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Exploring the Potential Mechanism of Buyang Huanwu Decoction Regulating Ferroptosis to Intervene in Secondary Brain Injury After Intracerebral Hemorrhage Based on Network Pharmacology and Molecular Docking

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Abstract: Intracerebral hemorrhage (ICH) is a serious type of acute stroke that can be very harmful to people. Secondary brain injury (SBI) is associated with a poor prognosis in isolated cerebral hemorrhage (ICH), and one of the reasons for this is iron-dependent ferroptosis. Buyang Huanwu Decoction (BYHWD) is an old Chinese medicine formula for qi-deficiency-blood-stasis stroke that has been applied in the rehabilitation of ICH, but its anti-ferroptosis mechanism is not yet known. Network pharmacology and molecular docking were used to identify the active compounds, the main targets, and the pathways of BYHWD against ICH-induced ferroptosis, etc. TCMSP was used to obtain the active compounds and their corresponding targets, and the ICH- and ferroptosis-related targets were obtained from GeneCards, OMIM, and DisGeNET. PPI, GO/KEGG enrichment analysis, and molecular docking were conducted. A total of 23 are active compounds, and 87 are general targets. AKT1, TP53, IL6, and TNF were the hub genes. Thus, BYHWD will disturb neuronal iron homeostasis, lipid peroxidation, oxidative stress, and neuroinflammation by affecting the PI3K-AKT pathway. Quercetin, Kaempferol, and Luteolin can all bind to GPX4, SLC7A11, and ACSL4. BYHWD can reduce post-ICH SBI by inhibiting neuronal ferroptosis through the following targets and pathways and is therefore a good drug for this disease.

Keywords: Buyang Huanwu Decoction; Intracerebral haemorrhage; Secondary brain injury; Ferroptosis; Network pharmacology; Molecular docking; Neuroprotection

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1. Introduction

ICH accounts for 20-30% of all strokes worldwide and is associated with a poor prognosis [1]. Hematoma-induced primary brain injury is usually irreversible, but prolonged progressive small-vessel

ischemia (SBI) caused by iron dysregulation, oxidative stress, and neuroinflammation has also been reported [2]. Ferroptosis is a type of iron-dependent regulated cell death caused by lipid peroxidation, and it has been induced in the event of intracranial hemorrhage (ICH) due to the release of iron from damaged blood vessels in the brain. Thus, one way to address ICH is to induce ferroptosis [3].

Buyang Huanwu Decoction (BYHWD) is a traditional Chinese medicine with low toxicity that has many targets in stroke, and it is often used in cases of ICH with qi deficiency and blood stasis syndrome [4]. Antioxidants, anti-inflammatory drugs, and so on can reduce neurological damage and brain swelling, but few systematic studies have been conducted to investigate the anti-ferroptosis mechanism of post-ICH SBI, and this has not been fully understood.

Network pharmacology and molecular docking are used to study the basis and molecular mechanisms of TCM prescriptions [5]. According to some clinical and basic studies, we have found that BYHWD regulates ferroptosis and reduces ICH-related SBI.

2. Materials and methods

2.1. Screening of BYHWD active ingredients and targets

All the chemical components of the seven herbal medicines in BYHWD have been obtained from the TCMSP database. The active substances have good general pharmacokinetic properties and have an oral bioavailability $\ge 30\%$ and drug-likeness ≥ 0.18. Based on the above, we predicted and normalized the corresponding target genes to human gene symbols in the UniProt database, then removed duplicates, invalid targets, and non-human targets, and obtained the final set of valid targets BYHWD [6].

2.2. Acquisition of disease-related targets

GeneCards, OMIM, and DisGeNET databases have all listed genes associated with the disease of ICH-induced secondary brain injury and ferroptosis. Summary and deduplication of the disease-target data have been carried out. Venn 2.1 was used to find the common targets of BYHWD and the disease, and then, among these genes in the intersection that can be regulated by ferroptosis to reduce post-ICH SBI, were selected as therapeutic targets.

2.3. PPI network construction

Import the overlapping therapeutic targets into the STRING database, set the species to Homo sapiens, and set a minimum interaction confidence score of 0.7. Delete the disconnected nodes and then optimize the overall arrangement of the network. Cytoscape 3.9.1 was used to construct and draw the PPI network. Topological sort is employed to find the degree of each node and the core hub genes.

2.4. GO and KEGG enrichment analysis

ClusterProfiler in the R language was used to perform function enrichment and KEGG pathway enrichment analysis. The three types of GO analysis are biological process, cellular component, and molecular function; the main biological functions of the target genes are listed below. KEGG analysis was carried out to find the corresponding regulatory signal pathways [7]. Set the statistical threshold of $P < 0.05$ and $FDR < 0.01$ to ensure that there are significant enrichments in the results.

2.5. Construction of regulatory network

After filtering the main active substances, we set the first therapeutic goals and greatly extended the paths, then built a regulatory network of drugs-ingredients-targets-paths in Cytoscape. A network can be constructed to organize the relationships among drugs, active ingredients, target genes, and signaling pathways in an organized way, and then all synergistic mechanisms of BYHWD against post-ICH SBI can be revealed.

2.6. Molecular docking verification

Based on the results of the network analysis, quercetin, kaempferol, and luteolin were selected as the ligand molecules, and GPX4, SLC7A11, and ACSL4 were chosen as the main ferroptosis-related receptor proteins. The 3D structures of the ligands were downloaded from the PubChem database, and the receptor crystal structures were obtained from the RCSB PDB database. PyMOL and AutoDockTools were used to prepare the structures, add water molecules, and calculate charges, etc. AutoDock Vina will be used in this paper. Binding affinity of -5.0 kJ/mol or lower is considered good binding, and at -7.0 kJ/mol or lower, it is a strong and stable molecular bond.

3. Result

3.1. Screening results of active ingredients and targets

A total of 23 qualified active compounds and 219 valid human target genes for BYHWD have been found. After removing duplicates, there are 1527 target genes for ICH- and ferroptosis-related diseases. Finally, it was decided that the first targets for the post-ICH SBI regulation of ferroptosis would be the 87 overlapping genes.

Table 1. Data sources and screening statistics of BYHWD active compounds and ferroptosis-related therapeutic targets for post-ICH secondary brain injury

Category	Item	Database / Tool	Screening criteria / Parameters	Number
Active compounds	Qualified active compounds of BYHWD	TCMSP	Oral bioavailability (OB) ≥ 30%; Drug-likeness (DL) ≥ 0.18	23
Drug targets	Valid human target genes	UniProt	Normalized to human gene symbols; duplicates, invalid, and non-human targets removed	219
Disease targets	ICH-related targets	GeneCards, OMIM, DisGeNET	Keywords: “intracerebral hemorrhage”, “secondary brain injury”	—
	Ferroptosis-related targets	GeneCards, OMIM, DisGeNET	Keyword: “ferroptosis”	—
	Deduped disease targets	—	Union and deduplication	1527
Intersection	Overlapping therapeutic targets	Venny 2.1	Intersection of BYHWD targets and ICH–ferroptosis disease targets	87
PPI network	Nodes / Edges	STRING + Cytoscape 3.9.1	Species: *Homo sapiens*; minimum interaction confidence ≥ 0.7; disconnected nodes removed	85 / 1247
Hub genes	Top 10 hub genes	Cytoscape topological analysis	Ranked by node degree	AKT1, TP53, VEGFA, IL6, TNF, JUN, CASP3, EGFR, MAPK3, MYC

Category	Item	Database / Tool	Screening criteria / Parameters	Number
Enrichment	KEGG enriched pathways	clusterProfiler (R)	$p < 0.05$; FDR < 0.01	137
Molecular docking	Ligand–receptor pairs	AutoDock Vina	Binding affinity ≤ −5.0 kJ/mol = good; ≤ −7.0 kJ/mol = strong	9 (3 ligands × 3 receptors)

3.2. Main hub gene screening

The optimized PPI network has 85 nodes and 1247 edges, and these are the functions of the therapeutic targets. The top 10 hub genes are AKT1, TP53, VEGFA, IL6, TNF, JUN, CASP3, EGFR, MAPK3 and MYC. All the above genes are necessary for neuronal iron metabolism, oxidative stress response, neuroinflammation, and programmed cell death, and are closely associated with post-ICH ferroptosis and the development of secondary brain injury.

3.3. GO enrichment analysis

GO enrichment analysis shows that BYHWD is involved in neuronal iron-ion homeostasis, suppresses lipid peroxidation, is induced by oxidative stress, and is associated with neuroinflammation. The basic units of the body are neuronal membranes, mitochondrial membranes, and the endoplasmic reticulum; all of them require iron metabolism and lipid peroxidation in the cell. First, it can bind to iron ions, and second, as an antioxidant, it reduces inflammation by binding to inflammatory cytokines and thus inhibits ferroptosis in neurons.

3.4. KEGG enrichment analysis

KEGG enrichment analysis shows that 137 pathways are enriched, and among them, the PI3K-AKT, TNF, HIF-1, and ferroptosis signaling pathways are the most closely related to the effect of post-ICH brain injury. Among the many ways neuronal survival and changes in the intracellular redox state are regulated after intracranial hemorrhage (ICH), one is PI3K-AKT signaling. Iron overload and oxidative stress in ICH reduce the PI3K-AKT/PKB pathway and cause lipid peroxidation and neuronal ferroptosis. HIF-1 is linked to cerebral hypoxia and dysregulation of iron metabolism, and the TNF pathway is associated with neuroinflammation. Neuroinflammation and ferroptosis will likely occur together and are thus more harmful to the brain in the long run. Therefore, BYHWD has several ways to inhibit ferroptosis, and these ways work together [8].

3.5. Network analysis

The constructed network of drug-ingredient-target-pathway has 130 nodes and 426 edges. Quercetin, Kaempferol, Luteolin, and Astragaloside IV were determined to be the main active components of BYHWD. The network consists of TCM compounds; that is to say, one component can regulate many disease genes, and a single core gene is co-regulated by all kinds of active ingredients. Quercetin had the highest node degree and was thus the main reason for the anti-ferroptosis and neuroprotection. Kaempferol and Luteolin reduce neuronal lipid peroxidation; Astragaloside IV improves cerebral microcirculation and reduces neuroinflammation. BYHWD has many ways to reduce harm to the brain, and it does not have to be any particular one.

3.6. Molecular docking results

According to molecular docking, many of the BYHWD compounds and the ferroptosis marker proteins have

relatively strong binding affinities. Quercetin-GPX4, kaempferol-SLC7A11, and luteolin-ACSL4 had the three smallest binding energies of -8.2 kJ/mol, -7.9 kJ/mol, and -7.6 kJ/mol, and could also form hydrogen bonds and hydrophobic interactions. SLC7A11 increases the uptake of extracellular cystine and therefore raises glutathione in neurons. Kaempferol reduces the activity of SLC7A11 and oxidative stress. Quercetin raises GPX4 to reduce harmful lipid peroxides and therefore inhibits ferroptosis. Luteolin decreases the expression of ACSL4 and therefore prevents the start of ferroptosis [9]. The docking results shown above are in line with the predictions of network pharmacology.

4. Discussion

Neuronal ferroptosis is one of the main reasons for the reduction of neurons and poor long-term outcomes after intracranial hemorrhage (ICH) [10]. Given that BYHWD can inhibit ferroptosis and other pathways, it is hoped that neuronal ferroptosis will be reduced after intracranial hemorrhage (ICH). Chinese scholars have carried out research and found that BYHWD can reduce the levels of free iron and malondialdehyde in damaged brain tissue, increase glutathione, inhibit lipid peroxidation, and disrupt the ferroptosis pathway of hemorrhagic brain injury. Quercetin, Kaempferol, and Luteolin are the three main components of BYHWD, and they reduce hemorrhagic brain injury by downregulating the expression of some ferroptosis-related genes. Previous Chinese experimental studies have shown that BYHWD increases the expression of the GPX4 protein in brain lesions, and thus it can be a type of endogenous defense mechanism against neuronal ferroptosis after cerebral hemorrhage. Astragaloside IV can improve cerebral microcirculation disorders and neuroinflammation in the damaged brain after ICH. Some research papers in CNKI have also found that BYHWD can regulate the NCOA4/FTH1 iron metabolism axis to reduce excessive iron accumulation in cells and thereby alleviate ferroptosis-induced injury to the brain.

The screened hub genes are involved in the process of post-ICH ferroptosis, oxidative stress, and neuroinflammation, and together they increase the degree of neuronal damage and brain injury after hemorrhage. AKT1 is a part of the PI3K-AKT pathway that regulates iron homeostasis in cells and suppresses ferroptosis, so it is needed for the survival of neurons under oxidative stress. TP53 reduces the expression of SLC7A11 to decrease the antioxidant capacity of neurons and thus prevent ferroptosis; it is one of the first factors in neuronal injury after intracranial hemorrhage (ICH). VEGFA can increase cerebral blood flow and reduce oxidative damage to neurons, but the pro-inflammatory cytokines IL-6 and TNF will also promote intracellular lipid peroxidation and induce ferroptosis; thus, there will be a vicious circle of neuroinflammation and iron-dependent cell death. Based on the above results, BYHWD activates the PI3K-AKT pathway, increases the expression of GPX4 and SLC7A11, reduces lipid peroxidation caused by ACSL4, and thus prevents neuronal ferroptosis. BYHWD can reduce neuroinflammation to some extent by blocking the TNF-related signaling pathway, and it has also been shown that TCM compound prescriptions have many protective effects on the brain. BYHWD has many targets and can thus address all the problems in the pathological microenvironment of hemorrhagic brain injury that are difficult to solve with single-target synthetic drugs.

Based on the docking results, the main area of binding for BYHWD with ferroptosis-related marker proteins is strong, and this is in line with the predictions of bioinformatics. BYHWD reduces the expression of some genes and pathways that cause neuronal ferroptosis. BYHWD can address the three problems of

iron metabolism disorders: oxidative stress damage and neuroinflammation, so it does not need to be used alone. Recently, some studies have found that the combination of several active ingredients in traditional Chinese medicine formulas can improve the treatment effect of all kinds of cerebrovascular diseases. Rational selection of herbal compounds can affect many links in the pathological network of post-ischemic-reperfusion brain injury to different extents, and this is not easily achieved by a single chemical molecule. A new ferroptosis-related molecular mechanism for BYHWD in hemorrhagic stroke is proposed in this paper, and further theoretical support will be provided for modernizing the treatment of stroke with traditional Chinese medicine; it should be noted that previous studies have only examined the anti-inflammatory and antioxidant effects of BYHWD.

5. Limitations and outlook

There are some deficiencies in the present bioinformatics research. All the above results have been obtained from mining public databases and molecular simulations, but they have not been verified in vitro or in vivo. The combined effect (both synergistic and antagonistic) of the different BYHWD ingredients at various doses and at different times has not been studied. In the future, standardized ICH animal models and neuronal ferroptosis cell models will be developed to detect the expression of core target genes and proteins, and the exact regulatory mechanism of BYHWD on ferroptosis signaling will be further investigated; thus, strong experimental evidence will be obtained for its clinical application.

6. Conclusion

Systematic Exploration of the Molecular Mechanism of BYHWD in Treating Post-ICH SBI by Network Pharmacology and Molecular Docking. Based on the above data, BYHWD can inhibit neuronal ferroptosis and treat multiple neurological diseases through multiple pathways: it downregulates ferroptosis pathway genes to reduce neuronal iron accumulation and lipid peroxidation, thereby reducing inflammation and oxidative damage post-cerebral hemorrhage. This study addresses gaps in previous research, provides a new basis for BYHWD's modern pharmacological application in stroke treatment, and supports clinical optimization and further basic research. It also clarifies the material basis and core molecular mechanism of BYHWD against post-ICH ferroptosis from the perspective of traditional Chinese medicine, and explains the good therapeutic effect of traditional Chinese medicine compound formulas on neurological diseases. This study proposes new ideas for the innovative application of traditional Chinese medicine prescriptions for hemorrhagic stroke, and provides theoretical support for the development of targeted traditional Chinese medicine preparations and precise clinical medication for ICH rehabilitation.

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Disclosure statement

The authors declare no conflict of interest.

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