Study on gene network mechanism of Qufeng Tongqiao Prescription in treating cerebral ischemia

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Research Article

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Abstract

Objective: To study the network mechanism of qufeng Tongqiao Prescription in the treatment of cerebral ischemia-reperfusion related genes through network screening and molecular docking verification.

Methods: Venny diagram, GO and KEGG analysis, PPI protein interaction, Hub gene screening, molecular verification and animal behavior experiments were performed to conduct inflammation through network pharmacology.

Results: There were 53 intersecting genes of Qufengtongqiao Recipe and cerebral ischemia reperfusion. GO analysis showed that the main BP was response to lipopolysaccharide, and the main CC process was membrane raft. Cytokine receptor binding is the most important MF process.Age-rage signaling pathway in diabetic complications is the most important signaling pathway in KEGG pathway.Through molecular docking, it was found that Astragalus membranaceus was docked with MAPK14, IL4, FOS, IL6 and JUN, pueraria membranaceus was directly docked with JUN and IL4, Acorus acorus was linked to JUN and MAPK14, Ganoderma ganoderma and human were involved in JUN docking, and Ligusticum chuanqi and pueraria could not be docked with MAPK14 respectively.

Conclusion: Qufeng Tongqiao prescription has therapeutic effect on cerebral ischemia reperfusion.

Introduction

Stroke is a major public health problem in the world and leads to neurological deficits. Ischemic stroke remains the leading causes of death or long-term disability, accounting for 87% of all strokes (Lee, Chen et al. 2009). Cerebral ischemic injury is one of the major causes of death and disability worldwide (Tu, Wang et al. 2013).Restoring blood flow to the ischemic brain is often used to treat patients in clinical trials. However, reperfusion itself may also produce additional damage in the ischemic brains due to excessive production of reactive oxygen species (ROS).Cerebral ischemia-reperfusion is the primary pathophysiological process of stroke and can cause severe and long-lasting sequelas, resulting in a significant medical burden internationally, which results in 6.5 million deaths per year(Ren, Xie et al. 2020, Trotman-Lucas and Gibson 2021) .Among them, cerebral ischemia reperfusion (IR) injury is caused by multiple causes, such as cardiac arrest, peripheral vascular insufficiency and stroke(Qin, Zhang et al. 2020) .Underlying pathological mechanisms of ischemia-reperfusion (I/R) injury include glutamate excitatory toxicity, calcium overload, nitric oxide (NO) production, oxidative stress, inflammation, and apoptosis, ultimately leading to cell death.Oxidative stress and apoptosis after BRAIN I/R are the two major processes that induce neuronal injury(Mattson, Duan et al. 2001, Zhao 2004) .Early reperfusion of ischemic brain tissue can lead to a variety of negative consequences, including disruption of the blood-brain barrier, which may lead to cerebral edema and/or cerebral hemorrhage, neurovascular injury and neuronal death(Shao, Bao et al. 2018) .In addition, inflammation is caused by reperfusion injury and may lead to subsequent oxidative damage, excitatory toxicity and neuronal cell death, negatively affecting long-term disease prognosis(McColl, Allan et al. 2009, Chamorro, Dirnagl et al. 2016).Cerebral ischemia/reperfusion is a complex multifactorial process, and its mechanism remains still unclear, which requires researchers to further explore and clarify its mechanism.

For more than 2,000 years, Traditional Chinese Medicine (TCM) has been used in many Asian countries to treat cerebrovascular diseases, including ischemic stroke.In China, compound TCM preparations have a long history in the treatment of cerebrovascular diseases(Sun, Fan et al. 2015) .Due to the systematic and holistic characteristics of TCM, the holistic view of TCM is consistent with the multi-component, multi-target and multi-pathway mechanism of TCM formula. Meanwhile, TCM has gained many advantages due to its multiple drug targets and few side effects(Yang, Chen et al. 2021) .Which functions: party, say again: puzzle particles (made from China shenzhen CRC san-jiu medicine trade co., LTD), is a compound Chinese medicine preparation, the main ingredients of radix astragali, radix puerariae, ganoderma lucidum, stone calamus, ginseng, musk, leech, earthworm and rhizoma ligustici wallichii. It is the efficacy of yiqi t2dm, eliminating phlegm to begin to understand, attending to each knot, phlegm and blood stasis Dizziness, unstable walking,

and cognitive impairment (memory loss, slow reaction) due to qi deficiency and decreased spinal cord.Among them, Astragalus membranaceus has the effects of supplementing qi and raising Yang, benefiting wei and solid surface, supporting toxin to nourish muscle, benefiting water and reducing swelling, etc. and are used for the treatment of qi deficiency and weakness, middle qi depression; Ginseng has the effect of replenishing vitality, solidifying and removing fluid, and calming the nerves. It is mainly used for treating serious illness, chronic illness, blood loss and dehydrating; Ganoderma lucidum has the effect of nourishing the heart and calming the spirit, nourishing the lung and yiqi, regulating qi and removing stasis, nourishing the liver and strengthening the spleen, and treating deficiency of fatigue and weak body; Pueraria has the effect of releasing muscles, rising Yang through rash, antipyretic and shengjin, treating external fever, head pain and other diseases; Stone calamus has expectorant kaiqiao, tranquilizing and other effects, treating spittoon after stroke, delirium; Ligusticum chuanxiong has the function of promoting blood circulation, and qi, dispelling wind and relieving pain, and is used for the treatment of headache and rheumatism. Musk, used for kaiqiao awakening, huoxue menstruation, has the effect of kaiqiao awakening, huoxue Sanjie, analgesic detumescence; Leech, common name leech and China's traditional special medicinal aquatic animal, dried products after processing into traditional Chinese medicine, with broken blood and blood stasis; Dispelling symptoms and other effects, is for the treatment of stroke and other diseases; Digilong, with the effect of channelling channels and activating collaterals, is often used to treat qi deficiency, blood stasis, unfavorable channels and collaterals, hemiplegia and aphasia. However, the mechanism of TCM therapy on cerebral ischemia-reperfusion is still unclear and further study by researchers is in demanded.

Network pharmacology is a network that has the ability to study the "complex protein/gene disease" pathway and to elucidate the complexity of biological systems, drugs and diseases from a systemic perspective(Hopkins 2007, Li 2016) .At the same time, network pharmacology is growing into a prospective strategy combining systematic approaches that explore the intermodulation of multiple information pathways, and contribute to the understanding of drug multipharmacology, improving drug efficacy and clinical trial success rate(Zhang, Jiang et al. 2019) .In conjunction with network pharmacology, TCM has adopted multi-compound, multi-target and multi-path approaches, especially in the studies related to the treatment of cerebral ischemia-reperfusion (Kibble, Saarinen et al. 2015, Poornima, Kumar et al. 2016, He, Liu et al. 2021), Network pharmacology and molecular docking techniques are used to determine the effective material basis and molecular mechanism of qufengtongqiao prescription.(Kibble, Saarinen et al. 2015, Poornima, Kumar et al. 2016, He, Liu et al. 2021)

1 material method

Materials And Methods

1.1 Collection and conversion of active ingredients and component targets of Qufeng Tongqiao Recipe

Traditional Chinese Medicine Systems Pharmacology: TCMSP) (https://tcmspw.com/index.php) website, in herb column name, the input functions: pharmaceutical ingredients, click its Latin name, Make a copy of the relevant data for the details and Targets Information. All the chemical components of Qufengtongqiao prescription were searched with the key words "Astragalus membranaceus", "Geroot", "Ganoderma lucidum", "gladiolus gramineus", "ginseng", "musk", "leech", "earthworm" and "Chuanxiong". According to the screening conditions of Oral bioavail ability (OB)≥ 30% and drug-likeness (DL)≥0.18 in the absorption, distribution, metabolism and excretion (ADME) parameters, the active ingredients of Qufengtongqiao prescription are screened out. At the same time, the active ingredients in TCMSP were searched for corresponding targets.

1.2 Target protein of qufengtongqiao prescription was transformed into gene name

Enter “https://www.uniprot.org/” in the browser, open the UniProt database, enter the full names of Gene targets obtained by TCMSP database in the search box one by one, search for their Gene names, and copy them to Excel.

1.3 Acquisition of cerebral ischemia-reperfusion disease targets

In the GeneCards database (https://www. Genecards.org), the keyword "Cerebral ischemia reperfusion" was searched, and the targets related to Cerebral ischemia reperfusion disease were retrieved and downloaded into the table. The larger the Relevance score was, the closer the Relevance of the gene target to cerebral ischemia reperfusion was in Genecards. The order was made according to the score value and saved in the Excel table.

1.4 Network construction and analysis of "active ingredients and key targets"

Using a browser search (https://bioinfogp.cnb.csic.es/tools/venny/) Venny2.1 database, input in list1 search to the functions of: side of the active ingredient in the score value is greater than 10 related targets for gene abbreviation, Enter target targets with score value greater than 10 in cerebral ischemia-reperfusion disease into list2, and the site will automatically generate the intersection of gene targets of the active ingredients of Qufeng Tongqiao prescription and related gene targets of cerebral ischemia-reperfusion. Click the intersection to obtain the key targets of the active ingredients of Qufeng Tongqiao Prescription in the treatment of cerebral ischemia-reperfusion. Name List1 and List2 as short names for drug and disease names, change the image to color, and download.

1.5 Construction and analysis of key target protein interaction (PPI) network

Open the String database (https://string-db.org/) with a browser, and enter the key gene targets for the treatment of cerebral ischemia-reperfusion of the effective components Of dispelling wind and Tonoqiao formula obtained from Venny2.1 into the List of Names in the String database. Select Homo Sapiens from the "Organism" box, search for the protein interaction map, export and download the map.

1.6 GO analysis and KEGG pathway analysis

All genes found in literatures were analyzed by Gene Ontology (GO) and KEGG.Gene Ontology (GO) analysis is an international standardized Gene function classification database established by the Gene Ontology Consortium, which defines and describes genes in biological process (BP), cell localization (CC) and molecular function (MF). to comprehensively describe the properties of genes and gene products in living organisms.KEGG is the most widely used Pathway database for systematic analysis of gene function, linked genomic information and functional information.In this study, all target genes were mapped to the GO item of the gene database (http://www.geneontology), and the number of genes for each term was calculated.Path-based analysis is used to characterize the biological function of the target.Pathway enrichment analysis found signal transduction pathways in gene targets in KEGG database (http://www. genome.jp/kegg/).In this study, R language was used for GO analysis and KEGG pathway analysis.The R software version used in this study is 3.6.0 (http:// www.r-project.org), You need to install RSQLite, clusterProfiler, org.hs. eg, DOSE, enrichPlot, GGploT2, Colorspace, Stringi, PathView and other R packages. Copy the scripts analyzed by GO, KEGG and KEGG paths, paste and analyze them in the R software window.Signaling Pathway map, Barplot and Bubble Diagram were plotted by using DB, Enrichment plot and GGploT2 in GO and KEGG enrichment analyses.

1.7 Molecular Docking

The functions of the various components of the effective: the material input PubChem database (https://pubchem.ncbi.nlm.nih.gov) to get the 2 d structure, the key protein import PDB database (https://www.rcsb.org), and Choose the right protein structure.The target protein structure was dehydrated with PyMol software and ligand and receptor were separated.With the target protein as the receptor and the active substance as the ligand, the active site of molecular docking was determined according to the coordinate of the ligand in the target protein complex. Gridbox coordinates and size were set according to the active pocket of the target protein. AutoDock Vina was used for molecular

docking, and the binding conformation with the lowest free binding energy was selected. PyMol software was used for visual processing.

1.8 "disease-drug-active ingredient-key target-KeGG pathway" network construction and analysis

An Excel table of disease-drug-active components-key target-KEgg pathway was established, and drugs, drug components, intersection targets of cerebral ischemia-reperfusion therapy, KEGG pathway and corresponding gene targets of pathway were input into the table in sequence, and the table was imported into Cytoscape3.9.1 software. Through a series of adjustments, The network analysis diagram of PPI protein interaction analysis was drawn and downloaded. Meanwhile, Hub genes were screened in terms of Degree value, and the histogram of the top five Hub genes was drawn according to Degree value.

1.9 Model of qufeng Tongqiao prescription for cerebral ischemia reperfusion in rats

1.9.1 Grouping and administration

Sham group: 8 rats

NS group: 6 rats

QFH group: 6

1.9.2 SD rats provided by the Department of Experimental Animals, Kunming Medical University with the production license no. : SCXK (Yunnan) K2020-0004 were fasted for 8-10 h, The animal ethics code is KMMU20220854,and then anesthetized with 3% sodium pentobarbital.The right common carotid artery, external carotid artery and internal carotid artery of rats were separated and re-exposed.An incision was made in the external carotid artery and the thread plug was inserted into the common carotid artery through the incision.The external carotid artery was dissociated, and then the thread plug was inserted into the internal carotid artery. When the insertion depth was 18 mm, the thin wire at the distal end of CCA was tightly fastened. Finally, the wound should be closed and observation of single cage feeding should be made. After an hour, pull the tether out.Two milliliters of qufeng tongqiao prescription were taken every day for treatment.

1.9.3 Animal behavior Experiment

According to THE NSS standard, three people scored the neurological severity score (mNSS) of the rats at 1, 5 and 10 days respectively. Finally, the data of the three people were summarized for statistical analysis.

Results

2.1 A total of 1493 genes for cerebral ischemia-reperfusion were found in Genecards, and some genes were shown in Table 1:

Table1 partial cerebral ischemia-reperfusion gene targets

Cerebral ischemia reperfusion partial genes							
APP	LPL	TGFBR2	PLAU	PRKCA	CNTF	IRF5	MMP10
KRIT1	EDNRA	TSPO	NES	TGFA	MAOB	SLC11A2	CCL20
CST3	EDNRB	HTRA2	SLC12A2	MT3	GCKR	IRAK4	ITGA6
F2	SMAD4	SLC9A1	SMAD2	ITGAL	NRG1	MIR544A	KCNK2
IL6	RELA	GPT	EGFR	TNFRSF12A	CSF1	NPR1	RYK
TNF	TGFBR1	SLC6A4	LEP	OPRK1	LIF	PPARD	CNRIP1
NOS3	ADA	CSF1R	NOTCH1	PRKAA1	CLDN5	ABCG1	CHRDL1
COL4A1	MIR155	PF4	BAD	AOC1	UCN	NMT1	WNT9A
ACE	ACTB	PTGS1	STAT1	GCLM	ADAMTSL1	MIR191	CARTPT
F5	DRD2	PRKCE	IL2	MIR211	IRF1	CAMK2N1	SERPING1
NOS2	TIMP3	PRKAA2	BCL2L1	DCN	NEFL	S100A6	KCNA5
ENG	MIR145	TOMM40	ESR1	RTN4	HSP90AA1	MAP2K7	MIR675
SOD1	PIK3CG	MIR34A	IKBKG	BCL2L11	CCK	MIR193A	MIR134
MTHFR	CDKN2A	PDP1	SMPD1	G6PC1	PRKCD	CYP2C9	TSPAN18
ICAM1	PPARA	REN	GATA4	S100A9	SLC1A3	TGM1	SLIT2
PIK3CA	NLRP3	PTK2B	IGF1R	NTS	IL5	APOO	PTP4A2
MPO	CDON	HP	HYOU1	CAST	C1S	AASS	CFP
GAD1	AGER	SELL	VIP	KIT	SLC22A3	PPP1CA	STIP1
TP53	FN1	ADORA3	LRP2	GUSB	MIR221	ERBB4	TIPARP
VEGFA	MYH7	TNFRSF1A	TRAF6	S100A8	CCND1	PPIG	MIR342
PTEN	CD40	IL17A	GCG	GPX3	CHGA	JUP	PRDX2
IL10	CNR1	SYNGAP1	MIR142	BRCA1	MMP14	ROCK2	CREB3L1
MEF2C	TTN	MME	FASLG	GSTM1	GRM5	E2F1	ACSS2
CASP3	STAT3	NGB	CTSG	SLC24A3	IDO1	FNDC3B	ESM1
PLAT	TNNI3	HTR2A	SULT1A3	LEPR	CHKA	CCL26	ROBO1
SMARCA4	MIF	THBS1	NT5E	C1R	GDF15	LBP	PIM1
MAPT	TNNT2	TGIF1	CAV1	PER2	TRPM7	AK1	HSPA1L
GFAP	PCNA	GDNF	GABBR2	PDE4A	ADK	S1PR2	TRPA1
EDN1	MBP	LAMB1	PLSCR1	CUL3	GJB1	COL6A1	CHEK1
PSEN1	NFKB1	HSPG2	OGG1	SIRT3	UCP2	ARG2	SLC3A2
SERPINC1	XIAP	MT-CO1	A2M	CYSLTR1	RETN	ZFYVE9	MYH10
TLR4	IL13	RPS27A	RHOB	ABCB7	YAP1	MIR130B	NCK1
HIF1A	KCNMA1	ADIPOQ	SLC29A1	ANO1	TNC	ADAMTS20	AOX1

PRNP	ABCA1	IL1A	PTGIR	MIR199A1	PNPO	NPR3	GBA3
CTNNB1	ITGB2	NCF1	GCLC	UGCG	IL1R1	MAPKAPK2	BIRC2
IL1B	PLA2G7	CDKN3	LOC111365141	SLC22A2	BMP7	ZFAS1	MEF2D
COL3A1	MAPK8IP1	SH2B3	APLNR	CXCL11	YY1	CYP2C8	SLC6A19
CRP	ITGB1	GAPDH	CIITA	RPS6KB1	CCN2	CPT1A	MCM6
COL4A2	CAMK2A	VCP	FGF7	AGRN	HABP2	HTR2B	PTHLH
BCL2	TERT	AOC3	SLC22A8	STK11	CASP7	DLD	THY1
BDNF	BMP6	SST	P2RY1	MMP13	MIR107	SMPD2	SLC25A3
XDH	DNM1L	GSS	CGB5	CAPN2	LGALS3	CA9	EIF4EBP1
NOS1	FABP3	FGFR2	CYGB	AIM2	APEX1	PRKAB1	SLC22A4
CCL2	F8	SDHB	SERPINA4	MAP2K4	ACE2	TBXAS1	STMN1
CBS	IGFBP3	COX5A	MIR143	TAT	MIAT	PPIA	ATP2A1
SLC1A2	IL12A	ARID1B	CD55	UNC5B	IL15	MCAM	ATG9A
HMOX1	CCL3	IL2RB	MIR186	FOXO1	MIR140	HSF1	SLC27A4
MMP9	CCL5	VIPR1	TRPV1	NDUFA13	BDKRB2	TNFRSF10B	EGR3
SELE	HSPB6	FKBP1A	KLK1	MIR24-1	NAXE	TXN2	ENTPD2
EPO	AIF1	WT1	SLC16A1	IL22	RIPK1	STAT5A	UCP1

2.2 A total of 194 genes related to the components of qufengtongqiao formula were found in Genecars.Some genes are shown in Table 2:

Table2 Gene targets of effective components of Qufeng Tongqiao Prescription

Gene targets of effective components of Qufeng Tongqiao Prescription						
PGR	MMP2	MET	COL3A1	F7	HSD3B1	AHR
NOS2	MMP9	PKIA	CXCL11	ACHE	CHRNA2	PSMD3
PTGS1	MAPK1	JUN	CXCL2	MAOB	PRKCB	SLC2A4
AR	IL10	IL4	DCAF5	RELA	BIRC5	NR1I3
SCN5A	EGF	ATP5F1B	CHEK2	NCF1	DUOX2	INSR
PTGS2	RB1	ND6	CLDN4	OLR1	NOS3	DIO1
ESR2	IL6	HSD3B2	PPARA	ADRB1	HSPB1	PPP3CA
CHEK1	TP63	IKBKB	PPARD	HTR3A	SULT1E1	GSTM1
PRSS1	ELK1	AKT1	HSF1	ADRA2C	MGAM	GSTM2
NCOA2	NFKBIA	BCL2	CRP	ADRB2	IL2	AKR1C3
CHRM3	POR	BAX	CXCL10	ADRA1D	CCNB1	SLPI
CHRM1	ODC1	TNFSF15	CHUK	OPRM1	PLAT	MMP3
CHRM2	CASP8	AHSA1	SPP1	KCNH2	THBD	EGFR
ADRA1B	TOP1	CASP3	RUNX2	CHRM5	SERPINE1	VEGFA
GABRA1	RAF1	MAPK8	RASSF1	CHRM4	COL1A1	CCND1
GRIA2	SOD1	MMP1	E2F1	OPRD1	IFNG	BCL2L1
ADH1B	PRKCA	STAT1	E2F2	ADRA1A	IL1A	FOS
ADH1C	HIF1A	HMOX1	ACPP	SLC6A3	MPO	CDKN1A
LYZ	RUNX1T1	CYP3A4	CTSD	SLC6A4	TOP2A	EIF6
RXRA	ERBB2	CYP1A2	IGFBP3	RXRB	ABCG2	CASP9
SLC6A2	ACACA	CYP1A1	IGF2	KDR	NFE2L2	PLAU
ESR1	CAV1	ICAM1	CD40LG	MAP2	NQO1	ADCYAP1
PPARG	MYC	SELE	IRF1	NR3C2	PARP1	RXRG
MAPK14	F3	VCAM1	ERBB3	ADRA2A	PSMG1	CASP1
GSK3B	GJA1	NR1I2	PON1	LTA4H	MAP2K4	HK2
CCNA2	IL1B	CYP1B1	PCOLCE	MAOA	NR3C1	RASA1
PYGM	CCL2	ALOX5	NPEPPS	CTRB1	CACNA1S	GSTP1
AKR1B1	PTGER3	HAS2	NCOA1	CXCL8		

Figure 2 Gene targets of Qufengtongqiao Prescription

2.3Venny diagram The crossover genes between cerebral ischemia reperfusion and qufeng Tongqiao formula were analyzed, and a total of 53 key targets of the intersection genes of Venny diagram were obtained, as shown in Figure 1:

The left circle is the target of qufengtongqiao prescription, the right one is the target of cerebral ischemia reperfusion, and the intersection between them in the middle is the key target of treatment.

Table 3 Intersection genes of cerebral ischemia-reperfusion qufengtongqiao prescription

Intersection gene of cerebral ischemia-reperfusion - Qufengtongqiao prescription						
NOS2	ALOX5	HSPB1	CASP3	F3	CRP	IL6
PTGS2	VEGFA	PLAT	MAPK8	GJA1	CTSD	ODC1
PPARG	FOS	THBD	HMOX1	IL1B	CD40LG	SOD1
MAPK14	CASP9	SERPINE1	ICAM1	CCL2	PON1	HIF1A
ACHE	MMP2	IFNG	SELE	CXCL8	MAP2	JUN
ADRB2	MMP9	MPO	VCAM1	NOS3	CASP1	IL4
SLC6A3	MAPK1	NFE2L2	IL10	PARP1	COL3A1	AKT1
KDR	BAX	BCL2	EGF			

2.4. GO analysis

In order to clarify the mechanism of qufeng Tongqiao prescription in the treatment of cerebral ischemia reperfusion, go enrichment analysis was performed.The top 10 biological processes (BPS) involved in go enrichment analysis are as follows: Response to lipopolysaccharide, and molecule of bacterial origin, cellular response to chemical stress, cellular Response to oxidative stress,-oxidative stress, and reactive oxygen species, reactive oxygen species Metabolic process, response to metal ion, cellular response to lipopolysaccharide, regulation of apoptotic signaling Pathway.

The top 10 factors involved in cell component (CC) in go enrichment analysis were: Raft, membrane microdomain, membrane region, Caveola, Plasma membrane raft, secretory granule lumen, cytoplasmic Vesicle Lumen, Vesicle Lumen, Neuron projection cytoplasm, Collagen-containing extracellular matrix.

The top 10 factors involved in molecular function (MF) in go enrichment analysis were as follows: Cytokine receptor binding, cytokine activity, DNA-binding transcription factor binding, RNA polymerase II-specific DNA-binding transcription factor binding, Receptor ligand activity, Signaling receptor Activator activity, Phosphatase Binding, Protease Binding, Cysteine-type Endopeptidase activity involved in apoptotic signaling pathway, growth factor receptor binding.

The main BP was response to lipopolysaccharide, the main CC process was membrane raft, and cytokine receptor binding was the main MF process.

2.5 KEGG pathway analysis

In order to clarify the mechanism of qufeng Tongqiao prescription in the treatment of cerebral ischemia reperfusion, we performed KEGG pathway analysis. The results suggested that the first ten pathways in KEGG pathway analysis were age-rage signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis Pathway and Atherosclerosis, TNF signaling Pathway, IL-17 signaling Pathway, Chagas disease, Pertussis, Malaria, Kaposi Sarcoma-associated Herpesvirus infection and HIF-1 signaling Pathway.

Table4 the top ten KEGG pathways

ID	Description	pvalue	p.adjust	qvalue	Count
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5.75 e-30	1.18 e-27	3.39 e-28	22
hsa05418	Fluid shear stress and atherosclerosis	7.59 e-25	7.81 e-23	2.24 e-23	21
hsa05417	Lipid and atherosclerosis	9.54 e-24	6.55 e-22	1.88 e-22	23
hsa04668	TNF signaling pathway	7.23 e-17	3.72 e-15	1.07 e-15	15
hsa04657	IL-17 signaling pathway	1.92 e-16	7.92 e-15	2.27 e-15	14
hsa05142	Chagas disease	6.34 e-16	2.18 e-14	6.23 e-15	14
hsa05133	Pertussis	1.69 e-14	4.97 e-13	1.42 e-13	12
hsa05144	Malaria	2.60 e-13	6.69 e-12	1.91 e-12	10
hsa05167	Kaposi sarcoma-associated herpesvirus infection	2.99 e-13	6.85 e-12	1.96 e-12	15
hsa04066	HIF-1 signaling pathway	1.48 e-12	3.04 e-11	8.70 e-12	12

2.6 PPI protein interaction and analysis

2.6.1 Open string(https://string-db.org/)After UniProt Transformation) and paste the intersection genes in List of Names. Choose HOMO sapiens to map the network of interactions between a molecule and a protein.AKT1 and MAPK14 can be known from the table.AKT1, HSPB1;AKT1, SOD1;AKT1, NOS3;AKT1, CASP3;AKT1, NOS2;AKT1, CASP9;AKT1, CD40LG;AKT1, JUN;AKT1 and BCL2 were the ten most important gene targets.

2.6.2 Screening Hub genes

The PPI interaction table was imported into Cytoscape to construct the network diagram. Sequencing was conducted according to the top 5 genes in Degree value, and the size of the circle was proportional to the that of Degree value. As shown in Figure 4B, the histogram of Degree value is made according to size, likewise in Figure 4C. The sequence of genes from large to small is: MAPK14, FOS, IL6, IL4 and JUN.

Table5: Relationship between cerebral ischemia reperfusion and effective components of Qufeng Tongqiao Prescription

Relationship between cerebral ischemia reperfusion and effective components of Qufeng Tongqiao Prescription						
node1	node2	coexpression	experimentally_ determined_ interaction	database_ annotated	automated_ textmining	combined_ score
AKT1	MAPK14	0.083	0.83	0.6	0.769	0.956
AKT1	HSPB1	0	0.76	0	0.982	0.995
AKT1	SOD1	0	0.09	0.9	0.569	0.957
AKT1	NOS3	0.049	0.879	0.9	0.988	0.999
AKT1	CASP3	0	0.475	0	0.882	0.935
AKT1	NOS2	0.063	0.077	0.9	0.646	0.965
AKT1	CASP9	0	0.231	0.9	0.814	0.984
AKT1	CD40LG	0	0.056	0.9	0.493	0.947
AKT1	JUN	0	0.057	0.8	0.877	0.974
AKT1	BCL2	0.062	0	0.9	0.457	0.944

2.7 Molecular docking results

Five HUB genes screened from Cytoscape were docked with each active ingredient in qufengtongqiao prescription. The structure of HUB gene was found in PDB database and downloaded. A series of operations were performed to docked hub gene protein with the active ingredient, and finally the ligand and the overall picture were derived.As shown in FIG. 5 and 6, FIG. 5A: IL6-huangqi, FIG. 5B:IL4-huangqi, FIG. 5C: FOs-huangqi, FIG. 5D: MapK14-huangqi, FIG. 5E: Jun-huangqi, FIG. 5F: Jun-gegen, FIG. 5G: Jun-shichangpu, Figure 6H: Jun-lingzhi;Figure 6I: Jun-Renshen, Figure 6J: IL4-Gegen, Figure 6K: MapK14-Shichangpu, Figure 6L: MapK14-Gegen, Figure 6M: mapK14-Chuanxiong.Figure 6L: MAPK14-Gegen, Figure 6M: MAPK14-Chuanxiong Target proteins in cannot docking with drug ligands.

2.8 Cytoscape mapping

The active components of qufengtongqiao prescription, the intersection genes of drugs and diseases, the table of genes in the first ten KEGG pathways related to them and key targets were imported into Cytoscape software, and the disease-drug active component-key target-KeGG pathway map was drawn through a series of operations.FIG. 7 shows that: Based on the screening of drug component targets, network interaction between related targets was found.

On the left is cerebral ischemia-reperfusion disease,the middle one is mol number of Hirulong Huoxue Tongyu Capsule and 56 active ingredients, and the outer blue circle is common gene of cerebral ischemia-reperfusion and Hirulong Huoxue Tongyu Capsule.The first 10 KEGG pathways are on the right.The relationship diagram of cerebral ischemia-reperfusion - Hirulong Huoxue Tongyu capsule -KEGG pathway network was constructed, which was connected by the close relationship in the diagram. The regulation effect of Hirulong Huoxue Tongyu capsule on cerebral ischemia-reperfusion was expounded.

2.9 Behavioral verification

MNSS neurobehavioral evaluation was performed on the rats of SHAM, NS and QFH groups that had been successfully modeled at day 1, 5 and 10, and SPSS analysis was performed on the data. The main clause was first analyzed into one-way anVARIANCE, and the results were statistically mapped and tabulated. It was found that: On the first day, the

neurological function of the SHAM group was statistically significant compared with that of the NS group, P <0.001;On the fifth day, the comparison between SHAM group and NS group, NS group and QFH group was statistically significant (P <0.001), p=0.029 in the latter group;On day 10, comparisons between SHAM group and NS group, NS group and QFH group were statistically significant, p<0.001 for the former group and P =0.015 for the latter group. Then, repeated measurement an OVA was used to conduct sphericity test for the data again, and it was found that P =0.048, which could not be analyzed by assuming sphericity. Instead, Greenhouse-Geisler was used for analysis, and it was found that time and Time *group were statistically significant, with p<0.001 and P =0.009, respectively.

Table6 behavioral score

Variables	DF	SS	MF	F	P
Intergroup error	2	342.1186	171.0593	98.66594	< 0.001
Time	17	29.47327	1.733722		
Time*Intervence	2	70.44492	35.22246	92.66389	< 0.001
Repeated measurement	4	7.151034	1.787758	4.703268	0.004
error	34	12.92374	0.38011		

Note: SS is the sum of squares, MF is the mean square, DF is the degree of freedom, P is the significance p-value, SS is the sum of squares.

Discussion

Network pharmacology is a new method for systematic analysis of drugs from the aspects of biology, pharmacology, network analysis and computer technology, and combines gene targets of drug active ingredients and disease targets from many aspects. To study whether drugs have therapeutic or other effects on diseases(Ning, Zhao et al. 2017, Yuan, Ma et al. 2017) .Recent studies have found that the same effect can be achieved with a lower effective dose than that of a single chemical ingredient because of the rich chemical components contained in a single Chinese medicine.Therefore, Traditional Chinese medicine is characterized by low dose, multiple targets, extremely low drug resistance and few side effects. Hence, Traditional Chinese medicine has unique advantages in treating diseases regulated by multiple genes or requiring long-term drug use. "multi-gene targeted drugs" based on molecular network pharmacology are emerging as a new direction for TCM treatment of disease(Hopkins 2008) .In recent years, the main active ingredients in Traditional Chinese medicine and their therapeutic effects under cell network have been studied through TCM network pharmacology, which can confirm or support the dialectical and comprehensive coordination view of TCM.We believe that elucidating the mechanism of TCM at the level of molecular network regulation will provide useful inspiration and evidence for us to discover multi-targeted drugs.It may also reverse the development of modern multicomponent new drugs from clinically effective TCM and the modernization of TCM will also play a positive role in the promotion of these drugs(Long, Yuan et al. 2019) .Network pharmacology of TCM has a broader development prospect and provides a more comprehensive and systematic theoretical guidance for compound drugs.

In GO analysis, the main BP was response to lipopolysaccharide, the main CC process was membrane raft, and cytokine receptor binding was the main MF process.The response to lipopolysaccharide has been proven to induce fever in many experimental animals (e.g., rats, guinea pigs, rabbits), and the typical inflammatory response in humans, and the nervous system inflammation will be induced from the acute pro-inflammatory stage to the anti-inflammatory stage. Then to complete regression, LPS can stimulate and increase the level of Fos nuclear protein, so that the inflammatory response induced by LPS can be alleviated by knocking down or lowering the level of Fos in vivo, and LPS can induce the activation of microglia. Microglia have anti-inflammatory effects(Calvano, Xiao et al. 2005, Li, Zhang et al. 2018) .Raft is a

heterogeneous and dynamic domain in which lipids are tightly packed and rich in cholesterol, sphinolipids and other proteins such as various cell signaling proteins.Therefore, membrane rafts play an important role in cellular signaling pathways. At the same time, the raft domain shows lower mobility than other domains due to its rich cholesterol, which may stimulate signaling pathways(Li, Zhang et al. 2018) .Sphingomyelin synthase (SMS) is the rate-limiting enzyme of sphingomyelin, and sphingomyelin is an indispensable component of lipid rafts.SMS2 is highly expressed in brain cells and is involved in the formation of sphingomyelin in the central nervous system.When SMS2 is deficient in organisms, sphingomyelin content in the plasma membrane is reduced, which may disrupt the formation of functional membrane rafts, that play an important role in cellular signaling pathways.More importantly, the lack of SMS2 leads to a decrease in the abundance of TLR4 / MD2 complexes on the surface of macrophages stimulated by LPS, thus playing an anti-inflammatory role(Xue, Yu et al. 2019) .Cytokines in the cytokine receptor binding are small secreted peptides that regulate and guide immune responses. After cerebral ischemia and reperfusion, a large number of immune cells will gather at the site of injury. At the same time, the accumulated immune cells in the microvessels will be activated and a large number of inflammatory mediators and inflammatory cytokines will be released outwards, which will cause the accumulation of immunogranulocytes, resulting in excessive local inflammatory response. Cytokines need to bind to corresponding receptors to play a regulatory role in the immune response.

The first ten pathways of KEGG were analyzed. Age-rage signaling pathway in diabetic complications include diabetes mellitus, nephropathy and vascular calcification.Diabetic nephropathy can lead to renal function damage, such as glomerular disease that leads to neuropathy, while vasosclerosis can induce signaling pathways, which leads to chronic inflammation. Beyond that, mitochondrial dysfunction can induce apoptosis, which leads to oxidative stress response.Age-rage signaling Pathway changes the immune response of the body, producing pro-inflammatory cytokines, reactive oxygen species and active nitrogen intermediates, thus inducing inflammation and immune suppression, and causing vascular endothelial injury.In addition, endometrial calcification is inextricably related to lipid deposition, inflammatory cells, and atherosclerosis(Lee, Lee et al. 2020) .Atherosclerosis is a chronic inflammatory disease caused by lipids, which can easily form atherosclerotic plaques at susceptible sites. The easily formed sites are often related to the profile of the disturbed fluid shear stress(Chen, Qin et al. 2019) .Moreover, fluid shear stress with extremely lower than average and reciprocating fluidity will lead to the atherosclerotic phenotype of endothelial cells, resulting in endothelial cell proliferation and inflammation, which can readily lead to atherosclerosis(Zhou, Li et al. 2014) .The third is Lipid and atherosclerosis.The change of lipid metabolism is one of the risk factors and characteristics of atherosclerosis, of which the pathogenesis of atherosclerosis is relatively complex, mainly the accumulation of lipid in the artery wall and the occurrence of chronic inflammation.In the early stages of atherosclerosis, also known as "adipose streaks", intracellular lipids of foam cells accumulate in the vascular wall, resulting in chronic endothelial cell damage that persists, and adipose streaks deteriorate into atherosclerotic lesions(Poznyak, Grechko et al. 2020) .The fourth pathway is TNF Signaling Pathway.Tumor necrosis factor (TNF), also known as TNFα, is a tumor suppressor and pro-inflammatory mediator, and participates in systemic inflammatory response, which is one of the cytokines responsible for the acute phase response.Cerebral ischemia-reperfusion injury can be reduced by inhibiting TNF-α(Wang, Du et al. 2022).Il-17 signaling Pathway il-17 is a key mediator of inflammation. Its core genes include pro-inflammatory cytokines, chemokines, antimicrobial peptides (AMP), and inflammatory effectors, as well as moderate activators of signal transduction.Thus, the ability of IL-17 to synergistically interact with other inflammatory signals produces an extremely important inflammatory effect(Amatya, Garg et al. 2017) .Chagas disease may cause inflammation in the acute phase.Pertussis can cause intracranial bleeding when severe cough occurs. Malaria Causes headaches, hemolysis, anemia, etc.Kaposi sarcoma-associated herpesvirus infects a variety of endothelial cells, monocytes, and B cells, resulting in oxidative stress.Hif-1 signaling Pathway (HIF-1 signaling pathway) predispositions oxidative stress response and inflammation.

The protein relationship between Qufeng Tongqiao Recipe and cerebral ischemia reperfusion was analyzed, and the top ten gene pairs were AKT1 and MAPK14.AKT1, HSPB1;AKT1, SOD1;AKT1, NOS3;AKT1, CASP3;AKT1, NOS2;AKT1, CASP9;AKT1, CD40LG;AKT1, JUN;AKT1 and BCL2.It was found that AKT1 may play an important role in the treatment of

cerebral ischemia reperfusion by Qufeng Tongqiao prescription.Five Hub genes screened by Degree value in Cytoscape were MAPK14, FOS, IL6, IL4 and JUN.It may be the core regulatory gene of drug formulation and disease.

Molecular docking of five Hub genes with drug active ingredients suggested that these five Hub genes were key genes in the treatment of cerebral ischemia reperfusion. It was found that the five Hub genes were docked with Astragalus membranaceus and MAPK14, IL4, FOS, IL6, JUN, and pueraria root were directly docked with JUN and IL4. Acorus acorus was docked with JUN and MAPK14, ganoderma lucidum and ginseng were docked with JUN respectively, and Ligusticum chuanqi and pueraria root could not docked with MAPK14 respectively.

Conclusion

Qufeng Tongqiao prescription has therapeutic effect on cerebral ischemia reperfusion.

Declarations

Ethical approval and consent for participation

All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University. Approved by the Animal Experiment Ethics Review Committee of Kunming Medical University, the approval number is KMMU20220854

Human and animal ethics

No human studies are involved

The animal ethics code is KMMU20220854

Public consent

I declare that all authors agree to publish.

Whether there is supporting data

I declare that the data and materials contained in this manuscript have not been published elsewhere and are available.

Conflict of interest

There is no conflict interest in this study

Money

Translational study of microrNA-target gene regulatory network in stroke and acute brain injury. Major Science and Technology Project of Sichuan Province (in the field of social development), Project No. 2020YFS0043, fund 1 million, period 2020.1-2021.12, principal: Wang Tinghua

Author's Contribution

WTH and BX conceived and designed the study. SJ and WJC conducted experiments. YSJ contributed the medicine. LN analyzed the data, LN wrote the manuscript. All authors read and approved the manuscript.

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Figures

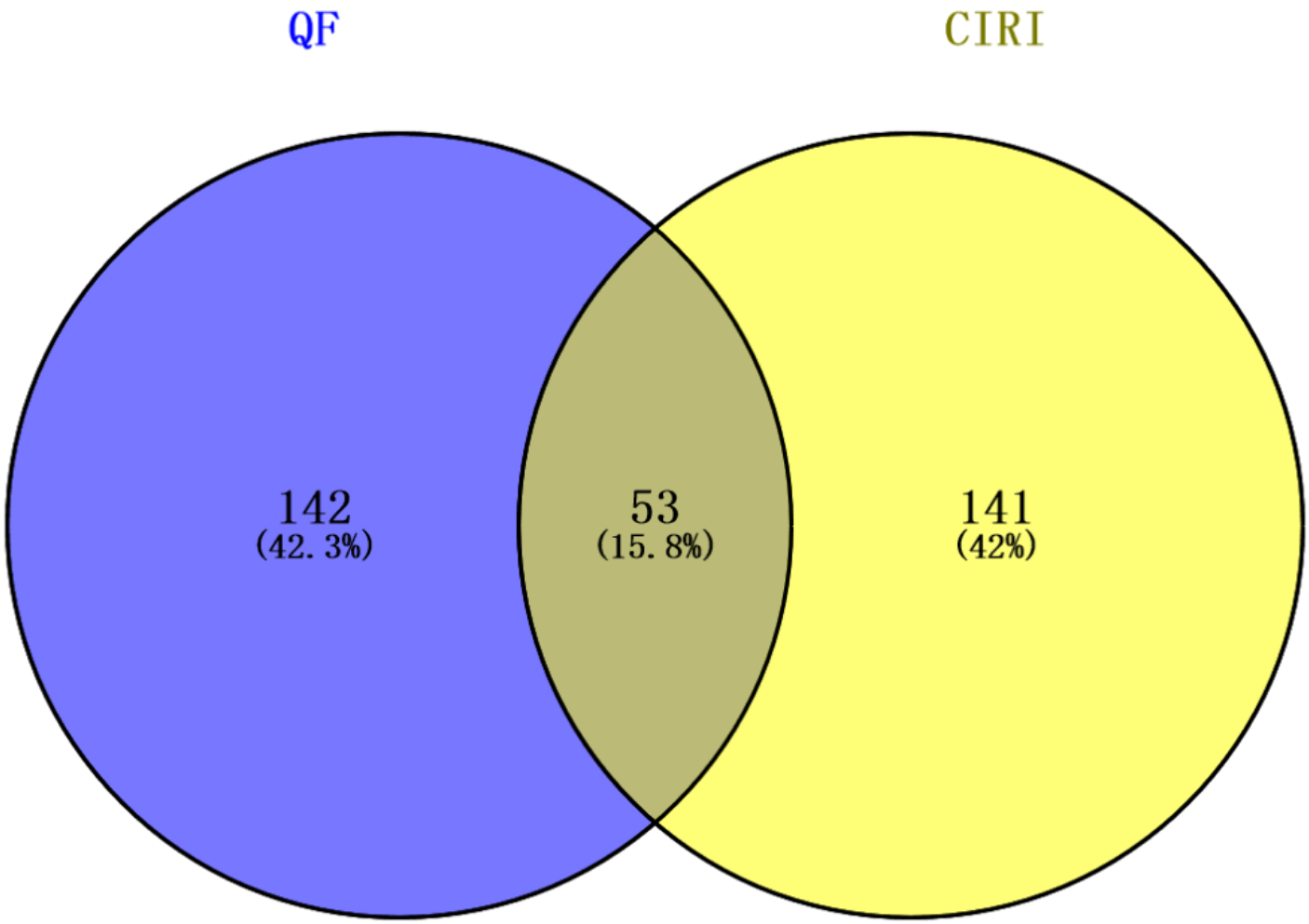

Figure 1

Venny diagram of key targets of cerebral ischemia-reperfusion qufengtongqiao formula

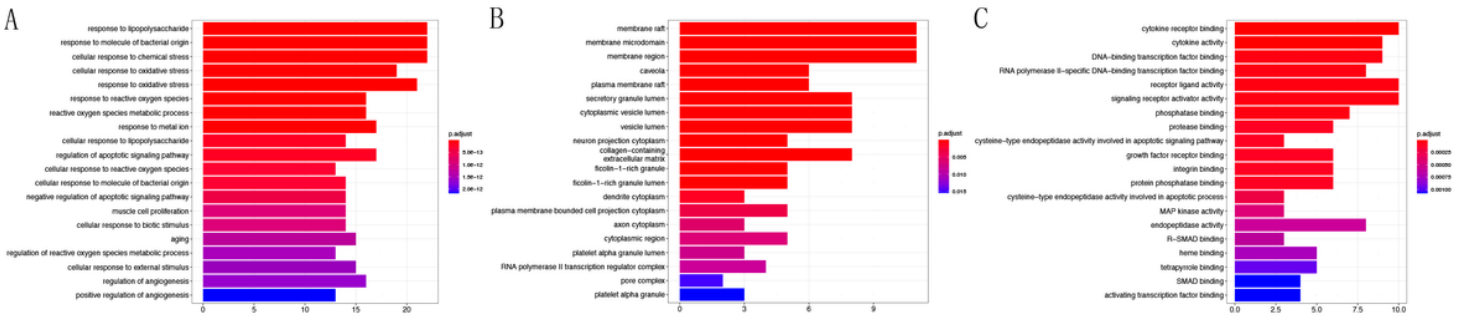

Figure 2

Enrichment diagram of Go analysis.A. Biological processes (BP), B. Cellular components (CC) and C are involved in enrichment analysis. Top 20 factors of molecular function (MF)

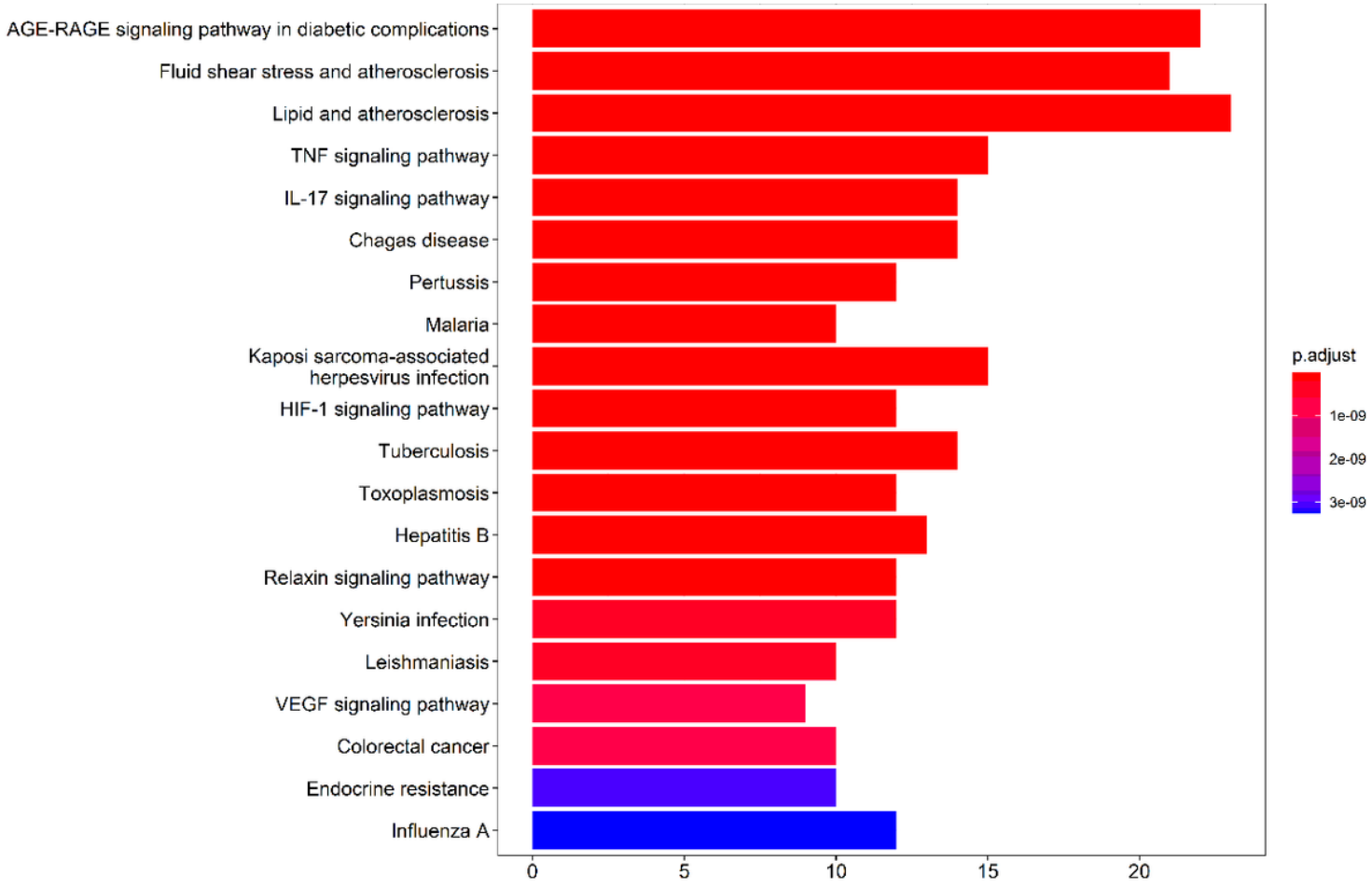

Figure 3

KEGG pathway analysis

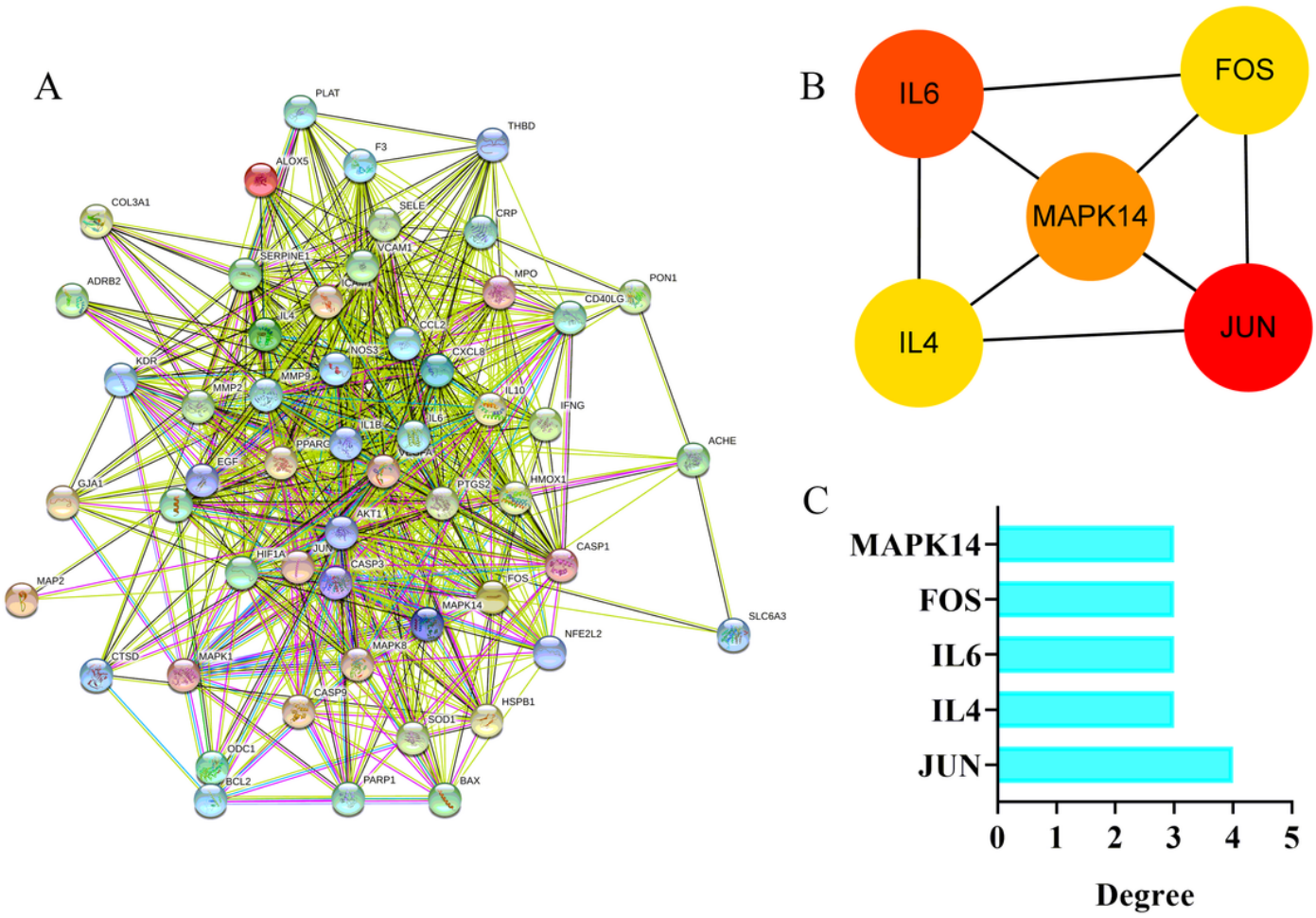

Figure 4

PPI networks of gene interactions

Figure A is the PPI network interaction analysis diagram.B is the first five hub genes;C is the histogram of HUB gene made according to Degree value.

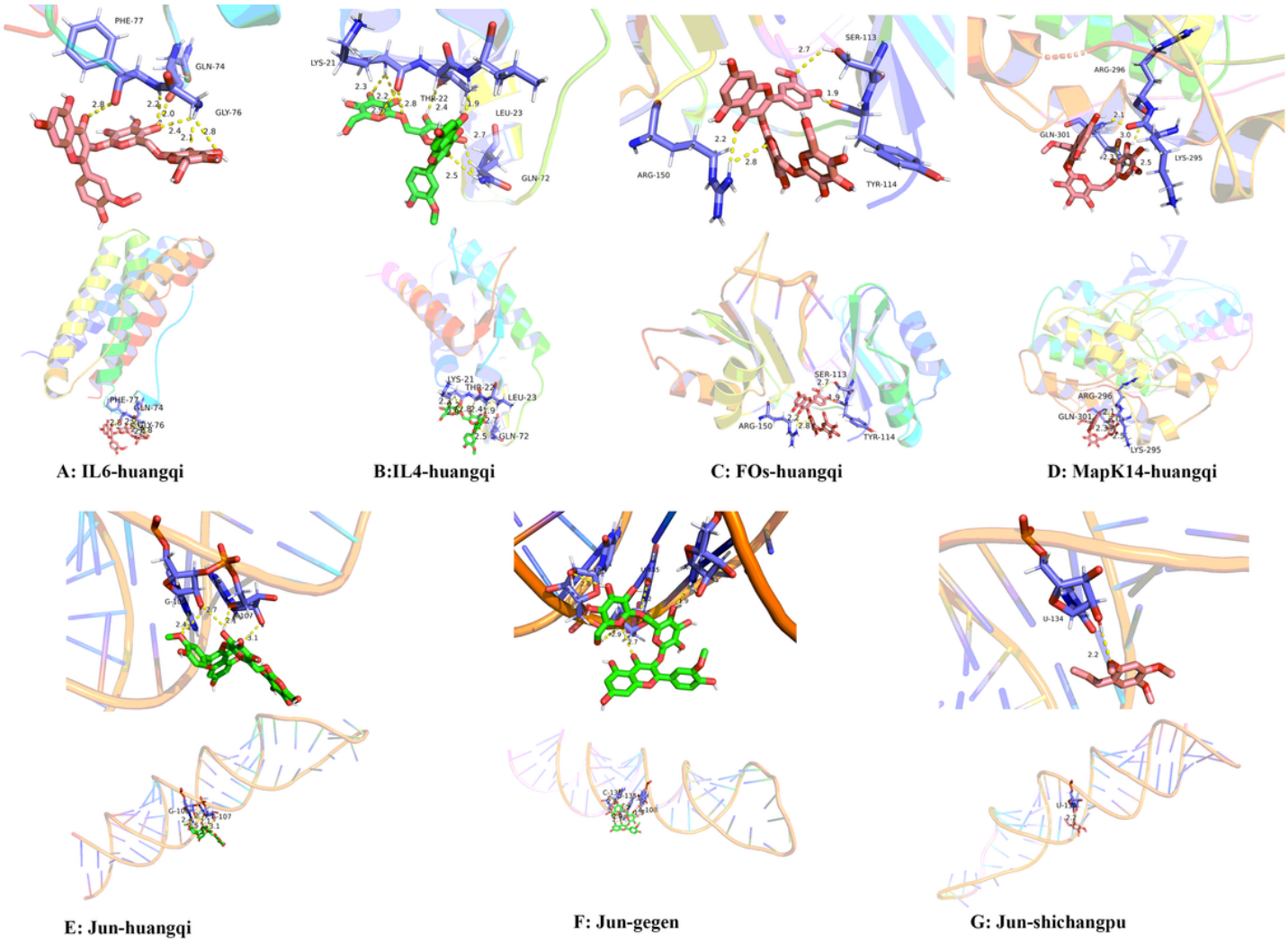

Figure 5

The effective component-related protein molecular docking of Qufengtongqiao Prescription

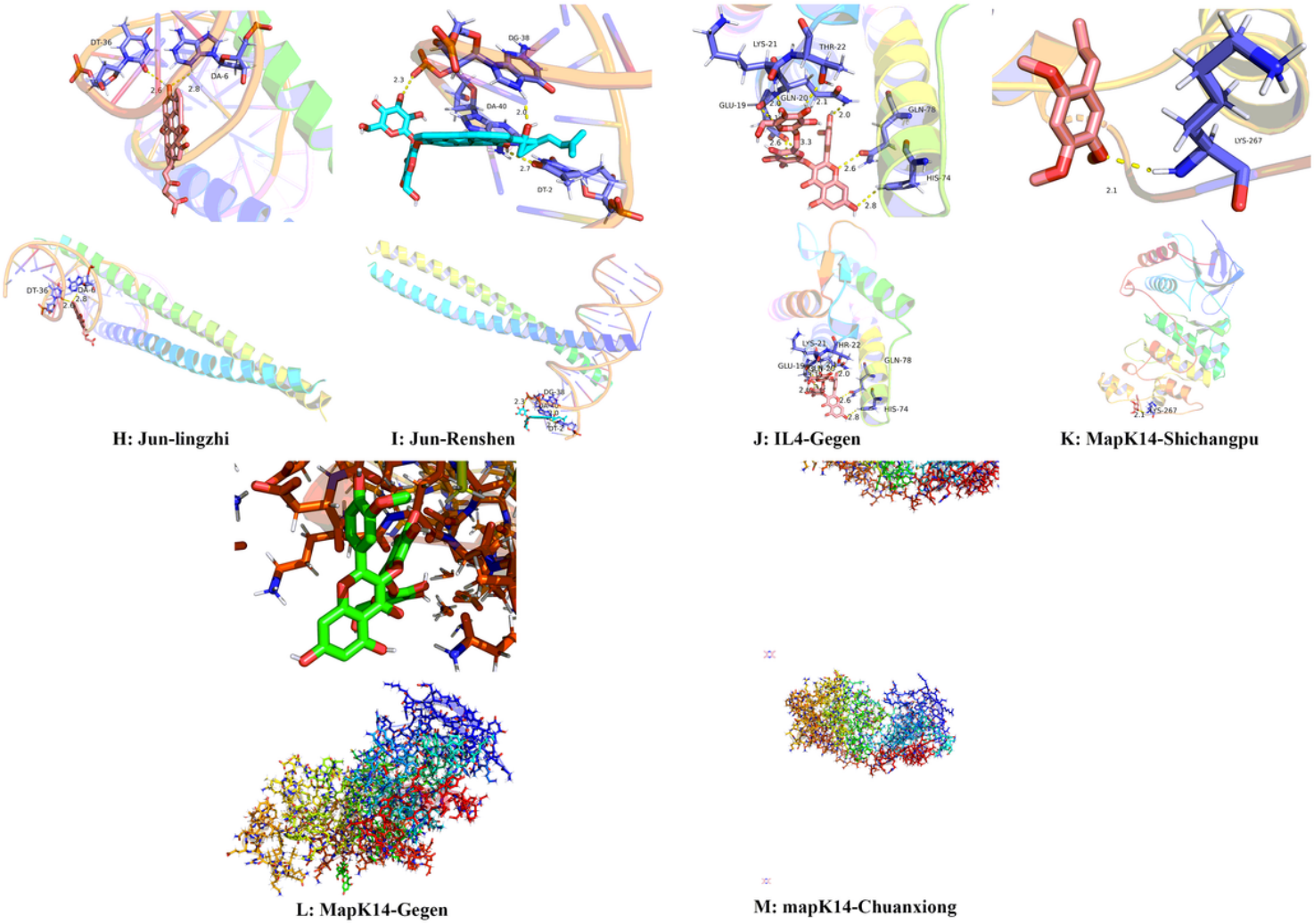

Figure 6

The effective component-related protein molecular docking of Qufengtongqiao Prescription

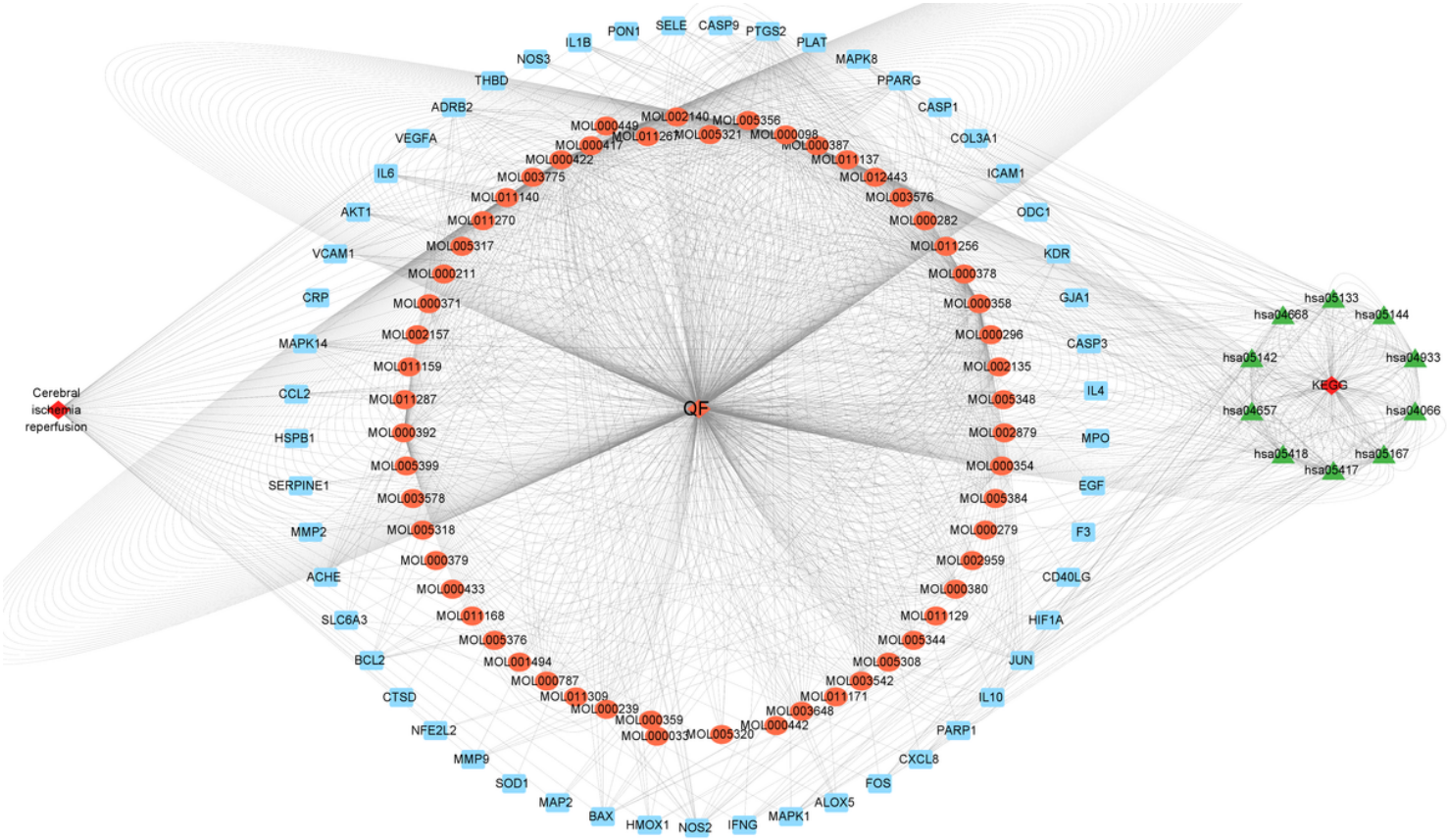

Figure 7

Cerebral ischemia-reperfusion - Qufengtongqiao formula -KEGG pathway network diagram

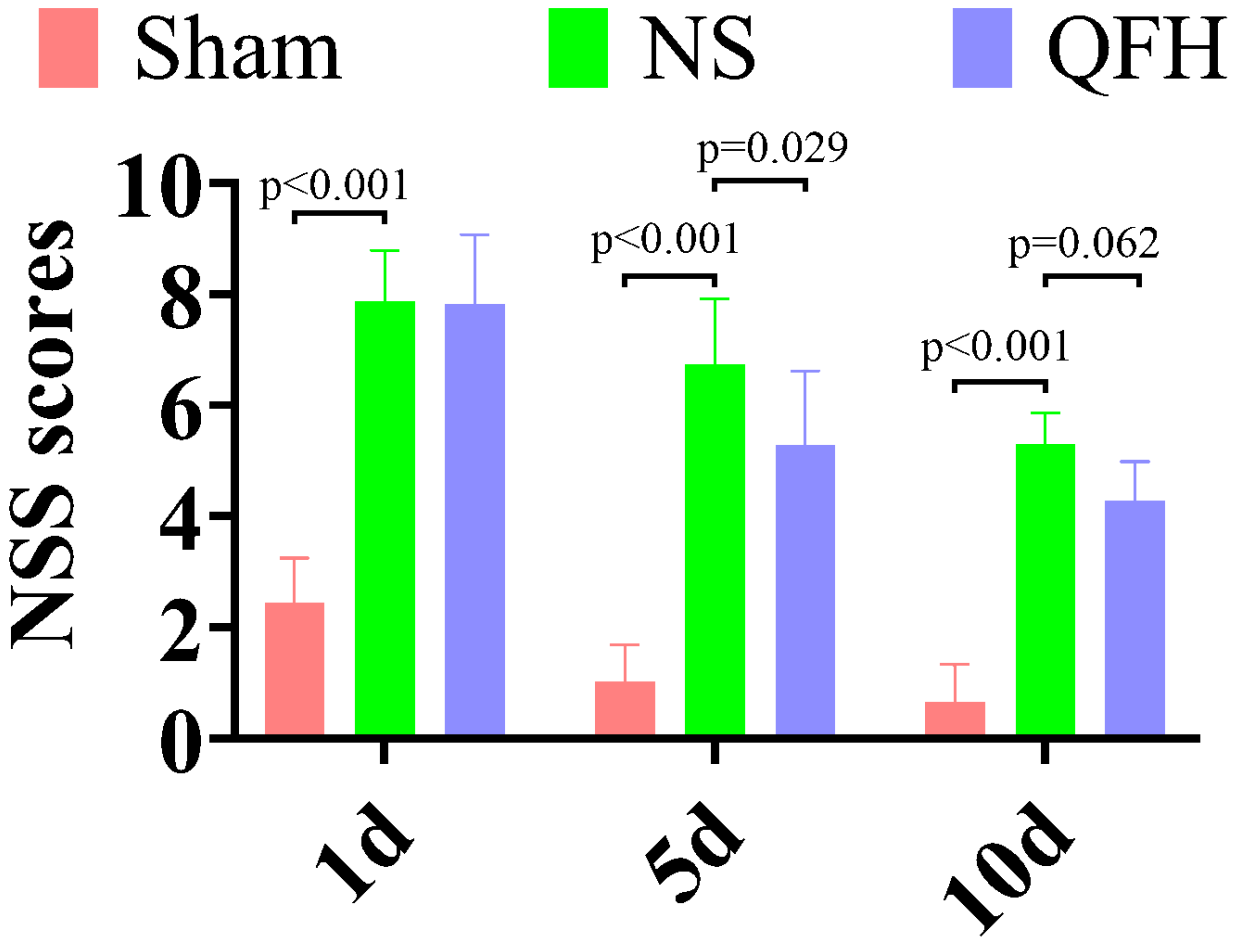

Figure 8

NSS scores of rats in each group at day 1, 5 and 10