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Therapeutic Effects and Potential Mechanisms of Glyasperin A against Myocardial Ischemia Based on Network Pharmacology and Molecular Docking

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Abstract [**Objectives**] To explore the therapeutic effects and potential mechanisms of Glyasperin A (GAA) on myocardial ischemia (MI) based on network pharmacology and molecular docking. [**Methods**] The molecular structure of GAA was downloaded from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP), and all targets of GAA were predicted by converting 3D model molecules into SMILES online tool and Swiss target prediction. Genecards database and DisGeNET database were used to find the targets related to MI, and then Venny 2.1.0 was used to generate the corresponding Wayne diagram, and then Cytoscape 3.9.1 software was used to construct the protein-protein interaction (PPI) network. With the help of DAVID database and Microbiology, the selected core targets were enriched and analyzed by gene ontology (GO), biological process (BP), and Kyoto Encyclopedia of Genes and Genomes (KEGG), and then the molecular docking between GAA and core targets was verified by AutoDock and Pymol software. [**Results**] A total of 1883 MI targets were screened, and in the protein-protein interaction network, AKT1, PTGS2, PPARG, ESR1, GSK3B were the proteins with higher values. Gene ontology and KEEG enrichment analysis showed that the biological processes involved mainly included inflammatory response, negative regulation of gene expression, and response to exogenous stimuli. Signaling pathways mainly include IL-17 signaling pathway, HIF-1 signaling pathway, and so on. The results of molecular docking showed that the binding energy of GAA and core protein was less than -5 Kcal/mol in four groups. These indicated that GAA with good binding had a certain therapeutic effect on myocardial ischemia. [**Conclusions**] Based on the systematic network pharmacology method, this study predicts the basic pharmacological effects and potential mechanisms of GAA in the treatment of MI, and reveals that GAA may treat MI through multiple targets and signaling pathways. It is expected to provide a basis for further study of its pharmacological mechanisms.

Key words Network pharmacology, Molecular docking, Glyasperin A (GAA), Myocardial ischemia (MI)

1 Introduction

At present, cardiovascular disease (CVD) is the main cause of death and morbidity worldwide, and ischemic heart disease is closely related to cardiovascular disease mortality, accounting for about 50% of the total cardiovascular disease mortality[1]. CVD is a risk factor for the development of cardiovascular disease[2]. Ischemic heart disease is a serious disease that endangers the human body. If myocardial ischemia (MI) necrosis is not intervened in time, it will lead to ventricular remodeling and further promote the occurrence of heart failure[3]. MI can lead to abnormal myocardial energy metabolism, consequently leading to failure to support the normal work of the heart[4], and pose a great threat to human health.

Glycyrrhizae Radix Et Rhizoma (Gancao) was first recorded in *Shen Nong's Herbal Classic*, also known as Fencao, Micao, and Meicao, honey grass and beauty grass[5]. Glycyrrhizae Radix Et Rhizoma is sweet and neutral in taste, enters the heart, lung, spleen and stomach meridians, and has the effects of relieving spasm and pain, eliminating phlegm and relieving cough, detoxifying and clearing heat, invigorating qi and tonifying the middle, and harmonizing various medicines[6]. "Nine Tenths prescriptions having Glycyrrhizae Radix Et Rhizoma", Glycyrrhizae Radix Et Rhizoma are commonly used medicines for the treatment of cardiovascular diseases, such as "Zhigancao Decoction"[7], "Tongmai Yangxin Pill"[8], "Xuefu Zhuyu Decoction"[9] and so on. Glyasperin A (GAA) is the effective component of Glycyrrhizae Radix Et Rhizoma, and GAA is a kind of flavonoid[10], which has many pharmacological activities such as lowering blood pressure, antitumor, anti-cardiovascular disease and so on[11]. In normal myocardial cells, mitochondria can maintain the vitality and function of myocardial cells by providing adenosine triphosphate (ATP), while myocardial ischemia can seriously affect the structure and function of ATP, resulting in insufficient supply of ATP, thus affecting myocardial function[12].

To sum up, it is speculated that GAA can intervene in myocardial ischemia, but the anti-MI mechanism of GAA is still unclear. This study is based on network pharmacology and molecular docking technology to explore the potential anti-MI effect and molecular mechanism of GAA, so it has certain theoretical value.

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2 Data and methods

2.1 Analysis method

2.1.1 GAA target gene acquisition. The compounds with the mol2 structure downloaded by GAA in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspnw.com) database were uploaded to a 3D model molecule to be converted into a SMILES online tool, and the corresponding Smiles number was obtained. Then the corresponding Smiles number was imported into the Swiss target prediction (http://www.swisstargetprediction.ch/) database to predict the corresponding target.

2.1.2 MI gene search. We entered "myocardial ischemia" to search for MI genes in the Genecards (http://www.genecards.org/) database and the DisGeNET (https://www.disgenet.org/) database.

2.1.3 Creation of Venn diagram and construction of protein-protein interaction (PPI) network. We used Venny 2.1.0 analysis tool to compare and analyze the GAA targets and MI related targets, and plot the Venn diagram, obtained the common targets of GAA and MI, and then imported 40 common targets into the STRING database. Setting the confidence level >0.400, we removed the free target protein, obtained the protein interaction information, and then imported the results into Cytoscape software for visualization and network topology analysis, constructed the PPI network, and screened out the core targets.

2.1.4 GO and KEGG enrichment analysis. The effective targets of GAA were uploaded to DAVID software, and human subjects were selected for gene ontology (GO) function enrichment analysis and visualization. GO analysis included biological process (BP), cell composition (CC) and molecular function (MF). At the same time, the main pathway of GAA in MI was obtained by KEGG pathway enrichment analysis, and the enrichment results were visualized by microbioinformatics (https://www.bioinformatics.com.cn).

2.1.5 Molecular docking verification. The top five core proteins in PPI network, AKT1 (1unp), PTGS2 (5f19), PPARG (1fm6), ESR1 (1uom) and GSK3B (1gng), were docked with GAA by Autodock 1.5.6 software. The PDF structure of the core protein was downloaded from RCSB PDB (https://www.rcsb.org/), and the MOL2 structure file of GAA was downloaded from TCMSP. Autodock 1.5.6 and pymol software were used to dehydrate and hydrogenate GAA. Operations such as torsion bond were checked, and then the treated protein and GAA were docked. The docking results were visualized using Pymol software.

3 Results and analysis

3.1 GAA target prediction Through the above related search and operation, the targets with probability greater than 0 were selected, and 100 targets were obtained.

3.2 Search of MI gene and protein The data retrieved from Genecrads database were screened with the median Relevance score of 2.091 066, and a total of 1 544 genes were obtained. 755 genes were retrieved from DisGeNET database, and 1 883 MI genes were obtained after removing duplicate values.

3.3 Construction of Venn diagram and PPI network The GAA and MI intersection genes obtained in Section **2.1.3** were introduced into Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/veeny/) to obtain the corresponding Venn diagram (Fig. 1). Then the results were imported into Cytoscape 3.9.1 software for visualization and network topology analysis, the free protein were hidden, and the PPI network was plotted, as shown in Fig. 2. The darker the color in the figure, the larger the value. The core targets included AKT1, PTGS2, PPARG, ESR1, GSK3B, *etc.*

3.4 GO enrichment analysis A total of 205 GO entries ($P<0.05$) were obtained from David database, including 121 biological process (BP) entries, 31 cell composition (CC) entries and 53 molecular function (MF) entries, as shown in Fig. 3. BP results include inflammatory response, response to exogenous stimuli, signal transduction, and negative regulation of gene expression. CC results include perinuclear region, cytosol, extracellular space, *etc.* MF results include protein binding, enzyme binding, arachidonic acid 12 lipoxygenase activity, *etc.*

3.5 KEGG enrichment analysis Through KEGG functional enrichment analysis in David database, 138 signal pathways were obtained. The X axis is the size of P, the Y axis is the name of the pathway, the size of the bubble represents the number of genes, and the darker the color, the greater the *P* value (confidence). The results of KEGG enrichment analysis are shown in Fig. 4. There are 64 pathways, mainly including IL-17 signaling pathway, VEGR signaling pathway, HIF-1 signaling pathway, *etc.*

3.6 Molecular docking Autodock molecular docking binding energies were -6.69, -9.78, -3.85, -5.05, -5.25 kcal/mol, respectively. The analysis of molecular docking results refers to the binding energy (ΔGbind). If the binding energy is less than 0, it indicates that the ligand and receptor can bind spontaneously. Binding affinity <-4.25 kcal/mol indicates that the two molecules have standard binding ability, <-5.0 kcal/mol indicates good binding, and <-7.0 kcal/mol indicates strong binding activity[13]. The four groups of results with the smallest binding energy were selected for PyMol visualization, as shown in Fig. 5. According to the results, the binding energy of three groups is less than -5 kcal/mol, and the binding energy of one group is less than -7 kcal/mol. The results showed that GAA could bind to the key targets AKT1, PTGS2, ESR1 and GSK3B, suggesting that GAA has a certain effect on MI.

4 Discussion and conclusions

4.1 Discussion The prevalence of cardiovascular disease in China is still rising, and ischemic heart disease is one of the main causes of death[14]. Clinically, the preferred treatment strategy for acute myocardial ischemia is to quickly restore coronary blood flow by reperfusion such as thrombolysis or intervention, but reperfusion is also prone to secondary myocardial injury, namely myocardial ischemia-reperfusion injury (MIRI)[15]. During MIRI, some

cellular functional metabolism and structural damage can activate inflammatory response and oxidative stress, leading to myocardial injury[16]. GAA is a chemical component of Glycyrrhizae Radix Et Rhizoma, which has a certain therapeutic effect on MIRI, but its mechanism is not clear.

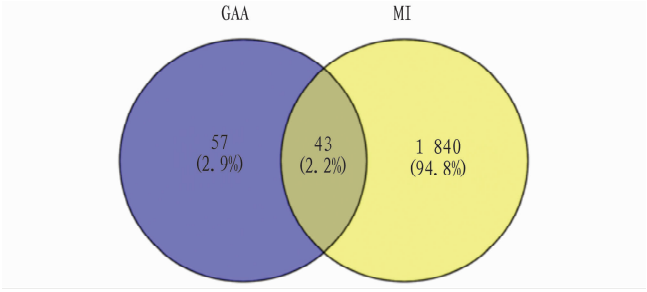

Fig. 1 Venn diagram

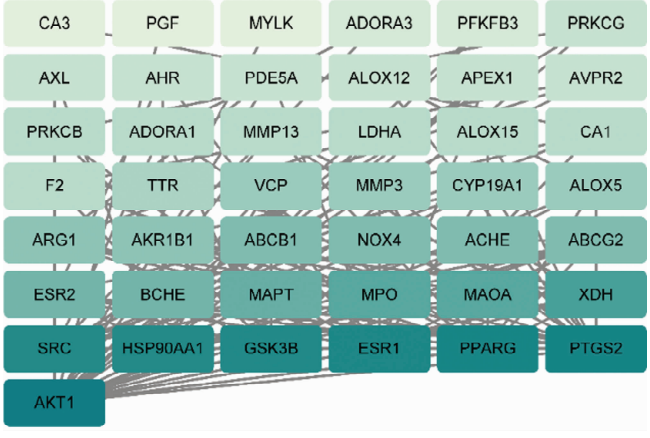

Fig. 2 PPI network

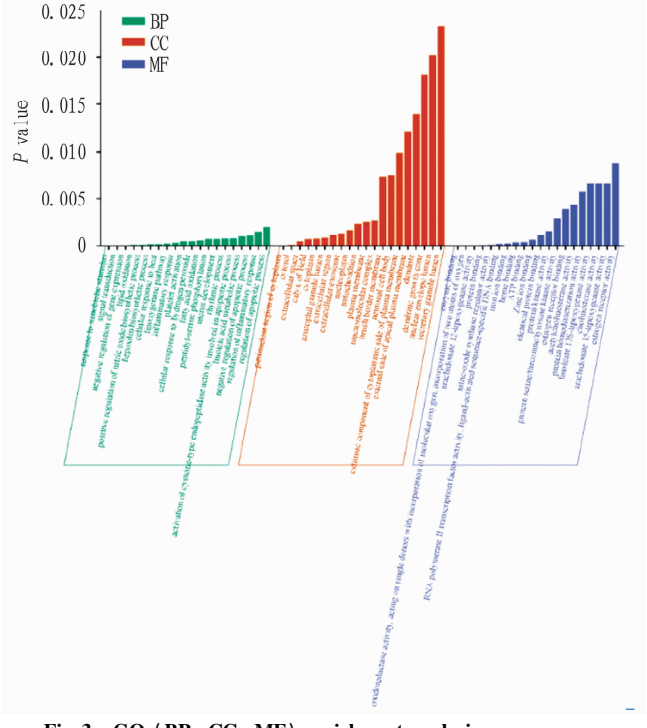

Fig. 3 GO (BP, CC, MF) enrichment analysis

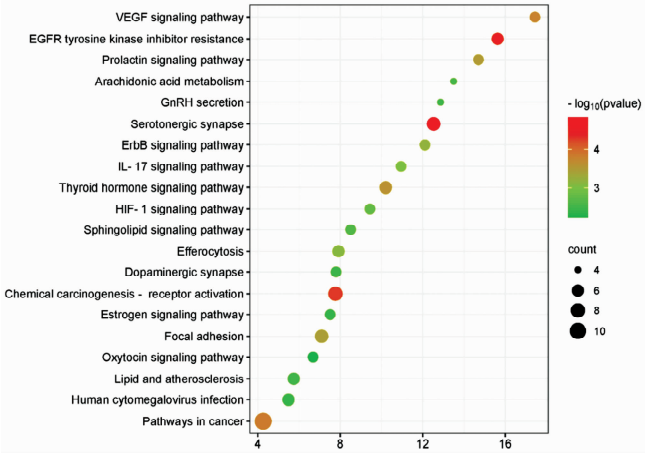

Fig. 4 KEGG enrichment analysis bubble diagram

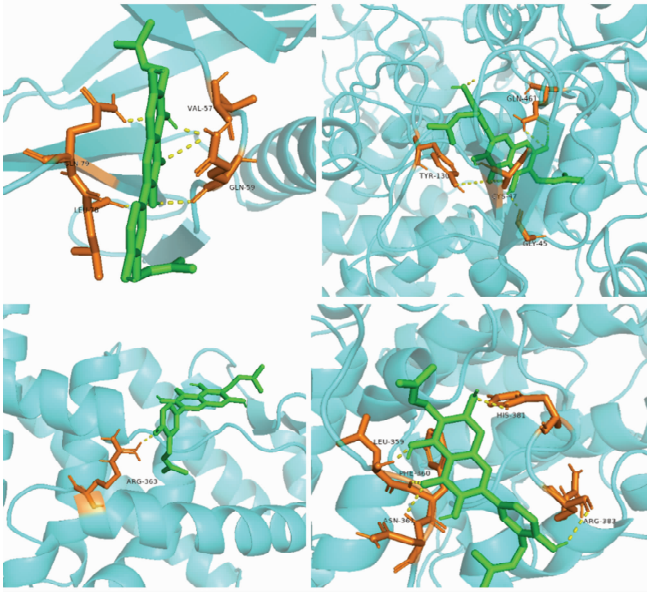

Fig. 5 PyMol visualization for GAA and AKT1, PTGS2, ESR1, GSK3BG

In this study, AKT1, PTGS2, ESR1 and GSK3B were found to be the key proteins of GAA against MI. AKT1 is a threonine kinase that is a key mediator of cell growth and survival processes, including glucose metabolism, apoptosis, transcription, cell proliferation, and migration[17-18], and phosphorylated threonine protein kinase (AKT) also regulates downstream factors mediating inflammatory responses[19]. PTGS2 is an enzyme that is released at the site of tissue injury to produce a hormone-like substance called prostaglandin E_2 that stimulates pain and inflammation[20]. ESR1 is a member of the nuclear hormone family of intracellular receptors. Studies have shown that excessive activation of ESR1 can induce increased secretion of IL-33 and aggravate bronchial asthma and inflammation[21]. SRC is a tyrosine protein kinase that regulates cell metabolism, proliferation, and inflammation[22]. Through KEGG pathway enrichment analysis, it was found that IL-17 signaling pathway, VEGR signaling pathway and HIF-1 signaling pathway were mainly enriched in many pathways related to

immunity and inflammation. The IL-17 signaling pathway is mainly mediated by the IL-17 family, and IL-17A and IL-17F are the major pro-inflammatory cytokines that bind to IL-17RA and IL-17RC, respectively, to form receptor complexes that generate transcriptional signals for inflammatory target genes[23]. HIF-1α is rapidly degraded under normoxic conditions, but can be synthesized in large quantities and induce the expression of VEGF under hypoxic conditions, and then VEGF promotes the activation of endothelial cells to induce inflammation, thereby promoting pathological angiogenesis and joint inflammation in rheumatoid arthritis[24]. Relevant studies have shown that inhibition of HIF-1α/VEGF signaling pathway can attenuate angiogenesis in rats with oxygen-induced retinopathy[25] and reduce lung inflammation in rats with acute lung injury[26]. In summary, GAA may regulate AKT1, PTGS2, SRC and other protein levels, and regulate IL-17 signaling pathway, VEGR signaling pathway and HIF-1 signaling pathway to treat related inflammation, from which it can be speculated that GAA has a certain anti-myocardial ischemia effect.

4.2 Conclusions In conclusion, GAA can act on AKT1, PTGS2, PPARG, ESR1 and other targets, and treat myocardial ischemia through cancer pathway, IL-17 signaling pathway, VEGR signaling pathway, HIF-1 signaling pathway and so on. In this study, the above results have been verified by molecular docking technology, but there is still a lack of animal, cell and other multi-level objective verification, coupled with the limitations of network pharmacology methods, the results predicted in this study need to be further verified by experiments.

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