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Network pharmacology study on the potential effect mechanism of Chuanzhi Tongluo Capsule in the treatment of cerebral infarction

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Abstract

Background: Chuanxiong Tongluo capsules have been widely used to treat recovered stroke and cerebral infarction, but their specific therapeutic mechanism is not well understood.

Methods: This study aims to investigate the mechanism of action for Chuanzhi Tongluo capsule on cerebral infarction based on a network pharmacology approach. The TCMSP platform collected the chemical composition of Chuanzhi Tongluo capsules. Its potential targets were predicted by Swiss target prediction and standardized using the Uniprot database for gene normalization. Meanwhile, the OMIM, Genecards, and TTD databases were used to obtain the targets related to cerebral infarction. The standard targets of Chuanzhi Tongluo capsule and cerebral infarction were uploaded to the STRING database to construct protein–protein interaction networks. Topological methods analyzed the key targets and components in the drug-component-disease-target network. Gene ontology function and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of the shared targets were performed using the DAVID database.

Results: A total of 105 active ingredients and 427 targets were associated with Chuanzhi Tongluo capsule, and there were 3055 targets related to cerebral infarction disease and 240 common targets between the two keywords. The key targets included INS, ALB, IL-6, VEGFA, TNF, and TP53. The conduction pathways involved include the calcium signaling pathway, cAMP signaling pathway, cGMP-PKG signaling pathway, and TNF signaling pathway.

Conclusion: The active ingredients in Chuanzhi Tongluo capsule may participate in the therapeutic process of cerebral infarction by regulating the calcium, cAMP, cGMP-PKG, and TNF signaling pathway through critical targets such as INS, ALB, IL-6, VEGFA, TNF, and TP53.

Abbreviations: ALB = albumin, APTT = activated partial thromboplastin time, cAMP = cyclic adenosine monophosphate, cGMP-PKG = cyclic guanosine monophosphate-dependent protein kinase G, CI = cerebral infarction, FDA = Food and Drug Administration, GO = gene ontology, IL-6 = interleukin-6, INS = insulin, OMIM = Online Mendelian Inheritance in Man, PKG = protein kinase G, PPI = protein protein interaction, PT = prothrombin time, TCM = traditional chinese medicine, TCMSP = traditional chinese medicine systems pharmacology database and analysis platform, TNF = tumor necrosis factor, TP53 = tumor protein 53, TT = thrombin Time, TTD = Therapeutic Target Database, VEGFA = vascular endothelial growth factor A.

Keywords: action mechanism, cerebral infarction, Chuanzhi Tongluo Capsule, network pharmacology

1. Introduction

Cerebral infarction, also known as ischemic stroke, is a common clinical cerebrovascular disease with a high mortality and disability rate in patients. Patients with this disease mostly have underlying diseases that induce local blood circulation disorders in the brain, such as hypertension and abnormal platelet function. These causes predispose patients to multiple ischemias, hypoxia, softening necrosis of tissues, and eventually neurological impairment.[1] Epidemiological data show that the disease is characterized by rapid onset, high disability, mortality, and recurrence rates, resulting in the highest rate of disability in patients caused by a single disease in the world.[2] Similar to atherosclerosis, hypertension, coronary heart disease, hyperlipidemia, diabetes mellitus, obesity, alcohol consumption, and smoking are common risk factors for cerebral infarction. The American Heart Association/American Stroke Association recommends that stroke prevention can be achieved by controlling the causative risk factors.[3,4] Currently, intravenous recombinant tissue fibrinogen activator is the only FDA-approved drug for the treatment of ischemic stroke. However, the drug must be administered within 4.5 hours of the patient's stroke onset, otherwise, there is a risk of intracranial hemorrhage.

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Intravascular mechanical embolization is one of the most important methods for the treatment of acute ischemic stroke in recent years and is a more effective treatment recommended in the guidelines for the treatment of this disease. However, the implementation of this method requires a more specialized team of physicians and a rigorous evaluation of the patient.[5] These drawbacks limit the clinical application of this treatment method. Current research shows that TCM can regulate the pathophysiological processes of patients with cerebral infarction through the advantages of multi-component, multi-target, and multi-effectiveness, and can play a more scientific, rational, and effective role in the purpose of comprehensive treatment.

In recent years, with the development of modern genomics, proteomics, metabolomics and other theories, as well as the introduction of a systems biology perspective and the application of bioinformatics, the concept of network pharmacology (Network Pharmacology) has been proposed.[6,7] The principle of network pharmacology is to transform the traditional "one drug, one target" model into a holistic "multi-component, multi-target" model by constructing a network relationship between active ingredients and targets. The approach of elaborating the systemic mechanism of action of complex drugs at the molecular level is in line with the holistic concept emphasized in Chinese medicine.[8] The complexity of compound components in traditional Chinese medicine formulations and the lack of research methods often make it difficult for researchers to achieve a detailed study of the mechanisms of the action exerted by its components. Therefore, the network pharmacology research for TCM provides a scientific basis for explaining the differences in the way of thinking between Chinese and Western medicine and points the way to the worldwide promotion of TCM.

The Chinese patent medicine Chuanzhi Tongluo Capsule is composed of the leech, Chuanxiong (Szechuan lovage rhizome), Danshen (Dan-Shen Root), and Huangqi (Milkvetch Root). This drug has the effect of clearing away heat and toxic material and is widely used in the treatment of stroke and cerebral infarction in the recovery period. The present study initially elucidated the mechanism of action of Chuanzhi Tongluo Capsule in the treatment of cerebral infarction and laid a good foundation for further research on the pharmacological basis and mechanism of action of this Chinese medicine.

2. Materials and Methods

2.1. Collection of active ingredients of Chuanzhi Tongluo Capsule

The compound components of Chuanzhi Tongluo Capsule were searched in the database of TCMSP and BATMAN with the keywords of "Huangqi", "Dan Shen", "leech", and "Chuanxiong". On the premise of pharmacokinetic principle, OB ≥ 30% and DL ≥ 0.18 were used as the screening conditions,[9] and the eligible components of Astragalus, Salvia, Leech, and Chuanxiong were selected as the active ingredients. This study is a summary of data from published articles and does not address issues related to patient ethics, etc. Therefore, this study does not require approval by the ethics committee.

2.2. Prediction of potential targets for the treatment of cerebral infarction with Chuanzhi Tongluo Capsules

A search was conducted in the human genetic database (Genecards https://www.genecards.org/) using the keyword "Cerebral Infarction, (CI)", and the disease-related genes were screened. The potential targets for the treatment of CI disease with Chuanzhi Tongluo Capsules were obtained by mapping the obtained targets to the compound targets screened in 1.1.

2.3. Target screening and construction of "drug-compound-target" network of Chuanzhi Tongluo Capsule

The active ingredients and their corresponding targets were searched and screened in the TCMSP database, and the gene names of the targets were corrected with the Uniprot database.[10] The target proteins were imported into Cytoscape 3.7.2 software to construct a "drug-compound-target" network for analysis.

2.4. Protein–protein interaction (PPI) network construction and analysis

The potential targets of the therapeutic CI effect of Chuanzhi Tongluo Capsule screened under 1.3 were entered into the STRING database, and the genus "Homo Sapiens" (Human) was set, and the protein interaction relationships were obtained. The data files were saved in TSV format. The obtained data were imported into Cytoscape 3.7.2 software to construct PPI networks and analyze the core targets of the conditional screening.

2.5. Gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

To gain insight into the functions of the potential target genes and their roles in the signaling pathways of the above screened CIs, we performed GO analysis using the ClueGO plug-in and KEGG pathway enrichment analysis using the DAVID database. We set the *P*-value < .05 and defined the species as "Homo Sapiens." The top 20 entries of the KEGG pathway were selected for visualization and ranked according to the number of enriched targets.

3. Results

3.1. Screening of active ingredients in Chuanhuitongluo capsules

A total of 105 compounds were screened by TCMSP and Batman-TCM databases in Chuanzhi Tongluo Capsules. Among them, 20 were from Astragalus, 65 from Salvia, 13 from the leech, and 7 from Chuanxiong. A total of 427 targets were predicted. For more information, see Table 1.

3.2. Prediction and screening of core targets for the treatment of cerebral infarction with Chuanzhi Tongluo Capsule

A total of 3055 potential CI targets were screened using the GeneCards database. The online tool Venny 2. 1 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to draw the Venn diagram (Fig. 1) of the active ingredient targets and CI targets of Chuanzhi Tongluo Capsule. A total of 240 potential predicted intersection targets were obtained for CI treatment with Chuanzhi Tongluo Capsule.

Table 1

The information on the active compounds and the targets of Chuanzhi Tongluo Capsules.

Drug ingredients	Chemical compound	Forecast targets	Database
Salvia miltiorrhiza	65	99	TCMSP
Milkvetch Root	20	126	TCMSP
Szechuan Lovage Rhizome	7	30	TCMSP
Leech	13	336	BATMAN-TCM

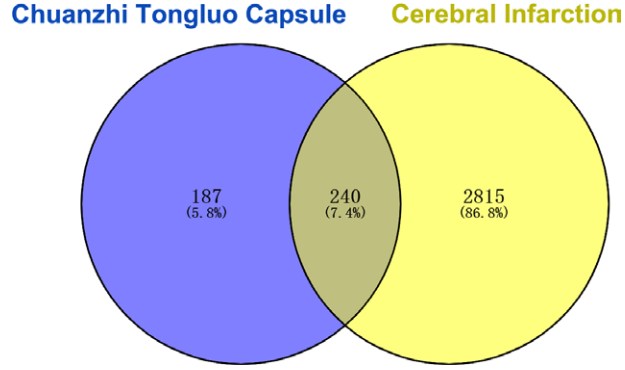

Figure 1. Venn diagram of the intersectional targets of Chuanzhi Tongluo Capsule for cerebral infarction.

3.3. Drug-component-target-disease network diagram construction

Cytoscape 3.7.2 software was used to construct the "drug-component-target-disease" network diagram (Fig. 2). A total of 550 nodes (including 4 drug nodes, 1 disease target, 441 targets, and 104 active compound nodes) and 956 edges were analyzed in the network diagram. In the network, triangles represent the drug and cerebral infarcts of Chuanzhi Tongluo Capsule, circles represent the compound active compounds, and target genes are represented by V-shaped. In the network, each edge represents the interactions between nodes, and the degree value represents the number of connections between nodes and other nodes. The data are analyzed using the "Network analyze" plug-in. The nodes with significant rank and centrality values in the network are screened, and the resulting nodes may play a key role in the network.[1] Thus, these multiple correspondences between the active compounds and the targets reflect the complexity of the herbal compound, while the

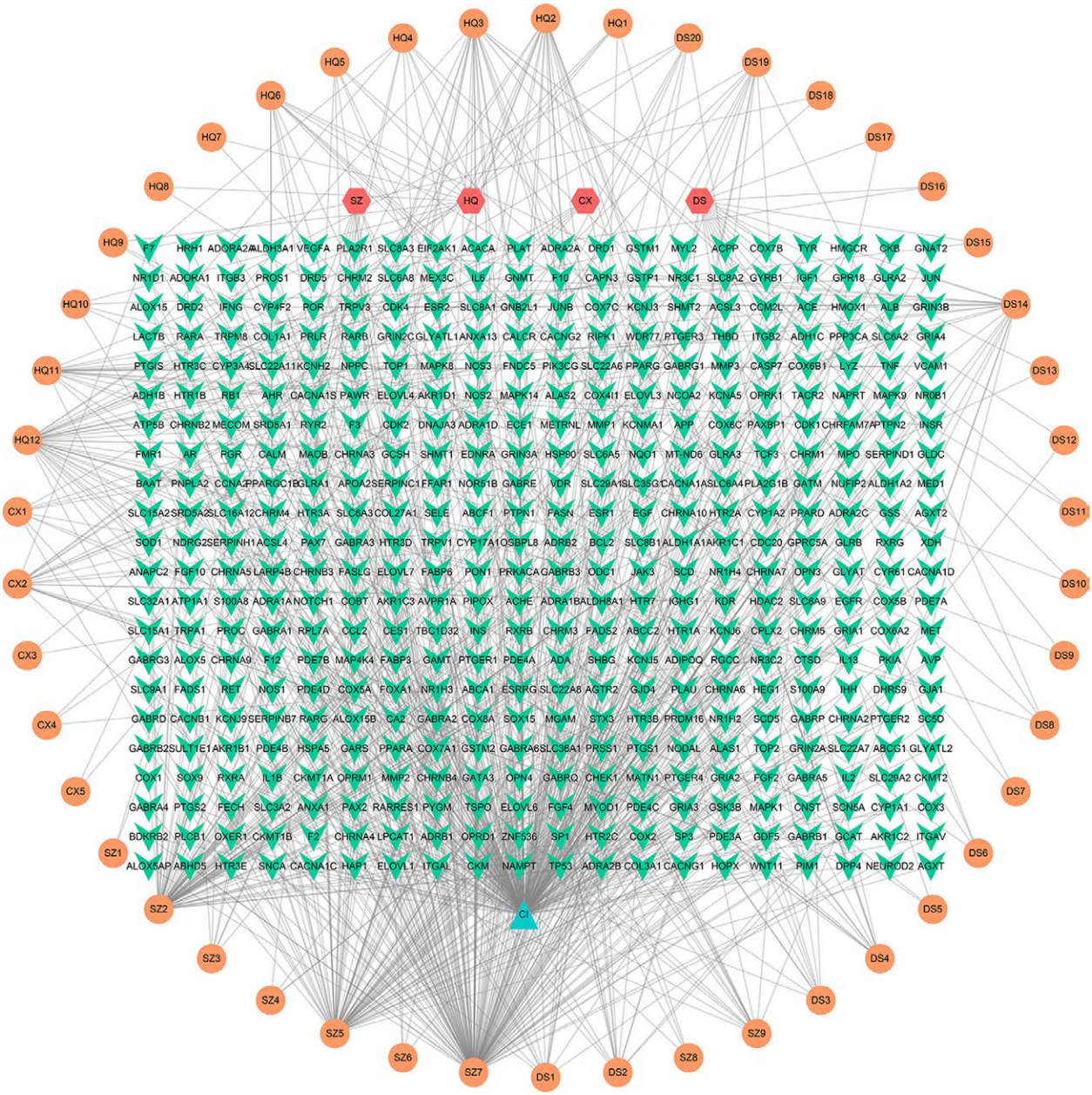

Figure 2. The drug-component-target-disease network diagram. For definition of abbreviations, see Appendix 1, Supplemental Digital Content, http://links.lww.com/MD/H448.

interaction between the components and the targets may be a reflection of the holistic therapeutic effect of the herbal compound. Based on the screening of the rank and centrality values of the active compounds, the top 5 active ingredients were crocetin, ursolic acid, D-Mannitol, quercetin, and hederagenin, respectively. This suggests that several of the above active ingredients may be key factors in the treatment of cerebral infarction with Chuanzhi Tongluo Capsule.

3.4. Prediction and analysis of core targets of Chuanzhi Tongluo Capsule for cerebral infarction

The intersecting targets of Chuanzhi Tongluo Capsule and cerebral infarction were imported into the STRING 11.0 database to obtain the protein interaction network map (Fig. 3). The obtained data were imported into Cytoscape 3.7.2 software in TSV format to construct the PPI network (Fig. 3). The network contains a total of 238 nodes and 3086 edges. The top 10 targets, such as INS, ALB, IL-6, VEGFA, TNF, TP53, APP, MAPK1, EGFR, and FTGS2, were ranked in descending order of degree value, and the top 10 targets may be the key targets for the treatment of cerebral infarction with Chuanzhi Tongluo Capsule (Table 2).

3.5. GO functional enrichment analysis

A total of 142 GO entries ($P < .05$) were obtained using the DAVID online platform for GO functional enrichment analysis of the intersecting targets. Among them, 96 entries were obtained for biological processes (Fig. 4A), mainly related to response to drug and response to hypoxia, and 21 entries were obtained for cell composition, mainly related to the plasma membrane and integral component of the plasma membrane (Fig. 4B); 25 articles of molecular function (Fig. 4C), mainly related to steroid hormone receptor activity (Fig. 5). The molecular functions are mainly related to steroid hormone receptor activity and drug binding.

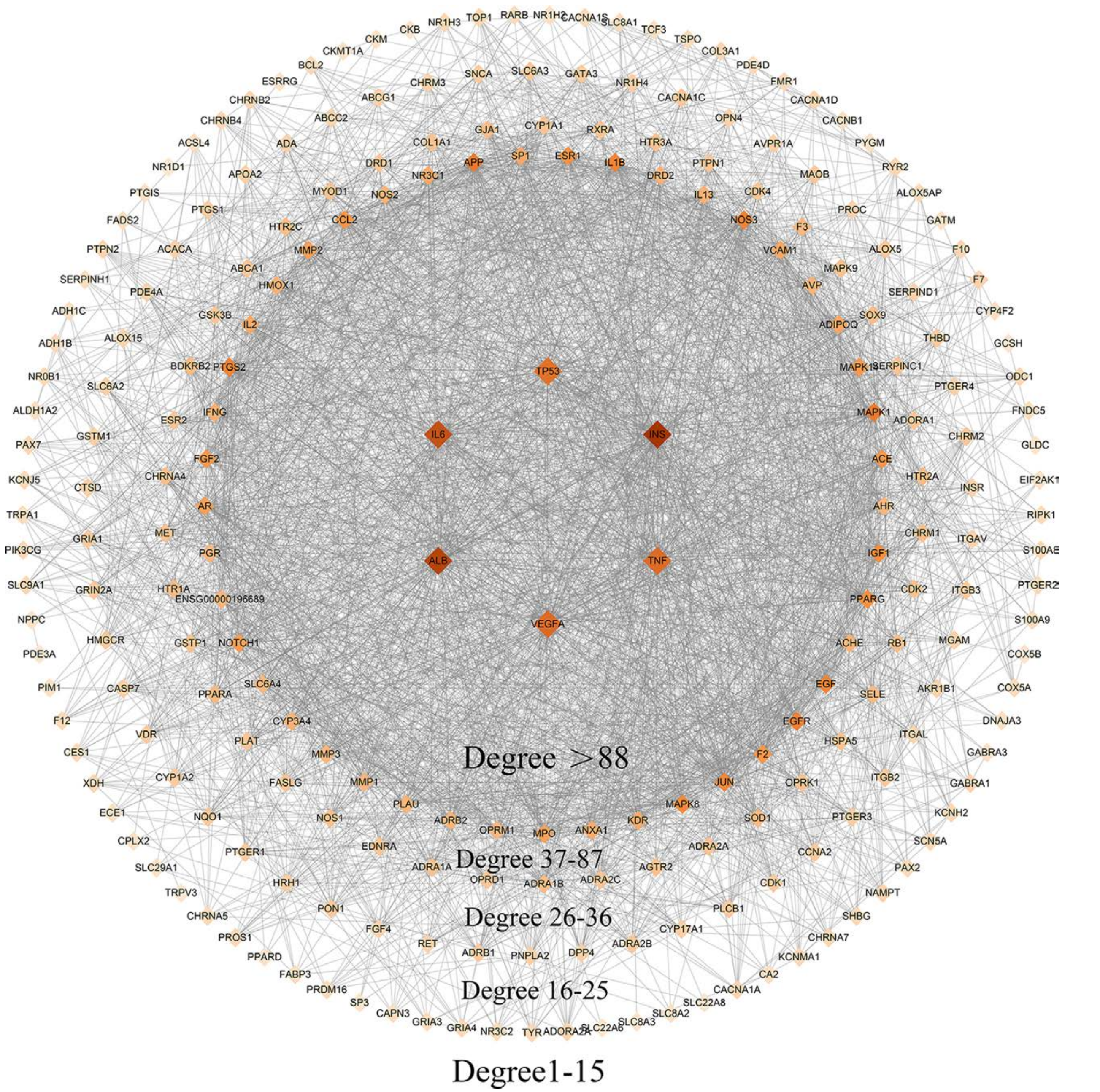

Figure 3. Protein–protein interaction network diagram. For definition of abbreviations, see Appendix 1, Supplemental Digital Content, http://links.lww.com/MD/H448.

3.6. KEGG pathway enrichment analysis

The DAVID database was used for enrichment analysis of the intersection targets, and $P < .05$ was used as the screening criterion. A total of 25 pathways were screened, mainly involving calcium signaling pathway, cAMP signaling pathway, cGMP-PKG signaling pathway, TNF signaling pathway, cGMP-PKG signaling pathway, TNF signaling pathway, and HIF-1 signaling pathway (Table 3). This suggests that the active ingredients in Chuanzhi Tongluo Capsule may be involved in the treatment of patients with cerebral infarction through the above signaling pathways.

4. Discussion

Cerebral infarction is one of the most common cerebrovascular diseases in clinical practice. The pathogenesis of this disease is mostly due to the obstruction of cerebrovascular blood circulation in patients, which causes the insufficient blood supply to brain tissue, thus triggering ischemia and hypoxia in brain tissue and leading to impaired brain function. The clinical symptoms of patients mainly include coma, unconsciousness, hemiplegia, and numbness of the limbs.[11,12] The etiology, pathological process and related pathogenic mechanisms of this disease are complex and diverse, and patients have a long course. Current studies have shown that Chinese medicine has multi-target and multi-faceted advantages in the treatment of this disease.[13]

In Chinese medicine, cerebral infarction is called "stroke." According to TCM, the pathogenesis of stroke patients is closely related to the pathological factors of wind, fire, phlegm, qi and stasis in TCM, among which "stasis" is involved in the whole process of ischemic stroke.[14] Patients with this disease are prone to sequelae during the recovery period due to an imbalance of Qi and blood, and blood vessels are not smooth. Therefore, activating blood circulation and removing blood stasis is quite important in the treatment of stroke disease.[15] Chuanzhi Tongluo Capsule is an enteric-soluble hard capsule of Chinese herbal medicine developed from four herbs, namely, Astragalus, Salvia, leech, and Chuanxiong, and supplemented by Buyang Huanwu Tang.[16] This Chinese medicine uses leech as the main drug component to release the patient's blood stasis; Chuanxiong is a supplementary drug that can work with a leech to reach the brain and activate blood circulation; it can also make the overall qi and blood flow smoothly and the functions of internal organs work well; as a supplementary drug, Huangqi's function of tonifying Qi can make the stagnant blood work again and maintain the meridians; Danshen has the function of activating blood to remove blood stasis, calming the mind and nourishing the heart; this Chinese medicine can be used for patients with cerebral infarction The herbal medicine can activate blood circulation, remove blood stasis, benefit qi, and promote circulation.[17] Studies have shown that quercetin, a major component of the Astragalus, can reduce neuronal cell damage through mechanisms such as inhibition of oxidative stress and inflammatory response, and play a role in protecting the nervous system in stroke.[18] Hirudin in leeches is the most potent natural thrombin inhibitor found to date, with significant anti-platelet aggregation, anticoagulation, antithrombotic, vasodilatory and viscosity-lowering effects.[19] Hirudin is less stable and its enteric capsules prevent the destruction of this active ingredient by gastric acid and pepsin, which further enhances its anticoagulant and antithrombotic effects.[20] Salvia can up-regulate the expression of fibroblast growth factor bFGF and has a protective effect on nerve cells undergoing ischemia-reperfusion; salvia can also further reduce PG concentration by reducing superoxide dismutase activity and mitigating ischemia-reperfusion injury.[21] Among them, danshenin can inhibit the release of thromboxane from endothelial cells.[22] Chuanxiongzin in chuanxiong can reduce ischemia-reperfusion injury in patients by reducing oxygen stress and promoting cellular mitochondrial energy metabolism.[23,24] In addition, Chuan Xiong has inhibited platelet aggregation and antithrombotic effects. Chuanxiongzin also reduces leukocyte adhesion to the venous wall, inhibits erythrocyte aggregation, accelerates erythrocyte electrophoresis, reduces platelet adhesion rate, and prevents elevated blood viscosity, among other effects.[25,26]

Based on the network diagram of drug-component-disease-target, the screening of key ingredients reveals that the main ingredients in Chuanzhi Tongluo Capsule for the treatment of cerebral infarction include, crocetin, ursolic acid, dextro-mannitol, quercetin, ivy saponin element (hederagenin), etc. There are reports in the literature showing various pharmacological effects

Table 2

Result of core target network information.

Name	Average shortest path length	Betweenness centrality	Closeness centrality	Degree	Radiality
INS	1.4556962	0.12366096	0.68695652	134	0.90886076
ALB	1.51476793	0.07540767	0.66016713	122	0.89704641
IL6	1.58649789	0.0511433	0.63031915	112	0.88270042
VEGFA	1.67932489	0.02430517	0.59547739	93	0.86413502
TNF	1.68776371	0.02564841	0.5925	92	0.86244726
TP53	1.70886076	0.02809122	0.58518519	88	0.85822785
MAPK1	1.71308017	0.02474894	0.58374384	81	0.85738397
APP	1.70042194	0.04541666	0.58808933	81	0.85991561
EGFR	1.74683544	0.01708144	0.57246377	76	0.85063291
PTGS2	1.76793249	0.02369004	0.56563246	75	0.8464135

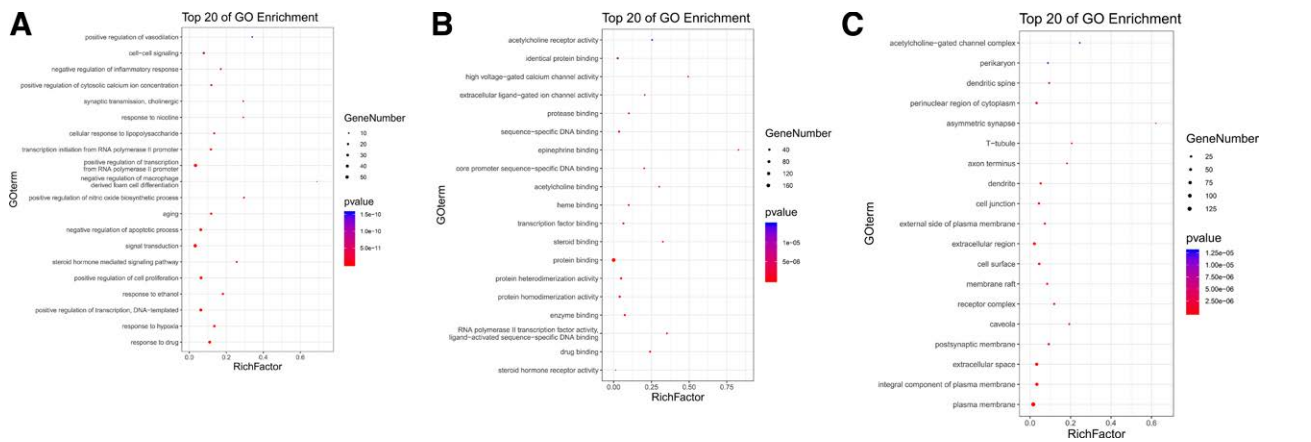

Figure 4. The chart of GO functional enrichment analysis. (A) Biological processes; (B) cell composition; and (C) molecular function. GO = gene ontology.

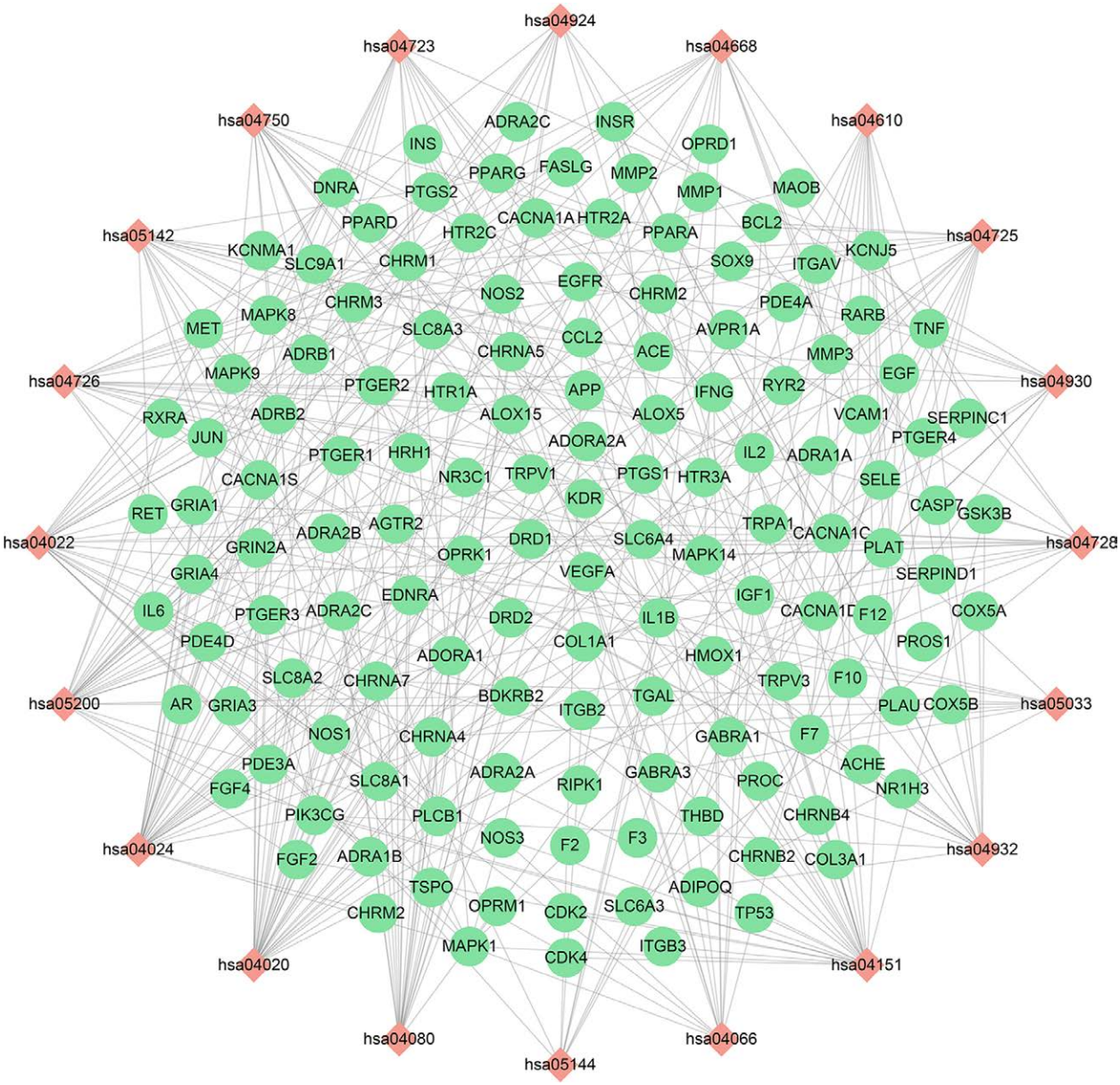

Figure 5. The target-pathway map of Chuanzhi Tongluo Capsule. For definition of abbreviations, see Appendix 1, Supplemental Digital Content, http://links.lww.com/MD/H448.

of saffron acid such as anti-tumor[27] and anti-atherosclerosis.[28] In addition, the experimental results of Zhang et al[29] showed that saffron acid could significantly prolong coagulation time in mice and tail bleeding time in mice; for in vitro thrombosis and venous thrombosis in rats, saffron acid significantly reduced the wet and dry mass of thrombus; and significantly prolonged TT, PT, and APTT in hemorrhagic rats. Studies showed that saffron glucoside was not absorbed in the small intestine of rats. The glycosides were also undetectable in the blood of rats, but their sapogenins, saffron acid, were detected.[30] These studies suggest that the anticoagulant and antithrombotic effects of saffron glucoside may be mediated through its sapogenin, saffron acid. In addition, saffron acid has several beneficial functions in the nervous system, such as neuronal survival, prominent plasticity, and microglia activation.[31] Microglia play a key regulatory role in the anti-inflammatory and immune response of the CNS. In the case of disease, the active function of the glial can help to restore homeostasis within the central nervous system. Ursolic acid, on the other hand, is a triterpenoid found in natural plants and has a variety of biological effects such as sedative, anti-inflammatory, antibacterial, anti-diabetic, anti-ulcer, and blood sugar lowering. Ursolic acid also has significant antioxidant properties. Bijuanjuan et al[32] reported that ursolic acid provides energy to cells by inducing the autophagic process, sustains their survival, and acts as a protective agent for vascular endothelial cells. Studies have shown that mannitol may contribute to the scavenging of oxidative free radicals from brain tissue in the ischemic zone and participate in altering the hemodynamics of the ischemic zone.[33] Also, neurons in the final area of the parietal nucleus of the cerebellum may be affected by the drug and thus involved in the regulation of cardiovascular function.[34,35] It has been shown that quercetin may improve its damaged nerves by inhibiting the release of inflammatory factors such as TNF-α and IL-1β in a rat model of stroke and further inhibiting apoptosis and oxidative stress levels of cells.[36] Ivy saponin elements are widely distributed in a variety of medicinal plants. It has been found that ivy saponin elements have various pharmacological effects such as antitumor, antidepressant, antibacterial

Table 3

Results of KEGG pathway enrichment analysis.

No.	Term	Count	Genes	*P* value
hsa04080	Neuroactive ligand-receptor interaction	44	OPRM1, TSPO, DRD1, TRPV1, ADORA2A, DRD2, OPRK1, NR3C1, BDKRB2, ADORA1, EDNRA, AGTR2, HRH1, HTR1A, CHRNA5, ADRA2A, CHRNA4, CHRNA7, ADRA2C, ADRA2B, PTGER1, PTGER2, GABRA1, PTGER3, PTGER4, GABRA3, GRIN2A, GRIA3, GRIA4, ADRB2, ADRB1, CHRM3, CHRM2, GRIA1, CHRM1, F2, CHRNB4, ADRA1B, AVPR1A, ADRA1A, CHRNB2, HTR2C, HTR2A, OPRD1	1.00E-19
hsa04020	Calcium signaling pathway	32	SLC8A3, DRD1, ADORA2A, BDKRB2, EDNRA, HRH1, CHRNA7, NOS3, NOS2, PLCB1, EGFR, PTGER1, SLC8A1, NOS1, SLC8A2, PTGER3, GRIN2A, CACNA1S, ADRB2, ADRB1, CHRM3, CHRM2, CHRM1, AVPR1A, ADRA1B, RYR2, ADRA1A, CACNA1C, CACNA1D, HTR2C, CACNA1A, HTR2A	1.08E-15
hsa04024	cAMP signaling pathway	31	PPARA, DRD1, ADORA2A, DRD2, SOX9, ADORA1, EDNRA, HTR1A, PDE4A, PIK3CG, PTGER2, PTGER3, GRIN2A, PDE3A, GRIA3, PDE4D, GRIA4, CACNA1S, MAPK1, ADRB2, ADRB1, CHRM2, GRIA1, JUN, CHRM1, MAPK9, RYR2, MAPK8, CACNA1C, CACNA1D, SLC9A1	1.38E-13
hsa05200	Pathways in cancer	39	PPARD, PTGS2, PPARG, FASLG, BDKRB2, MMP2, MMP1, EDNRA, BCL2, ITGAV, RARB, NOS2, PLCB1, EGF, FGF2, FGF4, EGFR, PIK3CG, PTGER1, AR, IL6, PTGER2, RET, PTGER3, PTGER4, RXRA, MET, TP53, IGF1, RB1, CDK4, CDK2, MAPK1, JUN, GSK3B, VEGFA, MAPK9, MAPK8, GSTP1	1.01E-10
hsa04022	cGMP-PKG signaling pathway	24	SLC8A3, KCNMA1, SLC8A1, SLC8A2, PDE3A, BDKRB2, CACNA1S, ADORA1, EDNRA, MAPK1, ADRB2, ADRB1, INS, ADRA2A, ADRA1B, ADRA1A, NOS3, ADRA2C, ADRA2B, PLCB1, CACNA1C, CACNA1D, INSR, OPRD1	2.76E-10
hsa04726	Serotonergic synapse	18	PTGS2, MAOB, PTGS1, SLC6A4, CACNA1S, KCNJ5, MAPK1, ALOX15, APP, HTR1A, ALOX5, PLCB1, CACNA1C, HTR3A, CACNA1D, HTR2C, CACNA1A, HTR2A	3.06E-08
hsa05142	Chagas disease (American trypanosomiasis)	17	PIK3CG, IL6, TNF, CCL2, FASLG, BDKRB2, MAPK1, ACE, JUN, MAPK14, IFNG, IL1B, MAPK9, MAPK8, NOS2, PLCB1, IL2	7.42E-08
hsa04750	Inflammatory mediator regulation of TRP channels	16	PIK3CG, PTGER2, PTGER4, TRPV1, TRPA1, TRPV3, IGF1, BDKRB2, HRH1, MAPK14, IL1B, MAPK9, MAPK8, PLCB1, HTR2C, HTR2A	2.06E-07
hsa04723	Retrograde endocannabinoid signaling	16	GABRA1, PTGS2, GABRA3, GRIA3, GRIA4, CACNA1S, KCNJ5, MAPK1, GRIA1, MAPK14, MAPK9, MAPK8, PLCB1, CACNA1C, CACNA1D, CACNA1A	3.10E-07
hsa04924	Renin secretion	13	KCNMA1, PTGER2, PTGER4, PDE3A, ADORA1, CACNA1S, EDNRA, ADRB2, ACE, ADRB1, CACNA1C, PLCB1, CACNA1D	3.68E-07
hsa04668	TNF signaling pathway	16	PIK3CG, IL6, TNF, CCL2, PTGS2, MMP3, VCAM1, MAPK1, CASP7, JUN, RIPK1, MAPK14, IL1B, MAPK9, MAPK8, SELE	6.69E-07
hsa04610	Complement and coagulation cascades	13	PLAT, F12, F10, BDKRB2, F7, PROC, THBD, F3, F2, SERPINC1, SERPIND1, PROS1, PLAU	8.65E-07
hsa04725	Cholinergic synapse	16	PIK3CG, ACHE, CACNA1S, MAPK1, CHRM3, CHRM2, CHRM1, BCL2, CHRNB4, CHRNA4, CHRNA7, CHRNB2, CACNA1C, PLCB1, CACNA1D, CACNA1A	1.08E-06
hsa04930	Type II diabetes mellitus	11	PIK3CG, MAPK1, TNF, INS, MAPK9, MAPK8, CACNA1C, CACNA1D, ADIPOQ, INSR, CACNA1A	1.28E-06
hsa04728	Dopaminergic synapse	17	DRD1, DRD2, SLC6A3, MAOB, GRIN2A, GRIA3, GRIA4, KCNJ5, GRIA1, GSK3B, MAPK14, MAPK9, MAPK8, PLCB1, CACNA1C, CACNA1D, CACNA1A	1.37E-06
hsa05033	Nicotine addiction	10	GABRA1, GRIA1, GABRA3, GRIN2A, CHRNA4, GRIA3, CHRNA7, CHRNB2, GRIA4, CACNA1A	2.24E-06
hsa04932	nonalcoholic fatty liver disease (NAFLD)	18	PIK3CG, PPARA, IL6, TNF, RXRA, FASLG, COX5A, ADIPOQ, COX5B, INS, CASP7, JUN, GSK3B, IL1B, MAPK9, MAPK8, INSR, NR1H3	2.78E-06
hsa04151	PI3K-Akt signaling pathway	28	PIK3CG, EGFR, IL6, RXRA, MET, COL3A1, TP53, FASLG, IGF1, ITGB3, CDK4, CDK2, KDR, MAPK1, CHRM2, INS, BCL2, CHRM1, ITGAV, GSK3B, VEGFA, NOS3, COL1A1, EGF, FGF2, INSR, IL2, FGF4	4.78E-06
hsa04066	HIF-1 signaling pathway	14	EGFR, PIK3CG, MAPK1, IL6, INS, BCL2, HMOX1, VEGFA, IFNG, IGF1, NOS3, NOS2, EGF, INSR	5.64E-06
hsa05144	Malaria	10	VCAM1, ITGAL, IL6, TNF, CCL2, IFNG, MET, IL1B, ITGB2, SELE	1.32E-05

KEGG = Kyoto Encyclopedia of Genes and Genomes.

and anti-inflammatory, and antidiabetic. Wu et al[37] showed that ivy saponin elements can improve motor impairment in PD mouse models through their neuroprotection. One study[38] found that ivy saponin elements had preventive effects on hyperlipidemia in both experimental rats and mice; also, its blood rheological properties of hyperlipidemia in experimental rats were significantly improved.

Topological analysis of the key targets showed that 10 targets such as INS, ALB, IL-6, VEGFA, TNF and TP53 were ranked among the top targets. This suggests that these targets may play a key role in the treatment of ischemic stroke with Chuanzhi Tongluo Capsule. Among them, insulin can achieve the function of dilating small pre-capillary arteries, enhancing the compliance of ductus arteriosus and expanding microvascular blood volume.[39] Albumin can maintain cellular metabolism by increasing the transport of pyruvate with neurons, thus achieving neuronal protection.[40] It has been shown that serum albumin levels gradually decrease in acute stroke patients; and the greater the decrease in albumin in patients, the larger the infarct area and the more the blood-brain barrier is disrupted.[41] interleukin-6 is one of the systemic inflammatory markers and plays an important role in the body's immune response against infection. il-6 may have a role in regulating the immune response, the acute phase response, and promoting hematopoiesis. Elevated IL-6 concentrations are closely associated with poor prognosis and high mortality in patients after stroke.[42] Rong Wei et al[43] demonstrated that the active ingredient in ginseng, ginsenoside Rg1, could achieve neuroprotective effects by decreasing the expression level of IL-6 in rats with ischemic stroke. VEGFA, a vascular endothelial growth factor, promotes cerebral neurovascular repair in the stroke region and improves the prognosis of stroke.[44]VEGFA has an important role in the process of angiogenesis after cerebral ischemia.[45] When VEGFA is given to the MCAO rat model, it increases the density of biological microvessels in the ischemic semidark zone and promotes angiogenesis.[46] TNF-α has the effect of activating microglia and promoting the expression of adhesion and chemokines. Improving the migration capacity of inflammation-related cells is one of the key causes of neuronal cell injury after ischemic stroke.[47] In addition, animal studies found that the volume of

cerebral infarcts and the degree of brain damage after cerebral ischemia were significantly higher in mice deficient in the TNF receptor gene than in wild-type mice. The above studies suggest that TNF has certain neuroprotective effects.[48] As one of the inflammatory cytokines, TNF enhances the degree of neuronal damage and thrombus formation in ischemic stroke. TP53 plays an important role in cell proliferation and apoptosis by regulating the synthesis of cell cycle-related proteins. In addition, there are reports in the literature showing that TP53 can be used as a genetic marker to predict the prognosis of stroke patients.[49]

The GO enrichment analysis of the targets of Chuanzhi Tongluo Capsule in the treatment of ischemic stroke suggests that biological processes such as drug response, response to hypoxia, the composition of the plasma membrane, steroid hormone receptor activity, and drug binding may play a major role. The KEGG enrichment pathway analysis showed that the potential targets of Chuanzhi Tongluo Capsules in the treatment of cerebral infarction mainly involve calcium signaling pathway, cAMP signaling pathway, cGMP-PKG signaling pathway, and TNF signaling pathway. Among them, the calcium signaling pathway stabilizes blood pressure by regulating Ca2+ channels, which can lead to an increase in peripheral vascular resistance, thus causing an increase in blood pressure. It has also been suggested that Ca2+ increases the stability of vascular smooth muscle cell membranes to alleviate stressful blood pressure elevation and decreases the endogenous vascular pressor response, which in turn promotes urinary sodium excretion and reduces blood volume; Ca2+ further enhances the active transport of sodium and potassium by relieving the inhibition of Na-K-ATPase activity, relaxes vascular smooth muscle, and achieves blood pressure reduction.[50] cAMP is one of the important intracellular second messengers that can exert a cerebral protective effect by activating the PKA signaling pathway and mediating the formation of neuronal regenerative synapses by binding its response element to the protein CREB.[51] In addition, it has been shown that the cGMP-PKG signaling pathway can be involved in regulating neurogenesis and cell proliferation processes.[52] Meanwhile, activation of the cGMP-PKG signaling pathway promotes neurogenesis and improves neural structure and function in post-ischemic mice.[53] And selective inhibition of the TNF signaling pathway has potential applications in reducing blood-brain barrier disruption and improving neurological prognosis.[47]

5. Conclusion

In summary, this paper investigated the pharmacological basis and potential biological mechanisms of Chuanzhi Tongluo Capsules in the treatment of cerebral infarction using a network pharmacology approach. The treatment of cerebral infarction with Chuanzhi Tongluo Capsule is a complex process involving multiple components, multiple targets and multiple pathways. The active ingredients in Chuanzhi Tongluo Capsule, such as saffron acid, ursolic acid, mannitol, quercetin, and ivy saponin element, may be involved in the therapeutic process of cerebral infarction by regulating calcium, cAMP, cGMP-PKG, and TNF signaling pathway through key targets, such as INS, ALB, IL-6, VEGFA, TNF, and TP53.

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