Network Pharmacology Integrated Molecular Docking Reveals the Mechanism of Zhongfeng Xingnao Formula against Intracerebral Hemorrhage

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Research

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Abstract

Background: Previous studies have shown that Zhongfeng Xingnao Formula (ZXF) can effectively reduce the mortality of intracerebral hemorrhage (ICH), but the underlying mechanism of the treatment remained still unexplored. This study aimed to expound the potential mechanism of ZXF in the treatment of ICH through network pharmacology and molecular docking.

Methods: The putative targets of ZXF were obtained from the TCMSP and Uniprot database, while the potential targets of ICH received from Drugbank, Genecards and OMIM database. Then through the Venn 2.1, the overlapping targets of disease and drug were gotten for the further study. The GO and KEGG enrichment analyses were performed by R version 4.0.2 software so that the signaling pathway was acquired to the subsequent analysis. Cytoscape was used to construct the drug-compound-target-pathway network and String was utilized for the protein-protein interaction network. What's more, the interaction between compound and target was verified by the AutoDockTools and Autodock Vina.

Results: There were a total of 166 ZXF-related targets and 1258 ICH-related targets obtained from the public databases. And 87 potential targets were both related to drug and disease. The GO enrichment analysis mainly involved receptor ligand activity, signaling receptor activator activity, and cytokine receptor binding, while the signaling pathway, such as Fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, were significantly enriched in the KEGG enrichment analysis. The molecular docking elucidated that the aloe-emodin, beta-sitosterol, quercetin could bound well to the top five targets sorted by degree value.

Conclusions: ZXF treated ICH through multiple compounds, multiple targets, and multiple pathways. The underlying mechanism of the treatment may be promoting angiogenesis, anti-inflammatory, anti-oxidative stress, and reversing atherosclerosis, which is of great significance for the treatment of ICH.

Introduction

Intracerebral hemorrhage (ICH) is a form of stroke with high morbidity, accounting for about 14.9% and 10% of all strokes in China and the United States respectively [1, 2]. What's worse, ICH is also a devastating disease with awfully high rate of disability and mortality [3]. However, there is almost no optimal treatment for ICH until now in the modern medicine field [4]. The Antihypertensive Treatment of Acute Cerebral Hemorrhage (ATACH-2) trial showed that intensive blood-pressure lowering cannot lead to a decline in mortality and disability [5]. And in the Intracerebral Haemorrhage Deferoxamine (i-DEF) trial, there was no significant evidence for the improvement in the 90-day good outcome [6]. In addition, the Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage (TICH-2) trial indicated that there was no significant difference in 90-day functional status between placebo-treated and tranexamic acid-treated patients [7]. Therefore, it is of great clinical importance to discover therapeutic drugs and formulations for the treatment of ICH.

In the field of traditional Chinese medicine (TCM), more and more attention was fixed on the therapy of stroke, especially hemorrhagic stroke [8]. Song et al found that Naoxueshu oral liquid can significantly reduce the inflammation and edema of ICH [9]. And Li et al. reported that tsRNAs may be the potential targets for Buyang Huanwu Decoction against ICH [10]. Moreover, the treatment of ICH by ZXF has been proven to effectively reduce the mortality, promote the absorption of hematoma, and improve the neurological deficits [11]. The Zhongfeng Xingnao Formula (ZXF), a widely acclaimed anti-ICH Chinese patent medicine, is composed of four Chinese herbs, such as Rhubarb (Dahuang in Chinese, DH), Panax Notoginseng (Sanqi in Chinese, SQ), Chuanxiong Rhizoma (Chuanxiong in Chinese, CX), Red Ginseng (Hongshen in Chinese, HS). It has the effects of replenishing qi, activating blood, resolving stasis, and stopping bleeding. Nevertheless, the underlying mechanism of ZXF in the treatment of ICH still remained unclear.

Network pharmacology is an emerging discipline combined with pharmacology and bioinformatics [12], which was first proposed by Hopkins in 2007 [13]. It believes that drugs exert effects through the interaction between multiple components and multiple targets, which coincides with the characteristics of traditional Chinese medicine [14]. Molecular docking is a widely used structure-based silico method, which is able to get the binding energy and binding mode of receptors and ligands through scoring function [15]. In this study, the network pharmacology and molecular docking were used to explore the active compounds of ZXF, the putative targets of ZXF against ICH, and the potential mechanism of ZXF in the treatment of ICH.

Materials And Methods

Collection and screening of active compounds

The compounds in ZXF were collected in Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) database [16](<https://tcmspw.com/tcmsp.php>) whose search terms were as follows: Dahuang (DH, rhubarb in English), Sanqi (SQ, sanchi in English), Chuanxiong (CX, szechwan lovage rhizome in English), Hongshen (HS, red ginseng in English). TCMSP database is an important pharmacology platform of Chinese herbs that provides critical data about pharmacokinetic properties, such as oral bioavailability (OB) [17], drug-likeness (DL) [18], intestinal epithelial permeability (IEP), blood-brain-barrier (BBB) and etc. Therefore, the compounds with $OB \geq 30\%$ and $DL \geq 0.18$ simultaneously in the TCMSP database were screened as active compounds for the further study.

Prediction of drug-related targets.

The validated target proteins of the active compounds were further predicted with the TCMSP database. And then the UniProt database (<https://www.uniprot.org/>) was utilized to convert the name of target protein into UniProt ID by the UniProt Knowledge Base (UniProtKB), with the organism limited to "Homo

sapiens” and the active compounds without UniProt ID eliminated. Ultimately the official Gene symbols were received from the abovementioned steps [19].

Identification of disease-related targets.

The OMIM database (<https://www.omim.org/>) [20], GeneCards database (<https://www.genecards.org/>) [21], DrugBank database (<https://go.drugbank.com/>) [22] were used to search for diseases-related targets with the keywords “Cerebral Hemorrhage”, “Intracerebral Hemorrhage”, and “Intracranial Hemorrhage”. And then the search results were summarized in an excel table and the duplicate data was removed from the table. Eventually the disease-related targets would be included in the subsequent analysis.

Acquisition of ZXF targets associated with ICH.

The drug-related targets and disease-related targets were both imported into Venn 2.1 (<https://bioinfo.gp.cnb.csic.es/tools/venny/index.html>), in which the Venn diagram was constructed. In addition, the intersection of the two kinds of targets was obtained from the website, which were the potential targets of ZXF against ICH.

Analysis of function and pathway Enrichment.

R version 4.0.2 software was used for Gene Ontology (GO) biological function enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway enrichment analysis [23, 24]. The threshold was set to $P.adjust \leq 0.05$. Through the GO enrichment analysis, the biological process (BP) terms, cell component (CC) terms, and molecular function (MF) terms which potential targets most highly enriched in could be obtained [25]. The top 10 terms sorted by gene ratio would be presented in the form of bubble plots. In addition, the signaling pathways for ZXF against ICH can be received from KEGG enrichment analysis [26]. The bar plots would be used to demonstrate the top 20 pathways sorted by P value.

Construction of drug-compound-target-pathway network

The Cytoscape 3.7.1 software was used to construct the drug-component-target-pathway network [27]. Nodes with different colors and shapes were used to represent drugs, compounds, potential targets, and signaling pathways respectively. The edges were used to connect the drug-compound, compound-target, and target-pathway. The top 20 signaling pathways were sorted by gene ratio and all potential targets were included in the network.

Establishment of protein-protein interaction

The potential targets were imported into the String database (<https://string-db.org/>) with the organism set to "Homo sapiens", the minimum required interaction score limited to "Highest confidence 0.400", and hiding the disconnected nodes [28]. Then the protein-protein interaction (PPI) network was established.

The PPI network was input to the Cytoscape 3.7.1 software, whose NetworkAnalyser function was used to calculate the degree value of the nodes. The degree value, one of the topological parameters, can reflect the significance of the node. Next, the Cytoscape software was used to visualize the degree value of the potential targets. The nodes with larger degree value were with larger size and darker color in the figure. In addition, the top 10 potential targets sorted by degree value, namely the core genes, also used the above method to construct a PPI network.

Verification by molecular docking.

Molecular docking is a widely used technology based on the theory of lock and key, revealing how the proteins and ligands interact with each other [29]. Therefore, the connections between the core targets and the active compounds were verified by molecular docking in our study. Firstly, the PDB format files of core targets were downloaded from the RCSB PDB database (<https://www.rcsb.org/>) [30], while the mol2 format files of the active compounds were downloaded from the TCMSP database. Secondly, the AutoDockTools 1.5.6 was used to pretreat the core targets and active compounds, including deleting water, adding hydrogens and computing charges. Also, the grid box was created by the AutoDockTools with coordinates and size of the box recorded [31]. Thirdly, the Autodock Vina 1.1.2 was used for docking operation to get the binding mode and binding affinity [32]. Last but not least, the PyMOL 2.4.0 was used to visualize the results of molecular docking to check the interaction between core targets and active compounds [33].

Results

Active compounds in ZXF.

After the collection and screening in the TCMSP database, a total of 16 active compounds were obtained in ZXF, including 6 Rhubarb, 3 Red Ginseng, 7 Panax Notoginseng, and 4 Chuanxiong Rhizoma. Three of sixteen active compounds were shared by two or three Chinese herbs, such as beta-sitosterol, ginsenoside rh2, Mandenol. The detailed information was shown in Table 1.

Table 1
Information on the active compounds in Zhongfeng Xingnao Formula

ID	Mol. ID	Chemical name	OB(%)	DL	Herbs
A	MOL000358	beta-sitosterol	36.91	0.75	HX, SQ, DH
B	MOL005344	ginsenoside rh2	36.32	0.56	HS, SQ
C	MOL001494	Mandenol	42	0.19	CX, SQ
CX1	MOL002135	Myricanone	40.6	0.51	CX
CX2	MOL002157	wallichilide	42.31	0.71	CX
CX3	MOL000359	Sitosterol	36.91	0.75	CX
DH1	MOL002235	EUPATIN	50.8	0.41	DH
DH2	MOL002268	Rhein	47.07	0.28	DH
DH3	MOL002281	Toralactone	46.46	0.24	DH
DH4	MOL002297	Daucosterol_qt	35.89	0.7	DH
DH5	MOL000471	aloe-emodin	83.38	0.24	DH
HS1	MOL002032	DNOP	40.59	0.4	HS
SQ1	MOL001792	DFV	32.76	0.18	SQ
SQ2	MOL002879	Diop	43.59	0.39	SQ
SQ3	MOL000449	Stigmasterol	43.83	0.76	SQ
SQ4	MOL000098	Quercetin	46.43	0.28	SQ

Putative targets of ZXF.

A total of 47 potential targets of Rhubarb, 36 potential targets of Red Ginseng, 154 potential targets of Panax Notoginseng, and 23 potential targets of Chuanxiong Rhizoma were obtained from the prediction of the TCMSP database and the conversion of the UniProt database. Among them, 32 potential targets were shared by two or more active compounds. Consequently there were 166 potential targets of ZXF in total for the following study.

Potential targets of ICH.

By searching the above databases, 36 potential targets were from OMIM database, while 1215 potential targets were from GeneCards database, and 24 potential targets were from DrugBank database. After

screening, sorting, checking, deduplicating, and unifying the search results, a total of 1258 potential targets related to ICH were collected.

Overlapping targets of ZXF and ICH.

In the Venn diagram, there were 166 ZXF-related targets, 1258 ICH-related targets and 87 overlapping targets in total. It was worth mentioning that the 87 overlapping targets may be the potential targets of ZXF against ICH, which may play a critical role in the treatment of ICH (Fig. 2 and Table 2).

Table 2
Potential targets of Zhongfeng Xingnao Formula against intracerebral hemorrhage

NO.	Gene	NO.	Gene	NO.	Gene	NO.	Gene
1	MMP2	23	CHRM3	45	RUNX2	67	IL2
2	PLAU	24	IGFBP3	46	PGR	68	ERBB3
3	NOS2	25	PTGS1	47	CHRM2	69	ERBB2
4	CCNB1	26	F3	48	ODC1	70	FOS
5	BIRC5	27	JUN	49	CXCL10	71	IFNG
6	PON1	28	ESR2	50	SERPINE1	72	ICAM1
7	CHEK1	29	CD40LG	51	VCAM1	73	BCL2L1
8	F7	30	RASSF1	52	TP53	74	CHEK2
9	CASP8	31	CYP3A4	53	CASP9	75	RELA
10	CHRM1	32	CYP1B1	54	CDKN1A	76	HIF1A
11	PPARG	33	PRSS1	55	RASA1	77	MAP2
12	CRP	34	MMP9	56	AKT1	78	NOS3
13	GSTP1	35	TOP1	57	IL1B	79	CASP3
14	CCL2	36	STAT1	58	IL6	80	ADRA1B
15	EGF	37	IL1A	59	MPO	81	NFE2L2
16	CXCL8	38	ADRA1A	60	SELE	82	ADCYAP1
17	KDR	39	HMOX1	61	MMP3	83	PTGS2
18	PCNA	40	CASP1	62	SPP1	84	MAPK14
19	THBD	41	NR3C1	63	CHRM4	85	PLAT
20	IL10	42	MAPK1	64	IGF2	86	MYC
21	CCND1	43	ESR1	65	VEGFA	87	RB1
22	TNF	44	EGFR	66	GSK3B		

GO and KEGG Enrichment Analysis.

In the GO enrichment analysis, 87 potential targets were significantly enriched in 1782 BP terms, 41 CC terms, 112 MF terms (Padjust $\leq$ 0.05). The terms were sorted in descending order from top to bottom by the gene ratio. As was shown in Fig. 3, receptor ligand activity (Padjust = 1.02E⁻⁰⁷), signaling receptor

activator activity ($P_{\text{adjust}} = 1.02E^{-07}$), and cytokine receptor binding ($P_{\text{adjust}} = 2.65E^{-09}$) were three most highly enriched GOMF terms. Among the 41 GOCC terms, membrane raft ($P_{\text{adjust}} = 1.34E^{-07}$), membrane microdomain ($P_{\text{adjust}} = 1.34E^{-07}$), and membrane region ($P_{\text{adjust}} = 1.40E^{-07}$) were three most closely related to cell component of ICH. In terms of biological processes, potential targets concentrated more in response to lipopolysaccharide ($P_{\text{adjust}} = 5.03E^{-23}$), response to molecule of bacterial origin ($P_{\text{adjust}} = 7.15E^{-23}$) and cellular response to drug ($P_{\text{adjust}} = 4.80E^{-19}$).

A total of 139 signaling pathways were obtained from the KEGG enrichment analysis ($P_{\text{adjust}} \leq 0.05$). Among them, Fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, Kaposi sarcoma-associated herpesvirus infection, Human cytomegalovirus infection pathway were the most relevant pathway to ZXF against ICH (Fig. 3 and Table 3).

Table 3
Information of signaling pathway obtained from KEGG enrichment analysis

ID	Description	p.adjust	Count
hsa05418	Fluid shear stress and atherosclerosis	1.25E-21	24
hsa04933	AGE-RAGE signaling pathway in diabetic complications	2.04E-23	23
hsa04151	PI3K-Akt signaling pathway	4.9E-12	23
hsa05167	Kaposi sarcoma-associated herpesvirus infection	4.41E-16	22
hsa05163	Human cytomegalovirus infection	9.97E-14	21
hsa05161	Hepatitis B	2.52E-15	20
hsa05205	Proteoglycans in cancer	1.91E-13	20
hsa04010	MAPK signaling pathway	6.47E-11	20
hsa04668	TNF signaling pathway	5.65E-17	19
hsa04657	IL-17 signaling pathway	5.65E-17	18
hsa05160	Hepatitis C	2.66E-13	18
hsa05164	Influenza A	9.06E-13	18
hsa05215	Prostate cancer	1.73E-15	17
hsa05142	Chagas disease	3.29E-15	17
hsa04218	Cellular senescence	2.32E-12	17
hsa05169	Epstein-Barr virus infection	9.58E-11	17
hsa05166	Human T-cell leukemia virus 1 infection	3.39E-10	17
hsa05165	Human papillomavirus infection	1.21E-07	17
hsa05162	Measles	4.9E-12	16
hsa05224	Breast cancer	1.08E-11	16

Drug-compound-target-pathway network

In the drug-compound-target-pathway network, 4 red triangle nodes represented the drugs, while 16 green octagon nodes were on behalf of the compounds, 87 orange V-shape nodes were in the name of the potential targets, and the signaling pathways were represented by 20 purple diamond nodes. Altogether,

there were 127 nodes in the network and 518 edges in the network, indicating that ZXF treated ICH with multiple compounds, multiple targets and multiple pathways.

As shown by topology analysis, the five compounds with the highest degree were Quercetin, beta-sitosterol, Mandenol, Myricanone, and aloe-emodin, suggesting that these five compounds may be the main compounds for ZXF against ICH. Also, the top five potential targets sorted by degree value were AKT1, RELA, MAPK1, TP53, TNF, indicating that these five targets may be the core targets in treating ICH by ZXF. In addition, the top five signaling pathways with the highest degree value are Fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, Kaposi sarcoma-associated herpesvirus infection, and Human cytomegalovirus infection, indicating that ZXF treated ICH mainly through these five signaling pathways.

PPI network

As was shown in Fig. 4(A), there were a total of 87 nodes and 1514 edges in the PPI network for potential targets. The average node degree was 34.8. Sorted by the degree value, the top 10 potential targets, considered as core genes, were AKT1 (degree=68), TP53 (degree=68), and VEGFA (degree=68). =67), IL6 (degree=67), CASP3 (degree=66), JUN (degree=64), EGF (degree=64), PTGS2 (degree=63), EGFR (degree=61). Besides, it can be seen from Fig. 4(B) that 10 nodes and 45 edges were included in the PPI network of the core genes with the average node degree was 9.

Molecular docking

As was shown in Table 4, the core targets (AKT1, MAPK1, RELA, TNF, TP53) and active compounds (beta-sitosterol, myricanone, aloe-emodin, quercetin, mandenol) obtained from drug-compound-target-pathway network were chosen to further verify compound-target interaction by molecular docking. In general, the lower the binding affinity was, the more effective the molecular docking was. And the binding affinity less than 5 kcal/mol would be regarded as effective molecular docking. According to the criteria, aloe-emodin, beta-sitosterol and quercetin would be three active compounds in ZXF. Additionally, MAPK1 and TNF may be therapeutic targets for ZXF against ICH. Furthermore, the hydrogen-bonding interaction between receptor (core target) and ligand (active compound) was shown in Fig. 6.

Table 4
Molecular docking of core targets and active compounds

Target	PDB ID	Affinity (kcal·mol ⁻¹)				
		Aloe_emodin	Beta_sitosterol	Mandenol	Myricanone	Quercetin
AKT1	1UNQ	-6.6	-7.4	-4.3	-4.2	-6.4
MAPK1	4IZ5	-8.1	-9.5	-5.0	-8.6	-8.6
RELA	1NFI	-8.3	-7.6	-4.8	-7.1	-8.4
TNF	5YOY	-8.2	-9.0	-5.5	-9.0	-8.4
TP53	3Q05	-9.0	-7.8	-4.9	-8.2	-9.1

Discussion

ICH is a cerebrovascular disease with extremely high morbidity and mortality. Most patients are left with severe neurological impairment symptoms and relevant complications, which brings serious economic and psychological burdens to the society and families [34]. To make matters worse, there is yet no optimal therapy for ICH, whether the surgical treatment or the conservative treatment [35]. Nowadays, more and more focus was fixed on the treatment of ICH in the field of traditional Chinese medicine [36–38]. Previous study has shown that ZXF can improve the functional outcome of patients with ICH [11]. But the mechanism how the ZXF takes effect has not been extensively verified. This study aimed to explore the potential mechanism of ZXF against ICH through network pharmacology and molecular docking. And the results suggested that ZXF treated ICH through multiple compounds, multiple targets, and multiple pathways.

Through the search and screening of the TCMSP database, a total of 16 active compounds of ZXF were finally obtained. By constructing the drug-compound-target-pathway network, the top five active compounds sorted by degree value were Quercetin, beta-sitosterol, Mandenol, Myricanone, and aloe-emodin. Quercetin has been proven to exert a variety of pharmacological effects related to cerebrovascular diseases, such as oxidative stress, atherosclerosis, ischemia-reperfusion injury, endothelial cell dysfunction [39]. Beta-sitosterol were proved to be effective in ICH mainly by reducing the risk of plasma cholesterol and atherosclerosis [40]. Several pharmacological properties on Mandenol have been reported including attenuating the expression of pro-inflammatory factors [41], Gudiya et al have shown that Myricanone was equipped with the biological functions of antioxidant, anti-inflammatory, and anti-ischemia-reperfusion injury [42]. In addition, aloe-emodin is a widely existing anthraquinone in nature, which has been found to lower cholesterol by inhibiting PCSK9 [43].

Through the Uniprot database, OMIM database, Genecards database, and Drugbank database, there were 166 targets related to ZXF and 1258 targets related to ICH obtained in total. Among them, 87 targets

shared by disease and drug altogether. In the drug-compound-target-pathway network, AKT1, MAPK1, RELA, TNF, and TP53 were the top five targets with the highest degree value, which were the core targets of ZXF against ICH. Huang et al. found that AKT1 can precisely regulate the synthesis of nitric oxide and further promote vascular remodeling [44]. In addition, AKT1 activation can promote the overexpression of glial cell-derived neurotrophic factor (GDNF) and thereby protect damaged neuronal cells [45]. Sun et al. indicated that RELA, a key transcription factor for hypoxic response, can promote the expression of CXCL12 under hypoxic conditions by combining with LINC01693 and thus promote hypoxia-induced angiogenesis [46]. MAPK1, belonging to the MAPK family, can be activated by LncRNA NEAT1 to enhance cell viability and inhibit cell apoptosis [47]. Wei et al. illustrated that the methylation of the TP53 promoter may be related to the occurrence of atherosclerosis, which has got wider audience in the research of atherosclerosis [48]. TNF has been shown to promote apoptosis of injured neurons by activating apoptosis signaling pathways, which has gained widespread attention in the field of neuroscience research [49].

According to the GO enrichment analysis, the function of potential targets were significantly enriched in a wealth of MFs, CCs and BPs. The receptor ligand activity, signaling receptor activator activity, and cytokine receptor binding were three MFs most closely related to treatment of ICH with ZXF. And the most important CCs involved in the ZXF against ICH were membrane raft, membrane microdomain, membrane region. Additionally, the response to lipopolysaccharide, response to molecule of bacterial origin, and cellular response to drug were the most significantly enriched BPs related to the treatment of ICH. KEGG enrichment analysis revealed that the overlapping targets of ZXF and ICH were mainly enriched in a number of signaling pathways, such as fluid shear stress and atherosclerosis, AGE-RAGE signaling pathway, PI3K-Akt signaling pathway, kaposi sarcoma-associated herpesvirus infection, and human cytomegalovirus infection. Fluid shear stress and atherosclerosis plays a vital role in maintaining cardiovascular and cerebrovascular homeostasis by keeping the balance of redox and thus can protect vascular endothelial cells from oxidative stress [50]. AGE-RAGE signaling pathway has been proved to play a role as a pro-inflammatory mediator, which can activate the transcription factor NF-Kb and trigger the inflammation and oxidative stress [51]. Luo et al suggested that PI3K-Akt signaling pathway can reduce the level of reactive oxygen species and lipid deposition, which can inhibit the formation of plaques and reverse the progress of atherosclerosis [52]. Sun et al illustrated that activating the PI3K-Akt signaling pathway can up-regulate the expression of MIAT and aggravate atherosclerotic injury [53]. Kaposi sarcoma-associated herpesvirus infection signaling pathway is mainly related to the secretion of pro-angiogenic cytokines and pro-inflammatory factors, which is able to promote angiogenesis and induce inflammation signals [54]. Human cytomegalovirus infection signaling pathway plays a vital role in controlling the differentiation, proliferation, migration, and angiogenesis of cells and production and function of cytokines [55].

In the present study, network pharmacology was used to discover the active compounds, potential targets, and signaling pathways of ZXF against ICH to clarify the possible mechanism of the treatment. Additionally, the molecular docking was utilized to further verify the interaction between the compounds and the targets. Moreover, ZXF was picked out based on the preliminary clinical research of our team,

which was much more reliable than those unproven drugs. However, the main limitations of our study needed to be acknowledged. This study was based on the data in the existing literature and the compatibility of traditional Chinese herbs in clinical practice has not been taken into consideration. Thus, more multicenter prospective randomized clinical trials and better basic researches will be needed to validate the effect in the future.

Conclusion

All in all, the study expounded that ZXF could exert pharmacological effects through promoting angiogenesis, anti-inflammatory, anti-oxidative stress, and reversing atherosclerosis, which played a vital role in the treatment of ICH. Both network pharmacology and molecular docking were used to reveal the underlying mechanism of the treatment, which provided theoretical guidance for the subsequent in-depth basic researches. Nevertheless, a large number of clinical trials and basic researches were still ought to be carried out to confirm these results.

Abbreviations

ZXF: Zhongfeng Xingnao Formula;

ICH: intracerebral hemorrhage;

ATACH-2 : Antihypertensive Treatment of Acute Cerebral Hemorrhage [1];

i-DEF: Intracerebral Haemorrhage Deferoxamine;

TICH-2: Tranexamic acid for hyperacute primary IntraCerebral Haemorrhage;

TCM: traditional Chinese medicine;

TCMSP: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform; DH: rhubarb;

SQ: sanchi;

CX: szechwan lovage rhizome;

HS: red ginseng;

OB: oral bioavailability;

DL: drug-likeness;

IEP: intestinal epithelial permeability;

BBB: blood-brain-barrier;

UniProtKB: UniProt Knowledge Base;

GO: Gene Ontology;

KEGG: Kyoto Encyclopedia of Genes and Genomes;

BP: biological process;

CC: cell component;

MF: molecular function;

PPI: protein-protein interaction

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials:

All data are available in the manuscript and they are showed in figures and tables

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions

Jian-wen Guo and Ye-feng Cai conceived and designed the research; Can Wan and Guang-yao Zhang collected and analyzed the data; Zi-yi Zhou and Yun Lu provided useful suggestions on the methodology;

Can Wan wrote the paper. All authors read and approved the final version of the manuscript.

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Figures

Figure 1

The sketch map for potential mechanism of Zhongfeng Xingnao Formula against intracerebral hemorrhage

Figure 2

Venn diagram of overlapping targets of Zhongfeng Xingnao Formula (ZXF) and intracerebral hemorrhage (ICH)

Figure 3

GO and KEGG enrichment analysis. (A) (B) (C) Bubble plots of GO enrichment analysis. The size of the bubble represented the number of enriched potential targets. The color of the bubble indicated the significance of enrichment. (D) Bar plots of KEGG enrichment analysis. The length of the bar showed the number of enriched potential genes. The color of the bar suggested the significance of the pathway.

Figure 4

Drug-compound-target-pathway network. Four drugs, sixteen compounds, eighty seven potential targets, twenty signaling pathways were included with the size of nodes represented the degree value.

Figure 5

PPI network of potential targets (A) and core genes (B) for Zhongfeng Xingnao Formula against intracerebral hemorrhage

Figure 6

Molecular docking of core targets and active compounds. (A) TNF and beta-sitosterol. (B) TNF and myricanone. (C) TP53 and aloe-emodin. (D) TP53 and quercetin.