806.70±63.07	416.20±58.07	396.90±38.52	374.70±65.23
Berberine	y = 0.2951x + 0.0378	R^2 = 0.9996	0.04-10	0.47±0.13	0.48±0.11	0.73±0.06	0.97±0.14	0.69±0.13	0.28±0.03	0.15±0.07	0.15±0.04	0.13±0.01
Palmatine	y = 0.2197x + 0.0063	R^2 = 0.9999	0.02-5	0.17±0.02	0.27±0.08	0.28±0.02	0.48±0.06	0.33±0.06	0.15±0.03	0.14±0.02	0.12±0.02	0.07±0.02
Tangeretin	y = 0.9618x + 0.3233	R^2 = 0.9994	0.04-10	0.31±0.01	0.32±0.02	0.40±0.12	1.02±0.01	0.76±0.01	0.44±0.07	0.19±0.06	0.18±0.02	0.08±0.01
Nobiletin	y = 1.0641x + 0.0464	R^2 = 0.9998	0.04-10	0.40±0.14	0.47±0.10	0.64±0.19	1.71±0.13	1.41±0.12	1.31±0.16	0.61±0.08	0.24±0.08	0.16±0.07

Table 3: Stability Measured concentration Extraction recovery and Matrix effect of 6 analytes in UHPLC-QQQ-MS/MS

Compounds	Stability RSD (%)	Concentration (ng/ml)	Intra-day (n=6)		Inter-day (n=18)		Extraction recovery	Matrix effect
			Measured concentration (ng/ml)	RSD (%)	Measured concentration (ng/ml)	RSD (%)	RSD (%)	RSD(%)
Sophocarpine	1.92	12.5	10.97±1.26	4.01	10.08±1.33	4.99	102.53	108.17
		31.25	30.24±0.89	3.74	29.51±1.33	3.54	103.61	101.41
		100	91.91±1.18	1.98	87.86±1.78	1.57	88.79	100.54
Matrine	4.62	100	100.40±3.23	7.78	93.76±2.20	5.98	99.04	101.47
		250	245.30±24.51	7.53	234.80±7.06	8.26	103.12	91.28
		800	717.70±18.54	7.42	713.90±7.42	6.49	92.95	99.65
Berberine	4.27	0.5	0.47±0.10	10.68	0.53±0.28	10.08	110.74	95.08
		1.25	1.26±0.04	3.72	1.19±0.03	3.35	95.73	91.88
		4	3.95±0.87	4.05	3.52±0.24	2.8	93.66	108.45
Palmatine	3.75	0.5	0.47±0.13	7.52	0.52±0.02	13.04	98.81	106.06
		1.25	1.21±0.03	4.46	1.11±0.02	3.64	101.45	100.41
		4	4.11±0.08	1.86	3.96±0.26	2.46	97	106.94
Tangeretin	3.34	1	1.01±0.04	2.71	0.96±0.31	4.59	110.73	110.31
		2.5	2.29±0.32	2.71	2.42±0.12	3.17	101.96	104.37
		8	8.75±0.16	2.96	7.74±0.23	2.14	87.8	109.96
Nobiletin	1.63	1	0.94±0.02	2.19	0.92±0.05	3.07	92.74	99.13
		2.5	2.44±0.06	2.31	2.47±0.13	3.39	103.3	109.38
		8	8.71±0.21	5.51	7.90±0.29	3.85	85.97	91.89

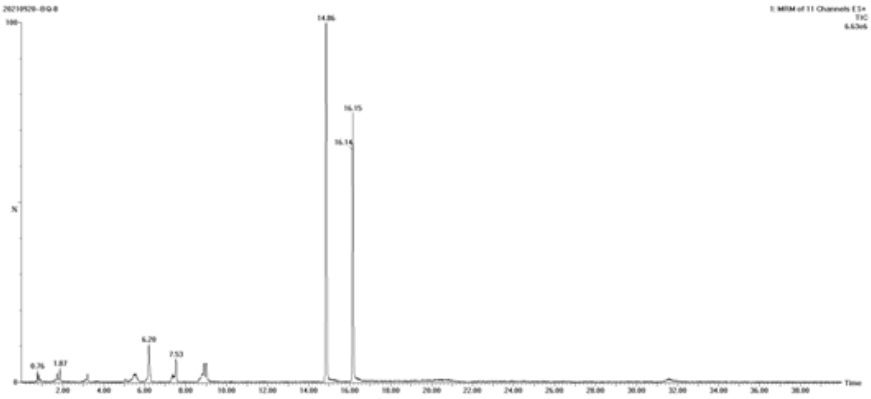

Figure 1: positive ion mode Xin-Su-Ning capsule TIC

4.2. Methodological Validation of Myocardial Tissue

LC-MS was used for the quantitative analysis of sophocarpine, matrine, berberine, Tangeratin, nobiletin. The 5 index components had a good linear relationship within the corresponding concentration range, and their R2 were all greater than 0.99, the results were shown in (Table 4). The relative standard deviation (RSD) values of stability, measured concentration were all less than 2.78 %, 14.03%, indicating that the instrument had good precision, the method had high repeatability, and the sample solution was stable for 24 h at room temperature. The sample extraction recovery and matrix effect RSD were between 85.05%-108.33%, indicating that the recovery rates of the 5 compounds in XSNC were good,the results were shown in (Table 5). Multiple reaction monitoring (MRM) is a highly specific and sensitive mass spectrometry technique for quantifying predefined compounds of interest. The UHPLC-MS/MS analysis method described above was subsequently used to simultaneously quantify 5 compounds of XSNC in rat plasma, the results were shown in (Table 4).

Table 4: Linearity, concentration of 5 compounds in Xin-Su-Ning capsule

Compounds	Regression equation	R2	Linearity range (ng/ml)	Compounds concentrations				
				0.25 (ng/ml)	0.5h (ng/ml)	1h (ng/ml)	2h (ng/ml)	4h (ng/ml)
Sophocarpine	y = 0.0559x + 0.9982	R^2 = 0.9934	0.9800—500	37.31±4.10	48.38±9.50	53.67±6.63	98.06±13.97	41.63±9.18
Matrine	y = 0.1219x + 1.6945	R^2 = 0.9974	1.9500—1000	152.80±23.76	176.40±7.67	184.8±8.15	217±6.43	177.5±9.31
Berberine	y = 1.5463x + 13.0140	R^2 = 0.9945	0.3900—200	2.11±0.34	3.21±0.99	3.71±0.85	3.31±0.72	1.45±0.72
Tangeretin	y = 4.6519x + 8.9937	R^2 = 0.9992	0.3900—200	1.16±0.28	2.04±0.30	1.72±0.19	1.49±0.01	1.36±0.14
Nobiletin	y = 1.9044x + 3.8478	R^2 = 0.9976	0.2000—100	1.23±0.21	1.88±0.16	1.78±0.10	0.64±0.28	0.56±0.23

Table 5: Stability Measured concentration Extraction recovery and Matrix effect of 5 analytes in UHPLC-QQQ MS/MS

Compounds	Stability (RSD%)	Concentration (ng/ml)	Intra-day		Inter-day		Extraction recovery	Matrix effect
			Measured concentration (ng/ml)	RSD (%)	Measured concentration (ng/ml)	RSD (%)		
Sophocarpine	2.11	50	49.25±4.61	4.11	47.33±3.75	6.79	103.31	103.45
		250	243.40±4.04	2.98	247.80±2.84	4.53	93.49	100.85
		400	388.00±25.84	1.55	372.90±16.01	13.38	85.09	88.45
Matrine	2.11	100	93.46±1.32	3.69	94.78±3.11	10.23	107.41	102.45
		500	476.30±35.99	1.42	488.60±20.21	6.85	107.48	107.97
		800	764.00±32.18	1.29	776.70±16.47	11.1	87.61	89.24
Berberine	2.78	20	17.48±0.53	0.64	18.08±2.25	7.62	101.77	108.33
		100	98.36±11.91	1.46	95.61±1.68	2.33	96.51	98.72
		160	149.30±13.87	1.22	156.00±2.86	3.47	91.9	90.15
Tangeretin	2.6	20	17.33±1.46	1.82	19.62±0.81	10.22	101.42	105.84
		100	99.00±11.07	0.8	92.92±4.21	6.51	104.81	106.98
		160	150.08±0.34	1.6	153.00±3.74	9.72	87.34	90.15
Nobiletin	2.24	10	9.80±0.89	1.04	9.95±0.87	10.18	105.07	107.39
		50	46.83±4.58	0.97	49.60±2.11	7.35	102.1	106.04
		80	74.90±0.44	1.93	78.09±4.94	14.03	85.54	85.42

4.3. Network Analysis

Six targets of XSNC in blood were predicted by the Swiss Target Predictionand TCMSP database, a total of 410 constituent targets were obtained by deleting the duplicated targets. With "Arrhythmia" as the key word, a total of 1672 arrhythmia disease-related targets were obtained from OMIM, TTD, PharmGKB, Genecard databases, 1564 relevant targets after removing the duplicate options. In Cytoscape 3.9.0 software, "drug targets-arrhythmia targets" was analyzed,the NetworkAnalyzer function was used to analyze the topological characteristic parameters in the network nodes,the key targets detected under the condition of degree more than double median, closeness more than one median and Closeness more than one median. Finally, 112 common targets were obtained. According to GO and KEGG enrichment analysis of 112 common targets, GO enrichment analysis was conducted on 112 common targets, and 122 biological processes (BP), 36 cellular components (CC), and 47 molecular functions (MF) were identified ($P<0.01$). According to the order of P value from small to large, the top 5 components were selected for analysis, and the 6 components were mainly through response to the drug, singal transduction, positive regulation of vasoconstriction; Plasma, Integral component of the plasma membrane, Dendrite; Heme binding, enzyme binding, drug binding, etc. played a role in the treatment of arrhythmia, as shown in (Figure 2).

The KEGG pathway analysis obtained 66 relevant pathways ($P < 0.05$), and the top 20 pathways were selected according to the order of P value from small to large.It can be concluded that the absolute refractory period and relative refractory period of cardiomyocytes can be prolonged by Adjusting Na+, K+, Ca2+ channels on cardiomyocytes, thereby slowing down information transmission, blocking excitatory reentry, and even reducing excitability, so as to play a role in the treatment of arrhythmia, and the results are shown in (Figure 3). The data of six blood components, key targets, and signaling pathways of XSNC were imported into Cytoscape 3.9.0 software for integration, that is, the "components-target-pathway" network diagram of XSNC was obtained, and the results are shown in (Figure 4).

Using String database, a PPI network with 111 nodes and 986 edges was obtained. The average degree value is 17.6. The PPI network was visualized using Cytoscape 3.9.0 software (Figure 5), where the size and color of the nodes represent the degree value of the node in the network. Larger and darker nodes indicate higher degree values, indicating that the node is more central and has more connections with other targets in the network. The occurrence of arrhythmia is mostly related to the ion channels on cardiomyocytes, which are mainly related to Na+, K+, and Ca2+ plasma channels. These three ion channels are in a dynamic equilibrium state maintained by the regular opening and closing of each ion channel on the cell membrane during different times of cardiac rhythm so that the ion signals inside and outside the cell are normally conducted.Therefore, the pathway related to Na+, K+, and Ca2+ channels in KEGG analysis results of the top 20 signaling pathways was Calcium signaling pathway, so the Calcium signaling pathway was selected as the main pathway of arrhythmia for verification.

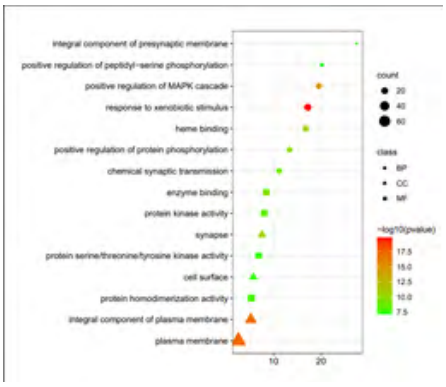

Figure 2: GO functional enrichment analysis

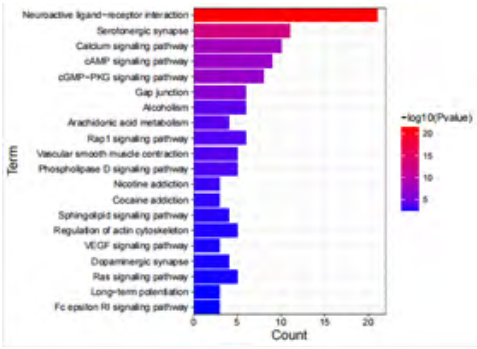

Figure 3: KEGG enrichment analysis

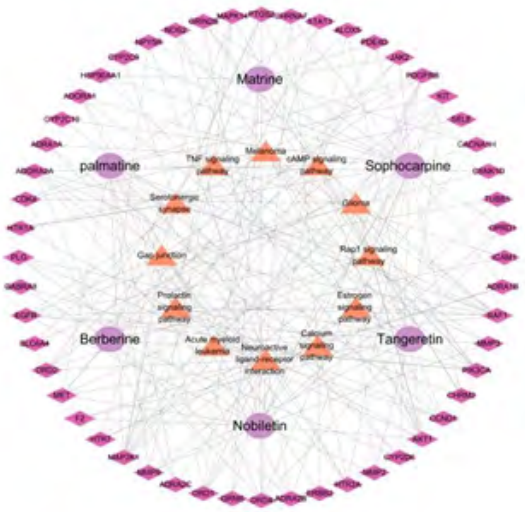

Figure 4: "Component-target-pathway" network diagram

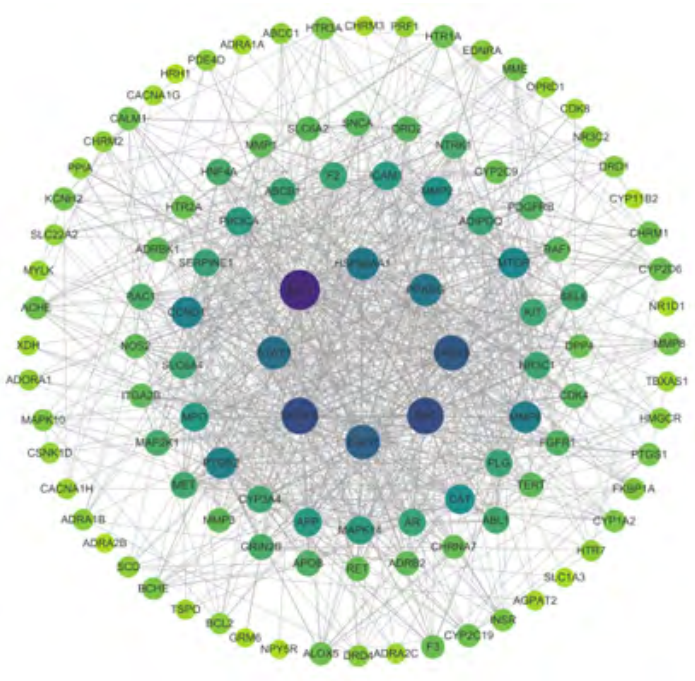

Figure 5: Protein-protein interaction network diagram

5. Discussion

Arrhythmia, which is mainly caused by increased Ca2+ concentration and decreased K+ concentration in ischemic myocardium, causes abnormal myocardial bioelectrical activity [12], Western medicine has limitations in drug treatment and non-drug treatment [13], in clinical practice, amiodarone and metoprolol are often used to treat arrhythmias and improve arrhythmias by inhibiting Ca2+ pathway, but their use effect is not good and will lead to adverse reactions. Long-term use of amiodarone will cause serious side effects on multiple organs and tissue types, including the heart,for example, the formation and conduction of sinus beats, the induction of significant bradycardia or torsclerotic ventricular tachycardia, etc [14]. Traditional Chinese medicine has potential advantages in the treatment of arrhythmia. XSNC consists of Coptidis Rhizoma, Pinelliae Rhizoma, Poria, Aurantii Fructus Immaturus, Dichroae Radix, Lotus Plumule, Sophorae Flavescentis Radix, Artemisiae Annuae Herba, Fructus Fructus Radix Ginseng Radixet Rhizoma, Ophiopogonis Radix, Glycyrrhizae Radixet Rhizoma, it is a good prescription for the treatment of phlegm heat disturbance arrhythmia. XSNC is antiarrhythmic by prolonging the duration and inhibiting the amplitude of action potentials in ventricular myocytes, and it is antiarrhythmic by blocking Na+ and K+ channels, and has the characteristics of class I and class III antiarrhythmic drugs [15]. In the current pharmacological study, XSNC significantly inhibited arrhythmias induced by chemical reagents, calcium chloride, chloroform, and isoproterenol. In cardiac ischemia-induced arrhythmias, XSNC could delay the onset of ventricular arrhythmias and shorten the duration of arrhythmias. XSNC also reduced total cholesterol levels and decreased blood viscosity, hematocrit values, and fibrinogen in normal rats [16].

In this study, by establishing the UHPLC- QQQ-MS /MS method, it was found that berberine and palmatine were from Rhizoma Coptidis, sophocarpine and matrine were from sophora flavescens, nobiletin and tangerine were from aurantii fructus immaturus. Many studies have shown that berberine exerts antiarrhythmic effects mainly by affecting K+ channels [17], almatine is similar to berberine in structure and has a high content in Coptis coptidis, which also has a protective effect on the heart [18], matrine and sophorine are alkaloids, which can exert their antiarrhythmic effects by affecting ion channels on cardiomyocytes and prolonging the duration of action potentials [19];tangerine has a protective effect on heart, and TAN can effectively reduce the size of myocardial infarction by regulating (upregulating) the protein expression of PI3K/Akt signaling pathway [20]; nobiletin attenuates myocardial I/R injury by activating Akt/GSK-3β pathway in H9c2 cardiomyocytes. Nobiletin has the effect of preventing myocardial ischemia-reperfusion injury and ischemic heart disease [21]. In this study, the pharmacodynamic material basis of XSNC was analyzed, and the composition and time change rule of XSNC in plasma and myocardial tissue were basically clarified, and the pathway of its components into plasma was predicted. Combined with the absorption of chemical components in plasma and myocardial tissue, the pharmacodynamic material basis of XSNC was further explained, and theoretical ideas were provided for the follow-up pharmacodynamic mechanism experiment.

6. Limitations and Prospects

For the material basic research of XSNC, the plasma and myocardial tissue components of XSNC were analyzed by UHPLC-QQQ-MS/MS technology and network pharmacology analysis, and the target sites were selected, but there are still some limitations. In this experiment, through the analysis of XSNC in rat plasma and myocardial tissue and the change of time, it is concluded that the components in plasma and myocardial tissue mainly come from coptis coptidis, sophora flavescens and fructus aurantii, which are less detected in other traditional chinese medicine, and there are shortcomings in this aspect. Although potential therapeutic targets and the therapeutic mechanism of drug active components have been selected, which further provides scientific basis for the treatment of arrhythmia, there is still a lack of protein expression to verify the accuracy of the mechanism. Based on the incomplete part, we will also continue to carry out further improvement research through multi-omics, large sample, multi-target, multifunctional verification and other methods in the future.

7. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

8. Funding

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9. Ethics Approval

The ethics involved in all animal experiments are in line with the Animal Ethics Committee of Tianjin University of Traditional Chinese Medicine (Ethical approval number: TCM-LAEC2021179).

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