To discuss the mechanism of colchicine in the treatment of acute cerebral infarction based on network pharmacology

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Abstract
To explore the mechanism of action of colchicine in the treatment of acute cerebral infarction (ACI) based on network pharmacology. The Swiss Target Prediction Database and CTD database were used to predict the target information of colchicine. ACI-related targets were retrieved using the GeneCards database, and the target protein interaction network (PPI) and active ingredient-target network were obtained by combining Cytoscape 3.7.1 software and R language. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis and gene function analysis (GO) enrichment analysis were performed using R language to preliminarily explore the multiple pharmacological mechanisms of action of colchicine. There were 200 targets identified by network parameter analysis; 958 ACI targets were identified. Overlapping comparisons allowed the extraction of 143 overlapping targets, and the top 30 targets were screened according to the topological isomerization parameters. Component-target networks were constructed. A PPI of overlapping targets was established to identify key targets. In addition, Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis and GO functional enrichment analysis were performed to explore the multiple mechanisms of action of colchicine in the treatment of ACI. Colchicine treatment of ACI is characterized by multi-component, multi-target and multi-pathway, and can exert complex network regulation through the interaction between different targets, providing a new idea and new basis for further exploration of the mechanism of action of colchicine in the treatment of ACI.

Abbreviations: ACI = acute cerebral infarction, GO = gene function, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein interaction network, VEGF = vascular endothelial growth factor.

Keywords: acute cerebral infarction, colchicine, network pharmacology, target

1. Introduction
Acute cerebral infarct is the loss of brain tissue caused by the sudden interruption of blood supply to the brain. It is usually mainly because the artery that supplies brain blood appears atherosclerosis and thrombosis, make lumen narrow and even occlude, bring about focal acute brain to supply blood insuf-iciency and come on; There are also abnormal objects (solid, liquid, gas) along the blood circulation into the cerebral artery or the supply of cerebral blood circulation of the neck artery, resulting in blood flow blocking or blood flow sudden reduction resulting in the corresponding control area of the brain tissue softening, necrosis.[1]

Studies have found that alkaloids extracted from natural plants have more medicinal value than the value of the plant itself. A typical example is colchicine, an alkaloid found in lil-ies. Colchicin is an important alkaloid originally found in the lily plant Colchicin. It is a kind of cover phenolone alkaloid, which can inhibit cell mitosis and inhibit the growth of cancer cells. It is clinically used to treat cancer, gout and other diseases. Colchicine is also a common reagent in cell biotechnol-ogy.[2] Through the combination of computer technology and biological technology, this study preliminarily discussed the relationship between colchicine and acute cerebral infarction (ACI) disease, and discussed the mechanism of colchicine in the treatment of ACI, which laid a foundation for the next experi-mental design.

2. Materials and Methods
2.1. Material
Toxicological Genome Database (CTD) (http://ctdbase.org/); swiss target prediction database (http://www.swisstargetpredic-tion.ch/); GENECARDS database (https://www.genecards.org/); STRING database (https://string-db.org/cgi/input.pl); KOBAS database (http://kobas.cbi.pku.edu.cn/kobas3); Cytoscape 3.7.1; R language.

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Ethical statement is not required for this article.

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2.2. Prediction of drug-related targets
The related targets of colchicine active constituents were predicted using Swiss database and CTD database, and then the corresponding standard gene names were searched in Uniprot online protein database with species “HOMOSAPIENS,” so as to get the related targets of colchicine active constituents.

2.3. Prediction of disease-related targets
GeneCards database and OMIM database were used to obtain the relevant content of ACI, and duplicate target genes were deleted to obtain the relevant targets of ACI.

2.4. Search for common targets of drugs and diseases
The target information of colchicine was mapped to the target information of ACI, and the overlapping target Venn diagram and overlapping target information could be obtained by R language operation.

2.5. Construction of drug network
The obtained target information of colchicine was sorted out, and Cytoscape3.7.1 software was used to draw the “component-target” network diagram of colchicine and ticagrelor.

2.6. Construction of drug-disease overlap target protein interaction network (PPI)
The overlapping targets of colchicine and ACI were imported into the String data analysis platform for calculation, and PPI could be constructed according to the strength of the interaction relationship between the targets.

2.7. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis
The overlapping target name of colchicine and ACI was converted into ENTREZ Gene ID by R language, and then the KEGG related information was obtained through analysis and operation with the help of KOBAS database. Then, P value was used as the reference value for screening, and then R language was used for analysis to obtain the relevant content of pathway enrichment analysis.

2.8. Gene function (GO) analysis
The overlapping target name of colchicine and ACI was converted into ENTREZ Gene ID by R language, and then the GO related information was obtained through analysis and operation with the help of KOBAS database. Then, P value was used as the reference value for screening, and then R language was used for analysis to obtain the relevant content of pathway enrichment analysis.

3. Results
3.1. Determination of drug action targets
Using PubChem (https://pubchem.ncbi.nlm.nih.gov/search/) and Search database, query the composition of the CAS number and the Canonical SMILES string is based on the submitted by small molecular compound structure, from the angle of the 2D and 3D structure similarity, indirect prediction submit targets for small molecule compounds. Input the query Canonical Smiles string into the Swiss Target Prediction website for Target docking, and obtain 100 potential Target genes of colchicine and ticagrelor respectively; At the same time, CTD (http://ctdbase.org/) database was used to retrieve the target information corresponding to colchicine, and the combined statistics of the two data information was used to identify the active ingredient target.

3.2. Prediction results of potential targets of drug therapy for diseases
According to the CTD database and Swiss database, there were 1054 and 100 targets of colchicine active ingredient, respectively, and the remaining 1141 target information after decontamination, ACI was retrieved through GeneCards database and OMIM database, and the results of GeneCards database were screened. The results showed that 738 and 270 targets of ACI disease were obtained in the two databases and 958 target information was obtained after deconduplication. A total of 14.3 targets were intersected by R language software between the corresponding targets of colchicine and the targets of ACI disease, and the Venn plot of the targets of colchicine and ticagrelor in the treatment of ACI could be obtained. Topological heterogeneity analysis was carried out on the overlapping targets, and the top 30 targets with degree value were screened for drawing analysis. Interleukin-6 (IL-6), tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) were relatively high degree targets among colchicine corresponding targets, which indicated that colchicine could play a role in the treatment of ACI through multiple targets.

3.3. PPI network construction
Thirty potential targets of colchicine in the treatment of ACI were analyzed in the STRING database, and the protein interaction network map was obtained. As shown in Figure 1, there are 301 nodes. In PPI network, “node” represents the action target and the Degree value is used to measure the importance of the target. The greater the value, the more the target is at the core of the network. “Edge” represents the association between acting targets, and its thickness represents the degree of combination between targets. It is expressed by the Combine Score value. The higher the value, the higher the degree of combination. Therefore, in this PPI, the core targets are IL-6, TNF, and VEGF. These proteins play a key role in the regulation of colchicine on ACI.

3.4. KEGG pathway enrichment analysis
Perl language was used to convert the overlapping target names into ENTREZ Gene ID, and KOBAS database was used to perform computation. The enrichment information of 100 KEGG pathways was obtained, indicating that the active component of colchicine can treat ACI through multiple pathways. According to P value, 30 pathways with low P value were selected, including TNF signaling pathway, Toll-like receptor signaling pathway, MAPK signaling pathway, etc. Then, R language is used to calculate the graph. The closer the color is, the higher the correlation is, as shown in Figure 2.

3.5. GO gene functional analysis
Perl language was used to convert the overlapping target names into ENTREZ Gene ID and KOBAS database was used for calculation. A total of 267 GO gene functional information were obtained, suggesting that colchicine and ticagrelor could be used to treat ACI by regulating multiple biological functions. According to P value, 30 functions with low P value were selected, including cell response to inflammatory stimulation, cell response to oxidative stimulation and other functions. Then, R language is used to calculate the graph. The closer the color is, the higher the correlation is, as shown in Figure 3.
4. Discussion
ACI is a clinically common disease with unusually high morbidity, disability and mortality. Patients suffering from ACI cannot receive timely treatment, which is easy to lead to disability and even death. In recent years, with the continuous development of society, people’s lifestyle has changed, and the incidence of ACI is also on the rise, which has a great impact on people’s physical and mental health as well as life safety. ACI has a high incidence of complications, and patients are prone to related complications if they cannot be treated in time and get good care.

Colchicine screened in this study plays an important role in the treatment of ACI. Colchicine has the effects of pain relief, detoxification, heat clearing, etc. Colchicine is an important
alkaloid originally found in the lily plant colchicine. It is a kind of cover phenolic ketone alkaloid, which can inhibit cell mitosis and inhibit the growth of cancer cells, and is clinically used to treat cancer, gout, and other diseases.\[7\]

In this study, by looking for the overlapping targets of colchicine and ACI, the potential targets of colchicine for the treatment of ACI were obtained, including IL-6, TNF, VEGF, Casp3, epidermal growth factor receptor (EGFR), Stat3, etc. Combined with the PPI network analysis diagram obtained in this study, it can be seen that colchicine affects the treatment of ACI through multiple targets and multiple signal pathways, and they play a synergistic role in various ways. Studies have shown that cysteine proteinase-3 (Gasp3), TNF, and EGFR play a central role in these targets and play an important role in the treatment of ACI. The physiological function of IL-6 is very complex. Normal nerve cells express low concentration of IL-6, which can not only be used for neuronutrition and neuroprotection, but also promote inflammation, demyelination, and glial hyperplasia.\[8,9\] Studies have found that significantly increased IL-6 level after ischemia/reperfusion can increase the expression of matrix metalloprotease-1 (MMP-1) and lead to tissue damage.\[10\] Studies have also confirmed that MMP-1 is involved in ischemic brain injury and the opening of the blood–brain barrier after reperfusion, and the destruction of the blood–brain barrier may aggravate the brain injury.\[11\] This suggests that high concentrations of IL-6 are involved in nerve damage after the onset of ACI. It has been confirmed experimentally that the serum TNF-α concentration increased significantly in the acute stage of cerebral infarction. TNF-α is one of the earliest inflammatory cytokines after cerebral ischemia. The concentration of inflammatory mediator TNF-α in peripheral blood in the acute stage of cerebral infarction is closely related to the activation of inflammatory cells in the central nervous system, which is similar to a reference index that can reflect the inflammatory course in the central nervous system and is regarded as a typical representative of inflammatory injury cytokines.\[12\] A large number of studies have confirmed that the activation of EGFR is related to the activation of the MAPK family and PI3K family.\[13\] Some studies have shown that the EGFR/ERK pathway plays a crucial role in cell proliferation, apoptosis and neuroprotection after cerebral infarction by affecting the cell cycle.\[14\] Experiments have shown that the JAK2/STAT3 pathway is closely related to the protective effect of myocardial ischemia–reperfusion.\[15\] After cerebral ischemia–reperfusion (I/R) injury, the JAK2/STAT3 pathway is activated, and EGF plays a protective role after I/R injury by activating the JAK2/STAT3 pathway.\[16\] Ginkgo biloba K promotes angiogenesis after ischemic stroke by activating the JAK2/STAT3 pathway and increasing the expression of HIF-1α/VEGF.\[17\] I/R injury can induce apoptosis, and activation of STAT3 can reduce apoptosis by up-regulation of Bcl-2 and down-regulation of Bax.\[18\]

In order to further analyze the signaling pathways and biological processes involved in the target of colchicine in the treatment of ACI, the KEGG pathway enrichment analysis and GO gene function analysis of the potential target of colchicine in the treatment of ACI were conducted in this study. Studies have shown that colchicine is involved in multiple KEGG signaling pathways in the treatment of ACI, including immune and anti-inflammatory signaling pathways. Biological effects induced by TNF are mediated by TNF-R1 and TNF-R2 receptors. TNF-R1 can activate NF-κB and induce apoptosis of cells,\[19\] TNF-R2 can activate NF-κB via TRAF2.\[20\] MAPK signaling pathway is closely associated with cerebral infarction and is involved in the regulation of neuronal differentiation and apoptosis in the pathological process of cerebral infarction, as well as other brain diseases.\[21\] Studies have verified that by reducing the activation of blood plates, inhibiting p-p38 MAPK to regulate the release of inflammation and reducing the death of macrophages, this may be one of the mechanisms of action in the treatment of cerebral infarction.\[22\]

| GO functional enrichment analysis. GO = gene function. |
|-------------------------------------------------------|
| Wound healing response to ethanol response to drug regulation of sequence-specific DNA binding transcription factor activity positive regulation of transcription, DNA-templated positive regulation of transcription from RNA polymerase II promoter positive regulation of smooth muscle cell proliferation positive regulation of peptidyl-tyrosine phosphorylation positive regulation of nitric oxide biosynthetic process positive regulation of NF-kappaB import into nucleus positive regulation of MAP kinase activity positive regulation of gene expression positive regulation of ESR1 and ESR2 cascade positive regulation of cell proliferation positive regulation of calcium homoeostasis activity negative regulation of cell proliferation negative regulation of apoptotic process MAPK cascade lipopolysaccharide-mediated signaling pathway inflammatory response immune response extracellular matrix organization cellular response to organic cyclic compound cellular response to mechanical stimulus cellular response to lipopolysaccharide cellular response to interleukin-1 cellular response to drug angiogenesis activation of MAPK activity |
| ![Figure 3. GO functional enrichment analysis. GO = gene function.](image-url) |
GO gene enrichment analysis showed that colchicine treatment of ACI involved multiple biological processes, such as cellular lipopolysaccharide response, positive regulation of ERK1 and ERK2 cascade, regulation of nitric oxide biosynthesis, and negative regulation of apoptosis process, which were all related to the pathogenesis of ACI. It plays an important role in the treatment of ACI.

5. Conclusion
To sum up, this study on the basis of network pharmacology, use all kinds of software and database, build the colchicine and for Greg “active ingredients—targets” network diagram, and the screening of potential targets for the treatment of ACI KEGG pathway enrichment analysis and GO gene function analysis, system this paper discusses the mechanism of action of colchicine treatment of ACI, for clinical colchicine can provide basis for treatment of ACI. However, the drawback of this study is that the network pharmacological analysis only provides a prediction, which needs to be validated by further experiments and clinical trials.

Author contributions
Qiaoxia Hu and Wenming He carried out the studies, participated in collecting data, and drafted the manuscript. Kena Luo and Puheng Liu performed the statistical analysis and participated in its design. All authors read and approved the final manuscript.

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