MEDS Chinese Medicine (2023)
Clausius Scientific Press, Canada

DOI: 10.23977/medcm.2023.050908
ISSN 2616-1753 Vol. 5 Num. 9

Exploration of the mechanism of action of turmeric in improving ischemic stroke based on network pharmacology

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Keywords: Network pharmacology; ischemic stroke; turmeric

Abstract: In order to preliminarily explore the molecular mechanism of turmeric to improve ischemic stroke with the help of network pharmacology. Based on the pharmacological analysis platform of traditional Chinese medicine system, we screened the effective ingredients of drugs and analyzed various effective ingredients of traditional Chinese medicine using the Swiss target prediction database. Based on STRING combined with Cytoscape 3.5.1, we conducted protein interaction analysis on cross targets, and then conducted GO function and pathway enrichment analysis on cross genes. The number of active ingredients screened for turmeric was 3, the number of targets was 107, and the number of targets for ischemic stroke was 1159.19 cross genes were obtained, among which NR1H3, ESR2, ESR1, NOS2, SREBF2, PPARG, PPARA, NOS2 may be key targets for the role of turmeric in ischemic stroke. Through GO function and pathway enrichment analysis, 10 pathways were identified. The PPAR signaling pathway, arachidonic acid metabolism, and endocrine factors regulate calcium reabsorption, endocrine resistance, insulin resistance, 5-hydroxytryptaminergic synapses, and estrogen signaling pathways ($p<0.05$). While arachidonic acid metabolism and endocrine factors regulate calcium reabsorption, 5-hydroxytryptaminergic synapses, and estrogen signaling pathways.

1. Introduction

Ischemic stroke, also known as cerebral infarction in clinical practice, generally refers to a kind of cerebrovascular disease due to the narrowing or occlusion of the arteries in the brain, leading to acute blood circulation disorders in the brain tissues, resulting in cerebral tissue ischemia and hypoxia, which causes a series of important dysfunctions, such as consciousness, speech, swallowing, and limb activities, etc. and it is characterized by three major features, namely, high incidence, high mortality, and high disability, which seriously jeopardizes the health of human beings [1]. With the continuous development of the national economy, the improvement of living conditions, the improvement of living standards and lifestyle changes as well as the prolongation of human life expectancy and the acceleration of the social aging process, the incidence of cerebrovascular diseases and the morbidity

and mortality rates in China have continued to increase, which seriously affects the health of China's residents, and has become a major public health problem hindering the development of social economy. Ischemic stroke belongs to the category of "stroke disease" in traditional Chinese medicine, which is one of the "Four Difficult Symptoms" in ancient Chinese medicine. It is mostly caused by excessive exertion and fatigue, overeating and satiety, loss of healthy movement of the spleen, spleen deficiency generating phlegm, phlegm-heat interlacing, liver wind entraining phlegm flowing through the meridians, or liver and kidney yin deficiency, liver yang overreacting, gas and blood weakening, wind and fire fanning each other, stagnation of blood stasis, and reversal of qi and blood against the brain and the onset of the disease. In short, the disease is located in the brain, and has a close relationship with the heart, liver, kidney and spleen; its main manifestations are hemiplegia, skin discomfort, crooked tongue, unfavorable language, and even sudden fainting and unconsciousness. According to Chinese medicine, stroke is a disease caused by labor, fatigue, internal injuries, worry, anger, etc. which leads to deficiency of qi and blood, disorder of yin and yang in internal organs, resulting in paralysis of cerebral veins or blood overflowing outside the cerebral veins. The disability and mortality rates of ischemic stroke patients are increasing year by year [2]. Therefore, the search for traditional Chinese medicine for ischemic stroke has become a hot topic in clinical practice.

Curcumin is a Chinese medicinal ingredient extracted from the rhizome of the turmeric plant, family Zingiberaceae, and is the main functional constituent of the Chinese medicinal herb turmeric, which was first recorded in the Tang Materia Medica, described as "bitter in taste, cold in nature, and non-toxic". Modern pharmacological research has found that curcumin not only has a wide range of pharmacological effects, but also is cheap and easy to obtain, with little adverse reactions, and plays a variety of pharmacological roles in anti-oxidative stress, anti-inflammatory choleretic, anti-apoptosis, anti-tumor, antibacterial, anti-virus, anti-ischemia-reperfusion injury, anti-vascular smooth muscle proliferation, as well as hypoglycemia and blood glucose and blood lipids, etc. And curcumin has been widely used in the treatment of diabetes mellitus, ischemic stroke, hemorrhagic stroke, epilepsy, as well as respiratory diseases, digestive diseases, cardiovascular diseases, peripheral arterial diseases, ophthalmic diseases, malignant tumors and many other diseases [3].

Curcumin is an orange crystalline powder at room temperature with a bitter taste because it contains 2 hydroxyl groups and is poorly soluble in water. When ph>8 points, curcumin turns from yellow to red. In addition, curcumin has strong coloring ability, not easy to fade, can be used as a dye. At present, curcumin is difficult to dissolve, difficult to extract, low absorption, fast metabolism, fear of light, fear of heat, sensitive to iron ions and other problems, resulting in low bioavailability of curcumin, limiting clinical applications. In recent years, the successful development of conductive drug systems with liposomes, microspheres, colloids, dendritic polymers and micromilk as carriers has significantly increased the solubility, stability, in vivo absorption and safety of curcumin, which can be used in the treatment of neurological disorders and pre greatly improved the biological activity of the defense, showing great possibilities for clinical application.

In this experiment, using turmeric as an entry point, the molecular mechanism of turmeric against stroke was clarified by enriching the herbal components, component targets, diseases, disease targets, protein interactions, GO functions and pathways by modern analytical software and database.

2. Database and Software

TCMSP (http://ibts.hkbu.edu.hk/LSP/.tcmspsearch/), Pubche (https://pubchem.ncbi.nlm.nih.gov), Swiss Target Prediction (http://www.swisstargetprediction.ch/), STRING (https://string-db.org/), DisGeNET (https://www.disgenet.org/).

3. Methods

3.1. Screening of active ingredients of turmeric

The chemical composition of turmeric was searched using the TCMSP database, and the oral bioavailability (OB) ≥ 30% and drug-like properties (DL) ≥ 0.18 were used as the screening conditions for the active ingredients, and three components were obtained

3.2. Screening of turmeric component targets

The SMILES name of each ingredient was queried using Pubchem database, and the target protein names and probability of action of the three ingredients were obtained separately using Swiss Target Prediction, and the target protein with probability 0 was removed.

3.3. Ischemic stroke target screening

We searched for "Ischemic stroke" in the DisGeNET database to obtain the target proteins for ischemic stroke.

3.4. Screening of intersecting genes

We used the "MicrolifeInfo" platform, the three active ingredients and the target proteins of ischemic stroke were analyzed, and 19 intersecting genes were obtained.

3.5. Intersection gene network

We Used Cytoscape software, the intersection gene network between turmeric active ingredient and ischemic stroke was mapped.

3.6. Protein Interaction Network

We Used STRING platform and analyzed the role and relationship between 19 intersectional target proteins.

3.7. Gene set function enrichment

The latest KEGG Pathway gene annotations were obtained using the KEGG rest API (https://www.kegg.jp/kegg/rest/keggapi.html) as the background, and the genes were mapped to the background set using the R package clusterProfiler (version 3.14.3) to perform enrichment analysis to obtain the results of gene set enrichment. A minimum gene set of 5 and a maximum gene set of 5000 were set, and a P value of < 0.05 and a FDR of < 0.25 were considered statistically significant.

4. Results

4.1. Screening of the active components of turmeric

We obtained three components, namely Stigmasterol, campesterol, Curcumae longae Rhizoma (CLR).

Stigmasterol is the major phytosterol in various herbs with strong pharmacological activities [4,5]. Studies have shown that Stigmasterol significantly ameliorates heart and brain I/R injury. Its effects

include reduction in infarct size, increase in myocardial and plasma superoxide dismutase (SOD) activity, scavenging of oxygen free radicals, calcium antagonism, improvement in microcirculation, reduction in brain water content, calcium ions, glutamate, aspartate, glycine, and malondialdehyde, a lipid peroxidation product.

Campesterol may be a key compound in turmeric [6]. Sesquiterpene compounds of turmeric have strong antimicrobial, anti-inflammatory, neuroprotective, anticancer, antiviral and antithrombotic activities [7]. Campesterol possesses anti-inflammatory, anti-angiogenic, anticancer, antioxidant and hypocholesterolemic properties [8,9].

Curcumae Longae Rhizoma, (CLR) is a traditional herbal medicine used for many years and contains mainly volatile oils and phenolics [10]. Several constituents have been identified in the volatile oil of Elsholtzia sp. mainly terpenoids, curcuminoids and curcuminoids. The main phenolic constituent of CLR is curcuminoids [10]. CLR also contains a new type of curcuminoids, 3-sitosterol, 3-sitosterol-3- o -carotene, and curcuminoid polysaccharides [11]. Pharmacological studies have shown that CLR has hepatoprotective, choleretic, antibacterial, anti-inflammatory, antitumor, hypolipidemic, and antipathogenic microbial effects [12]. It also protects the digestive system, enhances immune function, and improves coronary blood flow to the heart [13-17].

4.2. Screening of turmeric component targets and ischemic stroke target screening

There are 100 target proteins for Stigmasterol, of which 42 have a probability greater than 0. There are 100 target proteins for campesterol, of which 19 have a probability greater than 0. There are 100 target proteins for CLR, of which 46 have a probability greater than 0. There are 1159 target proteins for Ischemic stroke.

4.3. Screening of crossover genes

A total of 19 cross-linked genes were obtained from the "microbiology letter" platform, which are ACHE, AR, CYP2C19, ESR1, ESR2, G6PD, NOS2, NR1H3, PPARA, PPARD, PPARG, PTGER2, PTGES, SHH, SIGMAR1, SLC6A4, SREBF2, TBXAS1, VDR. (Figure 1)

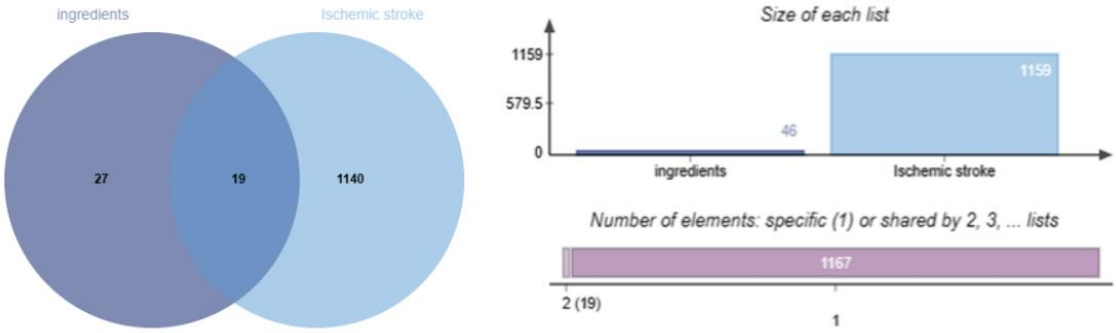

Figure 1: Screening of crossover genes

4.4. Intersecting gene network

This is a network diagram of the 19 intersecting genes drawn by using Cytoscape software. A, B and C represent the three active ingredients of Stigmasterol, campesterol and CLR turmeric in that order. (Figure 2)

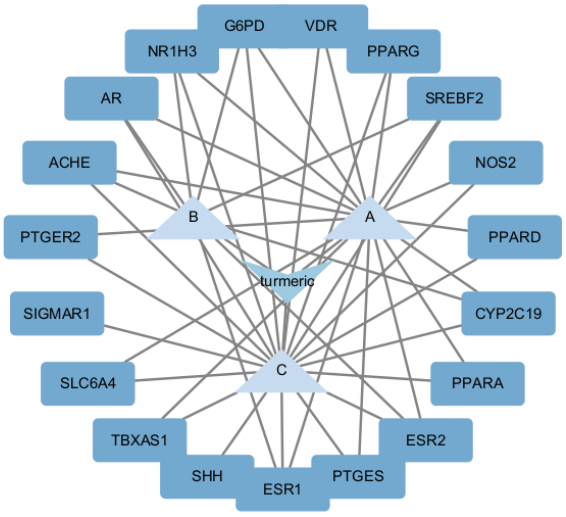

Figure 2: Network Diagram of Cross Genes

4.5. Protein interaction analysis and network construction

The 19 intersecting proteins were analyzed to obtain the relationship of their interactions by using STRING , in which the two highest scores, micro ESR1 and ESR2, had the highest scores and scores = 0.987, so we considered ESR1 and ESR2 as the most critical genes of turmeric to improve ischemic stroke. (Figure 3)

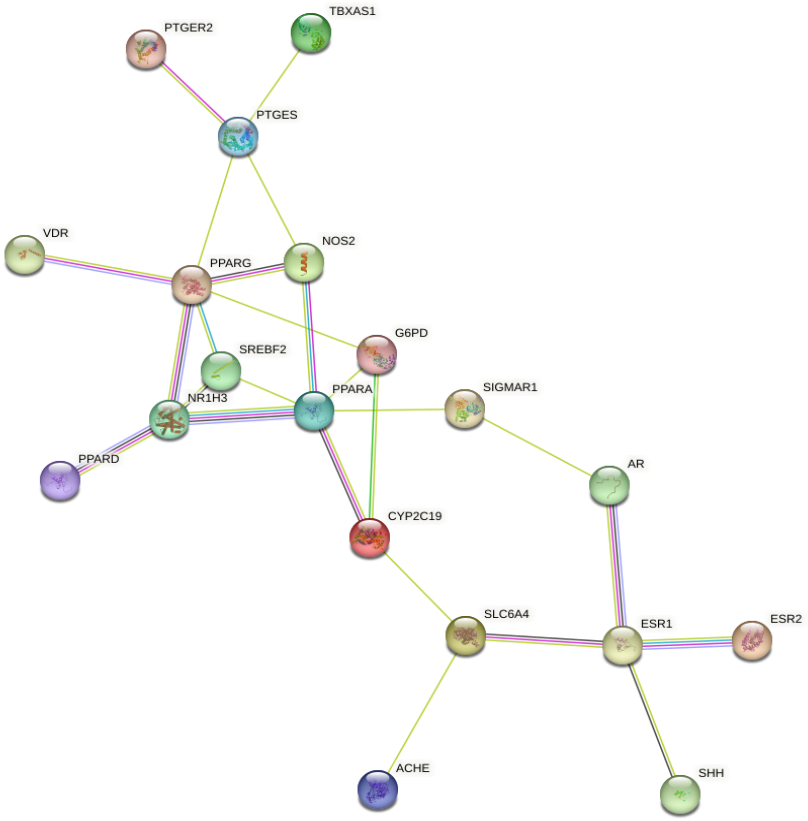

Figure 3: Protein interaction analysis and network construction

4.6. Pathway Enrichment

Pathway enrichment was performed on 19 intersecting genes, and we obtained 10 related pathways: Pathways in cancer, PPAR signaling pathway, Arachidonic acid metabolism, Endocrine and other factor-regulated calcium reabsorption, Prolactin, signaling pathway, Endocrine resistance, Insulin resistance, Serotonergic synapse, Estrogen signaling pathway, Breast cancer. (Figure 4 and Figure 5)

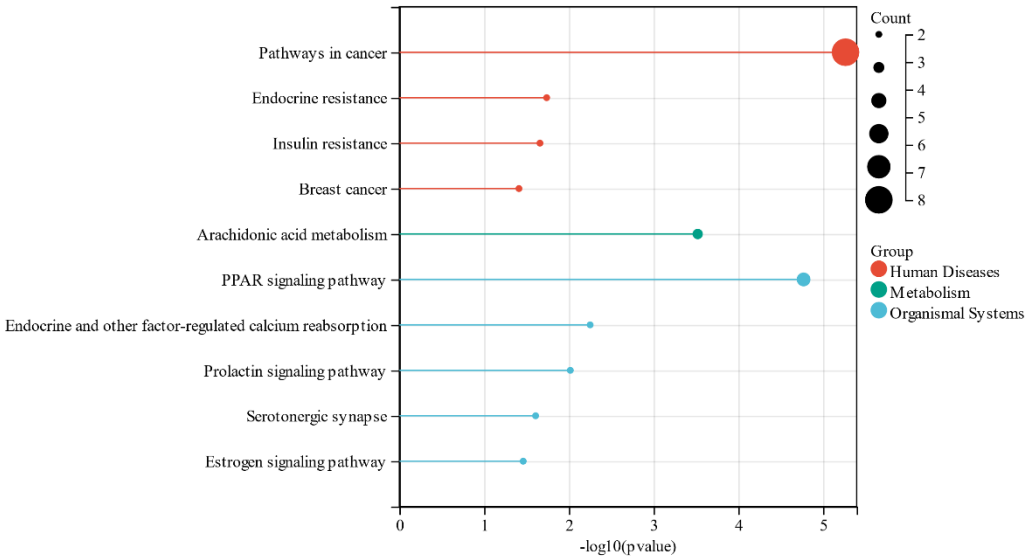

Figure 4: Pathway Enrichment

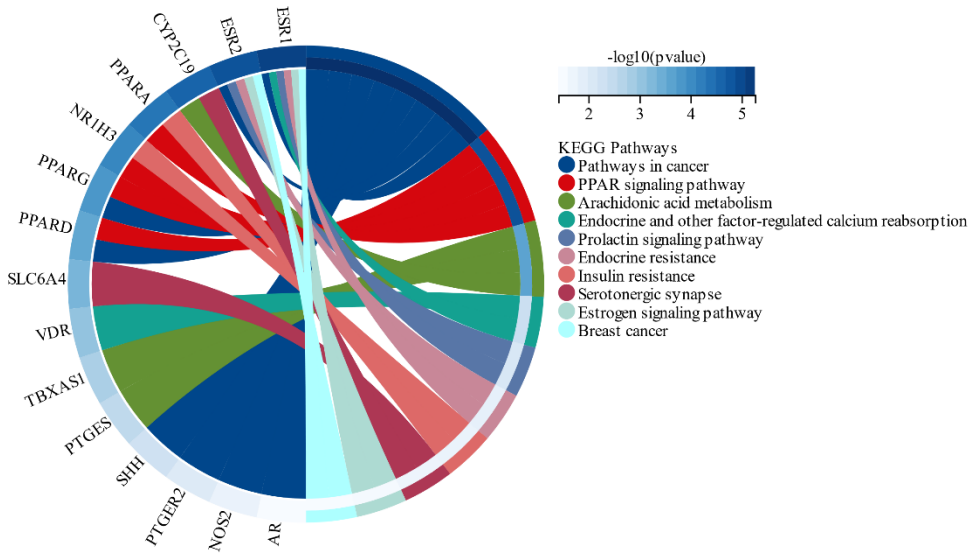

Figure 5: KEGG Pathways

5. Conclusion

Through network pharmacological analysis, we concluded that turmeric ameliorates ischemic stroke with eight main target proteins NR1H3, ESR2, ESR1, NOS2, SREBF2, PPARG, PPARA, NOS2 (score '0.9), of which ESR2 and ESR1 may be the core targets. The pathway of turmeric to improve ischemic stroke may be related to PPAR signaling pathway, arachidonic acid metabolism, calcium reabsorption regulated by endocrine and other factors, 5-hydroxytryptaminergic synapses,

and estrogen signaling pathway

6. Discussion

Ischemic stroke is a disease with high morbidity, pathogenicity and mortality, which poses a great threat to human health [18]. In recent years, more and more studies have pointed out the efficacy of traditional Chinese medicine in the treatment of ischemic stroke [19]. Curcumin and its analogs can play a neuroprotective role in ischemic stroke through a variety of pathways, including anti-inflammatory response, anti-oxidative stress, anti-apoptosis, and autophagy. Curcumin and its analogs can play a neuroprotective role in ischemic stroke through a variety of pathways, including anti-inflammatory response, anti-oxidative stress, anti-apoptosis and autophagy. And PPAR signaling pathway can achieve anti-ischemic stroke effects through various pathways such as inhibiting neuroinflammation, inhibiting neuronal apoptosis, inhibiting oxidative stress damage, and inhibiting neuronal autophagy. Arachidonic acid metabolism plays an important role in the pathogenesis of ischemic stroke. Due to the obstruction of cerebral blood flow in ischemic stroke, hypoxic brain tissue begins to rapidly cleave arachidonic acid from the membrane phospholipid bilayer, and arachidonic acid undergoes non-enzymatic-dependent and enzyme-mediated oxidative metabolism, resulting in the formation of a variety of biologically active metabolites, which contribute to the improvement of pathologic changes in ischemic stroke. It has been reported that stroke is closely related to estrogen levels, and estrogen has a better protective effect against stroke. We believe that curcumin may achieve therapeutic effects on ischemic stroke through several pathways mentioned above, showing great potential and application prospects in clinical disease prevention and treatment. This study suggests that curcumin is important for the treatment of ischemic stroke.

Chinese traditional culture has a long history, among which Chinese herbal medicine is an important component. Through thousands of years of theory and practice, Chinese herbal medicine has greatly protected the health of the people of this country, whether in the same disease with different treatments, the theory of medicine and food homology, or other traditional Chinese medicine theories. Although modern medicine has become the mainstream, there are still health problems that remain. Chinese medicine has the potential to bring breakthroughs in the health field. We hope Chinese medicine can bring more happiness to the people of the world.

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