The mechanism of Wenxin keli in treating ventricular premature beat based on network pharmacology and molecular docking

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Abstract: This study aims to investigate the molecular mechanism of Wenxin Keli (WXKL) in the treatment of Ventricular Premature Beats (VPB) using network pharmacology and molecular docking methods. The main active ingredients, related targets, and target genes of WXKL were obtained from the Traditional Chinese Medicine Systems Pharmacology (TCMSP) database and Uniprot. The target genes of VPB were retrieved from GeneCards and OMIM databases. The intersection of target genes was analyzed using Cytoscape to construct a drug-target network. A protein-protein interaction network (PPI) was built using the STRING database, and a "WXKL-active ingredients-key targets-significant pathways-disease" network was constructed. Further gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were analyzed, and molecular docking were conducted. A total of 41 compounds targeting 277 genes in WXKL. There were 671 genes associated with VPB, and WXKL shared 88 target genes with VPB, forming 1950 edges. Potential core targets for treatment were identified as STAT3, JUN, MAPK1, AKT1, TNF, MAPK14, IL6, and CAV1. The results of molecular docking showed that the core active components of WXKL were well combined with the core targets of ventricular premature beat.This study revealed the multi-component, multi-target, and multi-pathway characteristics of WXKL in treating VPB, providing a theoretical basis for further research on the mechanism of WXKL in treating VPB.

Keywords: Network pharmacology; Wenxin Keli; Ventricular Premature Beats; Molecular docking; Mechanism of action

1. Introduction

Ventricular premature beats (VPB), refer to the premature depolarization of the ventricular myocardium originating from an ectopic pacemaker below the atrioventricular (AV) node, before the impulse from the sinus node reaches the ventricles[1],PVBs are a common ECG finding in the general population and are recorded in up to 75% of healthy individuals undergoing 24-h ambulatory ECG monitoring, with an age-related increase in prevalence[2-4], and the incidence increases with age. In children younger than 11 years, the incidence is less than 1%, while in individuals older than 75, it can be as high as 69%[5].VPB can lead to malignant ventricular arrhythmias such as polymorphic ventricular tachycardia or ventricular fibrillation, which can result in severe consequences, including impaired ventricular contractile function, reduced cardiac function, arrhythmogenic cardiomyopathy[1], and even syncope or sudden cardiac death. The treatment of VPB primarily focuses on relieving symptoms and improving left ventricular function. Underlying factors such as hypertension, sleep apnea, hyperlipidemia, and electrolyte imbalances should also be actively managed. Currently, the

clinical management of VPB includes pharmacological treatment with antiarrhythmic drugs such as class I (mexiletine), class II (beta-blockers), and class III (non-dihydropyridine calcium channel blockers) agents. Non-pharmacological approaches, mainly catheter ablation, are also used. However, the effectiveness of drugs like mexiletine and beta-blockers are limited, and they often come with significant side effects[6], Moreover, some of these drugs can themselves induce arrhythmias. Catheter ablation[7], while effectivly, is an invasive procedure with associated costs and complexities, limiting its. Traditional Chinese medicine (TCM) has a long history of treating VPB, and recent randomized, double-blind, multicenter clinical studies have shown that WXKL and Ginseng Renshen Capsules can significantly reduce VPB and alleviategg clinical symptoms when compared to mexiletine or placebo[8, 9]. WXKL is the first approved traditional Chinese medicine for the treatment of arrhythmias in China. It has been shown to lower heart rate, reduce arrhythmias (VPB, ventricular tachycardia, ventricular fibrillation), and improve cardiac function when used in combination with Western medicine[10]. It is composed of five herbal ingredients: Codonopsis Radix (Dang Shen), Polygonati Rhizoma (HuangJing), Notoginseng Radix Et Rhizoma (San Qi), Ambrum (Hu Po), and Nardostachyos Radix Et Rhizoma (Gan Song). Animal studies have also demonstrated that WXKL can improve microcirculation and enhance myocardial contractility. The exact mechanisms of action of the main active components of WXKL and their interactions with target genes remain unclear. Therefore, this study aims to use network pharmacology techniques and comprehensive data platforms to explore potential target proteins for WXKL in the treatment of VPB and investigate the cellular signal pathways and mechanisms it affects.

2. Methods

2.1 Selection of Active Ingredients in WXKL and Target Genes for Active Ingredients

Active ingredients in WXKL were predicted based on their absorption, distribution, metabolism, and excretion (ADME) properties using the TCMSP database(https://tcmsp-e.com/tcmsp.php). Compounds with oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were selected. Human genes encoding these compounds were searched on the Uniprot database. The target genes for the active ingredients were retrieved from the TCMSP database. For compounds without target information in the TCMSP database, target prediction was performed using the PharmMapper database(http://www.lilab-ecust.cn/pharmmapper/). Target proteins were merged, and duplicate targets were removed. Standardized protein target and corresponding gene information were obtained from the Uniprot database (https: // www. uniprot. org/) . Target genes related to VPB were searched using the keywords "Ventricular premature beats" in the Genecards(https://www.genecards.org) and OMIM databases (https://omim.org/).

2.2 Construction of the Protein-Protein Interaction (PPI) Network and Selection of Core Targets

The intersection targets related to VPB were uploaded to the STRING database with species specified as "Homo sapiens" and a high confidence score threshold of 0.9. Independent targets were removed, and the resulting PPI network was analyzed using Cytoscape 3.9.1 software. Core targets were selected based on degree, betweenness centrality, and closeness centrality values.

2.3 GO and KEGG Pathway Enrichment Analysis of WXKL in Treating VPB

The intersection targets of WXKL and VPB were uploaded to the DAVID bioinformatics database, with "official gene symbol" selected as the identifier and "Homo sapiens" as the background database. GO and KEGG pathway enrichment analyses were performed with a significance threshold of $P < 0.05$. The top 10 enriched terms for each category (biological process, cellular component, molecular function) in GO analysis and the top 20 enriched pathways in KEGG analysis were visualized.

2.4 Construction of the "TCM-Active Ingredients-Key Targets-Significant Pathways-Biological Processes" Network

Combining the analysis results from Sections 1.4, 1.1, and 1.2, the PPI data for active ingredients in WXKL, potential target genes, and WXKL were imported into Cytoscape software to construct an interactive network diagram. Network Analyzer functionality was used to analyze the main active ingredients in the drug and identify core network molecular functional modules.

2.5 Molecular Docking Verification

Molecular docking was conducted using Sybyl-X2.1.1 software to assess the binding affinity between the active compounds in WXKL and the top three ranked core target proteins. The 2D molecular structure of WXKL was extracted from the PubChem database, and the protein structures of the top three core targets were obtained from the PDB (Protein Data Bank). Docking scores were used as an indicator to evaluate the binding capability between molecules. The binding modes were visualized using Discovery Studio 4.5 software.

3. Results

3.1 Selection of Active Ingredients in WXKL and Target Prediction

Based on the criteria of oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18, the main active ingredients of WXKL were obtained from the TCMSP database: 21 compounds from Codonopsis Radix (Dangshen), 12 from Ophiopogon japonicus (Maidong), 5 from Notoginseng Radix et Rhizoma (Sanqi), and 3 from Succinum (Hupo). Ambergris was excluded as it did not meet the selection criteria. After merging and removing duplicate compounds, a total of 41 active compounds were obtained, corresponding to 277 target genes.

3.2 Prediction of Target Genes for Active Ingredients in WXKL and Intersection with VPB-Related Target Genes

A total of 277 target genes were predicted for the active ingredients in WXKL. Additionally, 671 target genes were associated with VPB. The intersection of target genes between WXKL and VPB resulted in 88 potential target genes. After removing target nodes with no interaction relationships, the PPI network consisted of 115 nodes and 1950 edges, with an average degree value of 33.9. The PPI data were optimized and imported into Cytoscape 3.7.2 software(Figure 1). Based on this, it can be inferred that STAT3, JUN, MAPK1, AKT1, TNF, MAPK14, IL6, CAV1, and other target genes are potential core targets of WXKL for the treatment of VPB.

3.3 Construction of Drug-Ingredient-Target-Disease Network

The active ingredients and 88 potential target genes were input into the Cytoscape software to create the network, as shown in Figure 2. The network consists of 134 nodes, 437 edges, the nodes are sorted by degree values, and it is observed that among the 41 active components interacting with potential target genes, molecules like quercetin (MOL000098), luteolin (MOL000006), Chrysanthemaxanthin (MOL004492), 7-Methoxy-2-methyl isoflavone (MOL003896), among others, are connected to more than 10 target genes, suggesting they may be the main active ingredients. Additionally, targets such as PTGS2, PTGS1, AR, PGR, ADRB2, PPARG, CASP3, and ESR1 are connected to more than 10 components, indicating they might be the major target proteins involved in the action of WXKL.
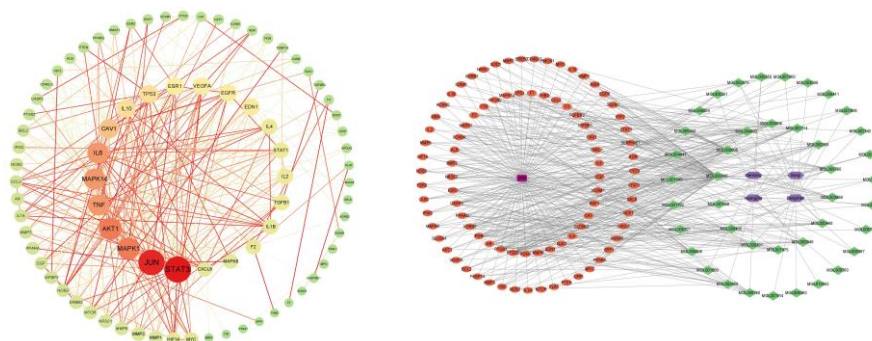

Figure 1: PPI network at the intersection of WXKL and PVB (Left)

Figure 2: Network of Components-Target-Pathways for Intersection Targets of WXKL in the Treatment of VPB (Right)

3.4 Enrichment Analysis

Using the DAVID database, GO and KEGG enrichment analyses were performed on the shared target genes between WXKL and VPB.Biological Processes (BP) with 527 entries, including positive regulation of gene expression, transcriptional positive regulation by RNA polymerase II promoter, positive regulation of DNA templated transcription, signal transduction, and more.For each category, the top 10 enriched results were selected for visualization analysis (Figure3). KEGG enrichment analysis identified a total of 145 related pathways, including the AGE-RAGE signaling pathway in diabetic complications, extracellular matrix-receptor interaction, fluid shear stress and atherosclerosis, lipid and atherosclerosis, MAPK signaling pathway, PI3K-AKT signaling pathway, tumor necrosis factor signaling pathway, relaxation pathway, HIF-1 signaling pathway, IL-17 signaling pathway, and more. This suggests that WXKL may have a multifaceted impact on processes such as cell apoptosis regulation, inhibition of inflammatory responses, and immune regulation in the treatment of VPB. The top 20 enriched pathways were selected for visualization analysis, resulting in the KEGG enrichment bubble plot(Figure 4).
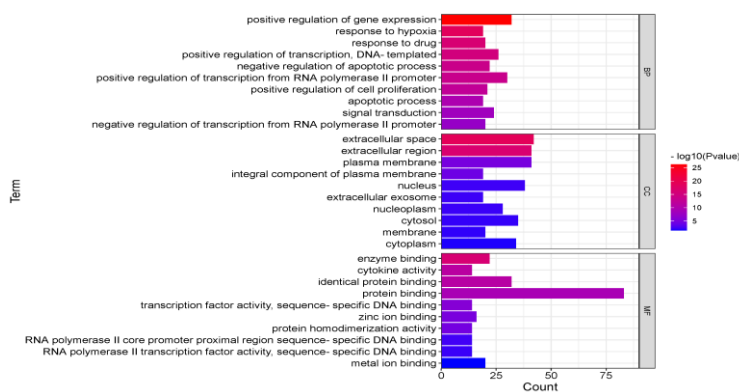

Figure 3: GO enrichment analyses of potential targets of the main active ingredients of WXKL
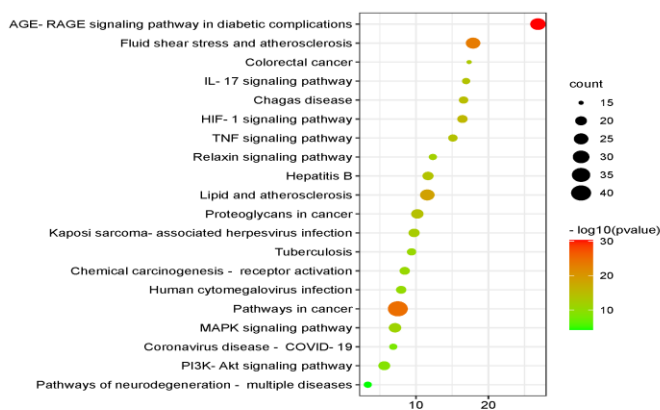

Figure 4: KEGG pathway enrichment analyses of potential targets of the main active ingredients of WXKL.

3.5 Molecular Docking

Table 1 displays the results of molecular docking between WXKL and various core target proteins, with docking scores all above 4.3. Among them, the binding affinity with MAPK1 is the highest, indicating that WXKL has a strong binding capability with core target proteins. The molecular docking mode between WXKL and core target proteins(Figure5). The small molecule ligand (WXKL) is shown in blue sticks, and the interactions between the ligand and the amino acids in the target protein are represented by green dashed lines. The results suggest that WXKL forms conventional hydrogen bonds, hydrophobic interactions, and van der Waals forces with the target proteins. Specifically, MOL000098 (quercetin) interacts with STAT3, MAPK1, and JUN; MOL000006 (luteolin) interacts with STAT3, MAPK1, and JUN; and MOL004492 (Chrysanthemaxanthin) interacts with STAT3, MAPK1, and JUN.

Table 1: Molecular docking score table

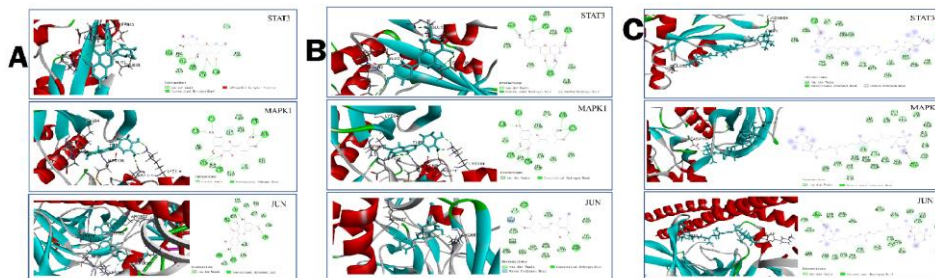|	STAT3	JUN	MAPK1
	6njs	1a02	6g54
MOL000098	5.62	6.02	7.18
MOL000006	5.06	6.16	7.32
MOL004492	4.51	6.10	4.39

Figure 5: The binding mode diagram of Wenxin Granules with core target proteins in molecular docking.

4. Discussion

Patients with anxiety and nervousness should be actively treated[11]. From the perspective of traditional Chinese medicine, VPB fall into the category of "palpitations"[11]. Traditional Chinese medicine like WXKL is a complex multi-component drug with various components that act on different targets related to VPB[12]. The common medications can achieve some therapeutic effects in the treatment of VPB, they often have limitations due to their single-target nature and significant adverse reactions. It is often initiated at low doses and gradually titrated upward based on individual patient responses until the desired therapeutic effect is achieved. Metoprolol tartrate has a strong tissue-penetrating ability and a very short half-life, which can lead to central nervous system side effects such as headaches, dizziness, and insomnia[13]. Codonopsis can enhance cardiac output, resist platelet aggregation, protect myocardial cells, and improve myocardial energy metabolism. However, it is an invasive treatment with high costs, and poor patient compliance limits its widespread use in clinical practice.

Compared to Western medicines with relatively single components, WXKL are complex, and their various components may target different aspects of ventricular arrhythmias. It has been reported that WXKL exert anti-arrhythmic effects by selectively inhibiting sodium currents (I_Na)[13]. The latest pharmacological research indicates that Codonopsis can enhance cardiac output, resist platelet aggregation, protect myocardial cells, and improve myocardial energy metabolism[14]. These herbs are integral components of traditional Chinese medicine and have been used for various health purposes. However, it's essential to approach herbal remedies with caution and consult a qualified healthcare practitioner, especially when using them to address specific health concerns. Additionally, scientific research and clinical studies are continuously being conducted to better understand the mechanisms and potential therapeutic applications of these herbs[15]. Sanqi can modulate cellular signaling pathways involving cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA), inhibiting calcium ion channels and preventing rapid arrhythmias[16, 17]. Total saponins R1 from Sanqi have been found to improve myocardial damage in rats with coronary heart disease, inhibit myocardial cell apoptosis, oxidative stress, and inflammatory reactions.[17]. These drugs complement each other, promoting blood circulation and eliminating blood stasis. In this study, a network pharmacology approach was used to predict the effective components and drug target proteins of WXKL in the treatment of VPB. Among the 7 main proteins, STAT3, JUN, MAPK1, AKT1, TNF, MAPK14, and IL6 are all closely related to VPB. Research has shown that STAT3 plays a key role in inflammation and can protect against myocardial ischemia-reperfusion injury by regulating the JAK2/STAT3 signaling pathway[18]. MAPK is a crucial signal transducer that conveys signals from the cell surface to the cell nucleus. It can induce pro-inflammatory effects on cells and regulate the cell cycle. Research has confirmed that AKT/mTORC1 is a significant regulatory pathway for de novo fatty acid synthesis. AKT, a downstream key regulator in the PI3K (Phosphoinositide 3-kinase) pathway, is involved in various life processes such as cell survival, insulin signaling, lipid metabolism reprogramming, angiogenesis, and tumorigenesis[19]. Recent research suggests that WXKL may potentially work by modulating

inflammation-related TGFβ-p38/JNK and MAPK signaling pathways. This modulation can lead to a reduction in extracellular matrix (ECM) collagen deposition. As a result, it can alter the structural characteristics of myocardial tissue and scars, thereby inhibiting myocardial tissue structural damage caused by myocardial ischemic injury and improving heart function[20]. Based on a literature review, it is known that WXKL play a crucial role in the treatment of arrhythmias: such as inflammation, immunity, ion transport, cell proliferation, and estrogen metabolism[21], In KEGG pathway enrichment analysis, it was found that WXKL exert their effects in treating VPB by participating in pathways related to complications of diabetes like the AGE-RAGE signaling pathway, extracellular space, fluid shear stress, and atherosclerosis signaling pathways, lipid and atherosclerosis signaling pathways, as well as the MAPK and PI3K-AKT signaling pathways. Therefore, it is believed that WXKL mainly achieve their effects in treating VPB by regulating multiple processes such as cell apoptosis, inhibition of inflammatory responses, and immune regulation. Using network pharmacology, it shifts the traditional single-target, single-pathway research perspective towards a multi-component, multi-target, and multi-pathway approach, allowing for a systematic analysis.

5. Conclusion

In this study, a network pharmacology analysis was conducted to systematically identify the effective components and mechanisms of action of WXKL in the treatment of VPB. This study provides a foundation for the clinical application of WXKL.

References

[1] Cardiac electrophysiology and pacing Branch of Chinese Medical Association. Chinese expert consensus on ventricular arrhythmia[J]. Chinese Journal of Arrhythmias, 2016,20(4): 279-326.
[2] KERR S R, PEARCE M S, BRAYNE C, et al. Carotid sinus hypersensitivity in asymptomatic older persons: implications for diagnosis of syncope and falls[J]. Arch Intern Med, 2006,166(5): 515-520.
[3] HINGORANI P, KARNAD D R, ROHEKAR P, et al. Arrhythmias Seen in Baseline 24-Hour Holter ECG Recordings in Healthy Normal   Volunteers During Phase 1 Clinical Trials[J]. J Clin Pharmacol, 2016,56(7): 885-893.
[4] von ROTZ M, AESCHBACHER S, BOSSARD M, et al. Risk factors for premature ventricular contractions in young and healthy adults[J]. Heart, 2017,103(9): 702-707.
[5] KENNEDY H L, WHITLOCK J A, SPRAGUE M K, et al. Long-term follow-up of asymptomatic healthy subjects with frequent and complex ventricular ectopy[J]. N Engl J Med, 1985,312(4): 193-197.
[6] KRITTAYAPHONG R, BHURIPANYO K, PUNLEE K, et al. Effect of atenolol on symptomatic ventricular arrhythmia without structural heart disease: a randomized placebo-controlled study[J]. Am Heart J, 2002,144(6): e10.
[7] Chen G, Fan X H, Zhang Kui-Jun, et al. Gender differences in the origin of idiopathic right ventricular outflow tract ventricular arrhythmia and the influencing factors of radiofrequency ablation[J]. Chinese Journal of Arrhythmias, 2018,22(02): 134-139.
[8] ZOU J G, ZHANG J, JIA Z H, et al. Evaluation of the traditional Chinese Medicine Shensongyangxin capsule on treating premature ventricular contractions: a randomized, double-blind, controlled multicenter trial[J]. Chin Med J (Engl), 2011,124(1): 76-83.
[9] HUA W, GAO R L, ZHAO B C, et al. The Efficacy and Safety of Wenxin Keli in Patients with Frequent Premature Ventricular Contractions: A Randomized, Double-blind, Placebo-controlled, Parallel-group, Multicenter Trial[J]. Chin Med J (Engl), 2015,128(19): 2557-2564.
[10] ZHENG R, TIAN G, ZHANG Q, et al. Clinical Safety and Efficacy of Wenxin Keli-Amiodarone Combination on Heart Failure Complicated by Ventricular Arrhythmia: A Systematic Review and Meta-analysis[J]. Front Physiol, 2018,9: 487.
[11] Chinese Medical Association ECG Physiology and Pacing Branch, Chinese Medical Association Arrhythmia Committee. 2020 China Expert Consensus on ventricular arrhythmia2016 Consensus upgrade version)[J]. Chinese Journal of Arrhythmias, 2020,24(03): 188-258.
[12] INVESTIGATORS T C A S. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction[J]. N Engl J Med, 1989,321(6): 406-412.
[13] HOU J W, LI W, GUO K, et al. Antiarrhythmic effects and potential mechanism of WenXin KeLi in cardiac Purkinje cells[J]. Heart Rhythm, 2016,13(4): 973-982.
[14] PENG J C, SU J F, HONG L P, et al. Effect of wenxin granule on plasma BNP and HRV of acute coronary syndrome   patients[J]. Zhongguo Zhong Xi Yi Jie He Za Zhi, 2013,33(8): 1046-1049.

[15] HE Y F, MA H T, WANG R N, et al. [Research progress on chemical constituents and pharmacological activities of Trigonella foenum-graecum][J]. Zhongguo Zhong Yao Za Zhi, 2021,46(16): 4069-4082.

[16] Zhang Y, Dai S Y, Xu B, et al. Progress in pharmacological research of total saponins of Panax notoginseng against myocardial ischemia-reperfusion injury [J]. Jiangsu Traditional Chinese Medicine, 2017,49(01): 82-85.

[17] Guo W W, Zhang X P, Li X, et al. Regulation of AMPK/Nrf-2/HO-1 signaling pathway by panax saponin R1 alleviates myocardial injury in rats with coronary heart disease[J]. Chinese modern applied pharmacy, 2021,38(01): 36-41.

[18] MAHDIANI S, OMIDKHODA N, REZAEE R, et al. Induction of JAK2/STAT3 pathway contributes to protective effects of different therapeutics against myocardial ischemia/reperfusion[J]. Biomed Pharmacother, 2022,155: 113751.

[19] LIEN E C, LYSSIOTIS C A, CANTLEY L C. Metabolic Reprogramming by the PI3K-Akt-mTOR Pathway in Cancer[J]. Recent Results Cancer Res, 2016,207: 39-72.

[20] HUANG Y, WU A, LOU L, et al. Wenxin Granules Influence the TGFbeta-P38/JNK MAPK Signaling Pathway and Attenuate the Collagen Deposition in the Left Ventricle of Myocardial Infarction Rats[J]. Cardiol Res Pract, 2019,2019: 3786024.

[21] TIAN G, SUN Y, LIU S, et al. Therapeutic Effects of Wenxin Keli in Cardiovascular Diseases: An Experimental and Mechanism Overview[J]. Front Pharmacol, 2018,9: 1005.