Study on the Clinical Efficacy and Mechanism of Tongnao Yin in Treating Acute Cerebral Infarction Based on Network Pharmacology and Molecular Docking Technology: Postprint

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Abstract

Background: Cerebral infarction is a disorder of regional blood supply to local brain tissue caused by various factors, and Tongnaoyin is an institutional formula used at Jiangsu Provincial Hospital of Chinese Medicine for its treatment. Objective: This study aims to elucidate the mechanism of Tongnaoyin in treating cerebral infarction through network pharmacology and clinical trials. Methods: A total of 199 patients with cerebral infarction admitted to Jiangsu Provincial Hospital of Chinese Medicine from January 2019 to June 2020 were enrolled. According to the random number table method, patients were divided into a control group (97 cases) and a trial group (102 cases). Both groups received standard treatment for stable cerebral infarction, while the trial group additionally received Tongnaoyin. Before treatment and at 2 weeks of treatment, both groups were assessed using the National Institutes of Health Stroke Scale (NIHSS) to evaluate the degree of functional impairment caused by stroke, and the modified Rankin Scale (mRS) to evaluate neurological function recovery. The chemical components of Tongnaoyin were screened from TCMSP and literature, and components meeting the requirements of oral bioavailability (OB) $\geq$ 30% and drug-likeness (DL) $\geq$ 0.18 were selected to identify the active ingredients of the formula. The molecular targets of Tongnaoyin for cerebral infarction were analyzed using the OMIM and GeneCards databases. After screening common targets, Cytoscape software and STRING database were used to construct network diagrams of compounds and target proteins, build protein-protein interaction (PPI) networks, and perform Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway enrichment analyses. Finally, molecular docking experiments were conducted to identify the main active components of Tongnaoyin for cerebral infarction. Results: After treatment, the NIHSS and mRS scores of the trial group were signifi-

cantly lower than those of the control group (P<0.05). A total of 60 active components of Tongnaoyin, 147 potential targets, 5,167 disease-related targets, and 121 intersecting targets between drug and disease were obtained. KEGG signaling pathway enrichment analysis identified prostate cancer, neuroactive ligand-receptor interaction, IL-17 signaling pathway, prolactin signaling pathway, PI3K-Akt signaling pathway, calcium signaling pathway, etc. Molecular docking revealed that the main active components of Tongnaoyin for stroke, β-sitosterol, kaempferol, and carotene, showed good binding affinity with the core protein androgen receptor (AR). Conclusion: Tongnaoyin may treat cerebral infarction by activating AR. The IL-17 signaling pathway, PI3K-Akt signaling pathway, and prolactin signaling pathway are also potential mechanisms.

Full Text

Clinical Efficacy and Mechanism of Action of Tongnao Decoction in Treating Acute Cerebral Infarction: A Study Based on Network Pharmacology and Molecular Docking

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Abstract

Background: Cerebral infarction is a disorder of blood supply to local brain tissue caused by various etiologies. Tongnao Decoction is an approved formula used at Jiangsu Province Hospital of Chinese Medicine for treating cerebral infarction.

Objective: This study aims to elucidate the mechanism of Tongnao Decoction in treating cerebral infarction through network pharmacology and clinical trials.

Methods: A total of 199 patients with cerebral infarction admitted to Jiangsu Province Hospital of Chinese Medicine between January 2019 and June 2020 were enrolled. Using a random number table method, patients were divided into a control group (97 cases) and an experimental group (102 cases). Both groups received standardized treatment for stable cerebral infarction, while the

experimental group additionally received Tongnao Decoction. The National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) were used to assess functional impairment and neurological recovery before treatment and at 2 weeks. Chemical compounds of Tongnao Decoction were screened from TCMSP and literature, selecting components with oral bioavailability (OB) $\geq$ 30% and drug-likeness (DL) $\geq$ 0.18. OMIM and GeneCards databases were used to analyze molecular targets. Common targets were screened to construct protein-protein interaction (PPI) networks, and gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using Cytoscape software and the String database. Molecular docking experiments identified the main active ingredients.

Results: After treatment, NIHSS and mRS scores in the experimental group were significantly lower than in the control group ($P<0.05$). A total of 60 active ingredients, 147 potential targets, 5,167 disease-related targets, and 121 intersection targets were identified. KEGG analysis revealed enrichment in prostate cancer, neuroactive ligand-receptor interaction, IL-17 signaling pathway, prolactin signaling pathway, PI3K-Akt signaling pathway, calcium signaling pathway, etc. Molecular docking showed that β-sitosterol, kaempferol, and carotene —the main active ingredients—demonstrated good binding affinity to the core protein androgen receptor (AR).

Conclusion: Tongnao Decoction may treat cerebral infarction by activating AR. The IL-17, PI3K-Akt, and prolactin signaling pathways represent potential mechanisms.

Keywords: Brain infarction; Tongnao decoction; Network pharmacology; Inflammation; Molecular docking

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Introduction

Stroke represents one of the most severe global public health challenges [1-2], with ischemic stroke accounting for 70% of cases. The most direct and effective treatments for ischemic stroke involve intravenous thrombolysis, arterial thrombolysis, and mechanical thrombectomy to rapidly reopen occluded vessels and promote revascularization, thereby rescuing neurons in the penumbra [3]. However, ischemia-reperfusion injury following revascularization can exacerbate brain damage. While neuroprotective agents such as free radical scavengers, opioid receptor antagonists, and excitatory amino acid receptor blockers have been developed to treat ischemia-reperfusion injury, sufficient clinical evidence to support their widespread use is lacking [4].

Tongnao Decoction can significantly improve neurological deficit symptoms in acute cerebral infarction patients, enhance daily living self-care ability, reduce disability rates to some extent, and improve cerebral blood flow and supply [5-6]. Additionally, it can exert synergistic effects with antiplatelet drugs in

the early stages of acute cerebral infarction, significantly inhibiting platelet aggregation and improving platelet-related parameters [7]. The formula contains *Chuanxiong* (CX), *Gouteng* (GT), *Tiannanxing* (TNX), *Hongjingtian* (HJT), *Jiujiechangpu* (JJCP), *Tianma* (TM), *Jiangcan* (JC), and *Shuizhi* (leech, SZ). Multi-herb compatibility represents the essence of Traditional Chinese Medicine (TCM) theory; however, elucidating its mechanisms using traditional methods is challenging due to complex components and numerous targets. Therefore, system-level investigation is necessary to reveal the therapeutic potential of Tongnao Decoction for cerebral infarction (CI).

Network pharmacology is a novel discipline based on systems biology theory, biological system network analysis, and multi-target drug molecular design for specific signaling nodes. It emphasizes multi-channel regulation of signaling pathways to improve therapeutic efficacy, reduce toxic side effects, increase success rates of clinical trials, and reduce drug development costs. With continuous innovation in systems biology and computer technology, network pharmacology has been recognized as a feasible approach to systematically elucidate the material composition and molecular mechanisms of TCM [7-8]. This method has been applied to study "compound protein/gene-disease"pathways, which can describe the complexity among biological systems, drugs, and diseases from a network perspective—sharing a similar holistic philosophy with TCM [9]. Applying systems biology methods to study the pharmacological effects, mechanisms, and safety of TCM is crucial for modern research and development. Consequently, a new interdisciplinary approach called TCM network pharmacology has emerged, pioneering a paradigm shift from experience-based to evidence-based medicine. The technical workflow of "classical literature screening—component selection—pharmacological evaluation—clinical validation" led by Academician Zhang Boli will provide a suitable platform for TCM modernization. Furthermore, recent advances in molecular biology and genomics have made increasing amounts of data available [10], such as TCMSP [11], STRING [12], OMIM [13], and DisGeNET [14-15].

Molecular docking is a theoretical simulation method that designs drugs based on receptor characteristics and drug-receptor interactions, primarily investigating interactions between molecules (such as ligands and receptors) and predicting their binding modes and affinities. This study employed network pharmacology to predict the potential mechanism of Tongnao Decoction in treating cerebral infarction. The workflow is shown in [Figure 1: see original paper].

Methods

1.1 Clinical Data A total of 199 patients with cerebral infarction admitted to Jiangsu Province Hospital of Chinese Medicine between January 2019 and June 2020 were enrolled. Using a random number table method, patients were divided into a control group (97 cases) and an experimental group (102 cases).

This study was approved by the Ethics Committee of Jiangsu Province Hospital of Chinese Medicine (Ethics No.: 2017NL-012-01). Before enrollment, patients and their families were informed of all study contents and related rights, and signed informed consent forms under the premise of voluntary participation. During treatment, one case in the control group did not cooperate, resulting in 96 valid cases in the control group and 102 in the experimental group. The majority of confirmed patients had lacunar infarction.

1.2 Inclusion and Exclusion Criteria 1.2.1 Inclusion Criteria: (1) Meeting diagnostic criteria from the *Chinese Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke* [16] and confirmed cerebral infarction by cranial CT and MRI within 48 hours; (2) Carotid intima-media thickness (CA-IMT) $\geq$ 1.2 mm determined by carotid color Doppler ultrasound; (3) Voluntary participation with good compliance and ability to maintain follow-up.

1.2.2 Exclusion Criteria: (1) Severe blood flow obstruction due to lumen plaques shown by carotid ultrasound; (2) Severe cardiopulmonary insufficiency or severe arrhythmia; (3) Severe liver or kidney dysfunction; (4) Severe infection or malignant tumor infection; (5) Acute coronary syndrome; (6) Drug allergy or allergic constitution; (7) Pregnant or lactating women.

1.2.3 Dropout and Termination Criteria: (1) Severe adverse reactions to study medications; (2) Non-compliance or incomplete treatment due to various reasons; (3) Occurrence of severe disease during treatment preventing continuation; (4) Missing data identified during processing.

1.3 Treatment Methods After admission, both groups received standardized treatment for stable cerebral infarction, including antiplatelet aggregation (aspirin 0.1 g orally once daily; clopidogrel 75 mg once daily, alone or in combination), anti-atherosclerosis and plaque stabilization (atorvastatin calcium 20 mg qd or rosuvastatin 10 g qd), free radical scavenging, etc. The experimental group additionally received Tongnao Decoction [*Zhinanxing* 10 g, *Chuanxiong* 10 g, *Tianma* 10 g, *Gouteng* (added later) 30 g, *Hongjingtian* 15 g, *Shuizhi* 5 g, *Baijiangcan* 10 g, *Jiujiechangpu* 10 g] decocted in water, one dose daily for 2 weeks.

1.4 Observation Indicators Both groups received 2 weeks of treatment. NIHSS and mRS scores were assessed at admission and at 2 weeks. NIHSS evaluates functional impairment caused by stroke through 11 items (score 0-42), with higher scores indicating more severe stroke and brain injury [17]. mRS measures post-stroke neurological recovery (score 0-5), with lower scores indicating better recovery [18].

1.5 Network Pharmacology Analysis 1.5.1 Chemical Component Screening and Target Prediction: Chemical compounds of Tongnao Decoction were screened from TCMSP (http://lsp.nwu.edu.cn/tcmsp.php)

and literature [19]. TCMSP is a traditional Chinese medicine systems pharmacology platform that reveals relationships among drugs, targets, and diseases. Compounds were filtered based on pharmacokinetic ADME (absorption, distribution, metabolism, excretion) processes. TCMSP describes ADME parameters including oral bioavailability (OB), drug-likeness (DL), and blood-brain barrier (BBB) permeability. OB indicates oral availability, while DL indicates similarity to known drugs. Components meeting OB $\geq$ 30% and DL $\geq$ 0.18 were selected as active ingredients.

1.5.2 Component Target Prediction: Active ingredients were input into the TCMSP database to obtain known targets, and Cytoscape 3.8.2 was used to construct compound-target protein network diagrams.

1.5.3 Disease-Related Target Identification: Using "cerebral infarction" as the keyword, OMIM (http://www.omim.org/) and GeneCards (http://www.genecards.org/) were searched to identify known disease targets, with duplicates removed. The UniProt knowledgebase [20-21] (http://www.uniprot.org/) was used to obtain standardized target names, with *Homo sapiens* selected as the organism.

1.5.4 Common and Key Target Analysis: Online tools were used to generate Venn diagrams showing relationships between different groups, identifying common targets between drug and disease. Intersection targets were used as drug effect targets, and Cytoscape 3.8.2 constructed drug-effect target-component interaction networks. Network analysis obtained Degree values reflecting target importance—higher values indicating more important drug-target interactions, yielding key drug-effect targets.

1.5.5 GO and KEGG Enrichment Analysis: To understand biological processes and signaling pathways, the Database for Annotation, Visualization and Integrated Discovery (DAVID) was used for pathway enrichment analysis. Tongnao Decoction target genes for cerebral infarction were input into DAVID for GO biological process analysis and KEGG pathway analysis. GO biological processes with P $\leq$ 0.01 and KEGG pathways with P $\leq$ 0.01 were considered significantly enriched.

1.5.6 Molecular Docking: Molecular docking was performed between active ingredients and the androgen receptor (AR) protein. Active ingredients in SDF format were downloaded from PubChem, then energy-minimized using Chem 3D and converted to MOL2 format. Target protein PDB structure files were downloaded from the RCSB PDB database. AutoDock Tools removed heteroatoms and calculated charges, followed by AutoDock Vina docking. Results were visualized using CB-Dock (http://clab.labshare.cn/cb-dock).

1.6 Statistical Methods SPSS 25.0 software was used for data processing and analysis. Categorical and ordinal data were analyzed using ^{2} tests or non-parametric tests. Non-normally distributed continuous data were expressed

as M(P_{25}, P_{75}) and compared between groups using Mann-Whitney U tests. $P<0.05$ was considered statistically significant.

Results

2.1 Clinical Trial Results Before treatment, no significant differences existed in NIHSS and mRS scores between groups ($P>0.05$). After treatment, NIHSS and mRS scores in the experimental group were significantly lower than in the control group ($P<0.05$). Both groups showed significantly lower post-treatment scores compared to pre-treatment ($P<0.05$). Results are shown in .

2.2 Network Pharmacology Analysis Results **2.2.1 Active Ingredient and Target Screening:** Active ingredients in Tongnao Decoction were screened from the TCMSP database and literature (), and an active ingredient-target interaction network was constructed using Cytoscape 3.8.2 ([Figure 2: see original paper]). Cerebral infarction-related targets were obtained from GeneCards and OMIM databases using keyword searches. A Venn diagram constructed the intersection between active ingredient targets and disease targets ([Figure 3: see original paper]), yielding 134 overlapping targets for subsequent network pharmacology analysis.

2.2.2 Key Target Identification and PPI Network Construction: Intersection targets were used as drug-effect targets to construct a drug-effect target-component interaction network using Cytoscape 3.8.2 ([Figure 4: see original paper]). Network analysis obtained Degree values, with the top 20 pharmacodynamic targets including estrogen receptor (ESR1), androgen receptor (AR), cyclooxygenase-2 (PTGS2), cyclin-dependent kinase 2 (CDK2), etc. (). A PPI network diagram displayed relationships among targets ([Figure 5: see original paper]).

2.2.3 GO and KEGG Enrichment Analysis: GO analysis revealed important biological processes including cellular response to lipids, cellular localization regulation, signal transduction, cellular response to stimuli, positive regulation of biosynthetic processes, regulation of signaling, blood pressure regulation, regulation of transport, and positive regulation of reactive oxygen species metabolic processes ([Figure 6: see original paper]), suggesting Tongnao Decoction is closely related to inflammatory responses in cerebral infarction treatment. KEGG analysis identified 20 pathways including prostate cancer, neuroactive ligand-receptor interaction, IL-17 signaling pathway, prolactin signaling pathway, PI3K-Akt signaling pathway, and calcium signaling pathway ([Figure 7: see original paper]).

2.2.4 Molecular Docking Results: Molecular docking between AR and the three main active ingredients showed AR bound most stably to β-sitosterol (-

10.1 kJ/mol), followed by kaempferol (-7.5 kJ/mol) and carotene (-7.02 kJ/mol). Three-dimensional views are shown in [Figure 8: see original paper].

Discussion

3.1 Overview Cerebral infarction primarily results from atherosclerosis and thrombosis in cerebral arteries, causing luminal stenosis or occlusion and focal acute cerebral insufficiency. Emboli (solid, liquid, or gas) entering cerebral arteries or carotid arteries can also obstruct blood flow, causing brain tissue softening and damage. In this study, Tongnao Decoction was used to treat cerebral infarction. However, elucidating its complex mechanisms using traditional methods is challenging. Therefore, integrating network pharmacology based on big data bioinformatics into TCM molecular mechanism research is essential [22-23]. This study employed network pharmacology to explore the material basis and molecular mechanisms of Tongnao Decoction for cerebral infarction.

Current Western medications for cerebral infarction have many adverse effects including respiratory difficulty and gastrointestinal reactions [24-26]. TCM features multi-target effects and fewer adverse reactions. This study identified 121 common drug-disease targets that may represent therapeutic targets for this formula. Based on topological analysis, 23 key targets were identified from these 121 common targets, including ESR1, AR, DPP-4, PTGS2, CDK2, etc. Among them, HSP90 is a ubiquitous molecular chaperone [27], and HSP90 inhibitors demonstrate significant anti-inflammatory effects [28-29]. The combination of GO analysis, clinical trials, and other modern studies confirms the important role of inflammation in cerebral infarction. KEGG enrichment analysis highlights the significance of the IL-17 signaling pathway in cerebral infarction, with HSP90 playing a crucial role in these pathways.

This study focused on AR as a key node in the sub-network. Molecular docking was performed between small molecules and the AR protein. Three molecules were found to directly interact with AR: β-sitosterol, kaempferol, and carotene. Future research should evaluate synergistic effects among these molecules.

3.2 Clinical Significance The approved formula Tongnao Decoction can significantly improve patients' quality of life.

3.3 Limitations The key targets and/or pathways identified through network pharmacology have not been validated in clinical trials. However, this study' s clinical results demonstrate significant improvements in NIHSS and mRS scores before and after Tongnao Decoction use, proving its effectiveness in reducing functional impairment and providing neuroprotective effects while inhibiting inflammation. Nevertheless, this study has not explored specific mechanisms or conducted experimental validation. Even with network pharmacology and

molecular docking results, the exact therapeutic mechanism of Tongnao Decoction remains incompletely understood. Future research will conduct further experimental validation based on these predictions.

Conclusion

This study combined network pharmacology and clinical trials to investigate Tongnao Decoction' s mechanism in treating cerebral infarction. Results suggest Tongnao Decoction may exert anti-inflammatory effects through HSP90. Additionally, IL-17, prolactin, and PI3K-Akt signaling pathways represent potential mechanisms. This study demonstrates that adding Tongnao Decoction to standard Western medical treatment significantly improves symptoms in cerebral infarction patients, suggesting Tongnao Decoction could be considered a complementary or alternative therapy. Furthermore, computational biology may provide a pathway for modern TCM research.

Author Contributions

WU Minghua proposed the research concept, designed the study, and formulated research questions. ZHANG Lin conducted experiments/investigations, selected subjects, collected samples, and performed testing. ZHANG Lin and GAO Jin collected, cleaned, and statistically analyzed data and prepared figures. GAO Jin drafted the manuscript. WANG Guangmei revised the final version and took responsibility for the paper.

Conflict of Interest: None declared.

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Note: Figure translations are in progress. See original paper for figures.