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Exploring the mechanism of Pujin oral liquid in the treatment of preterm white matter injury using network pharmacology and molecular docking

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Abstract

We aimed to elucidate the pharmacological mechanisms of Pujin oral liquid in treating preterm white matter injury (PWMI). The Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform was used to identify Pujin oral liquid's active ingredients and predict their targets. The known targets related to treating PWMI were identified from the GeneCards, Online Mendelian Inheritance in Man, DisGeNet, PharmGKB, and CTD databases. A drug–disease intersecting protein–protein interaction network using a STRING database was built; gene ontology function and Kyoto Encyclopedia of Genes and Genomes signaling pathway enrichment analyses were performed on common target genes using the Metascape database. Molecular docking of the active ingredients and key targets was validated using the AutoDock Vina software. In total, 470 Pujin oral liquid targets and 13,290 disease targets were screened from multiple databases, and Venn analysis identified 407 common targets. Protein–protein interaction analysis showed that Pujin oral liquid may impact SRC, MAPK3, MAPK1, TP53, STAT3, AKT1, PIK3R1, JUN, RELA, CTNNB1, and ESR1. Moreover, gene ontology functional analysis revealed processes such as the response to inorganic substances, cellular response to organic cyclic compounds, response to xenobiotic stimuli, regulation of system processes, and protein phosphorylation. The main signaling pathways were neuroactive ligand–receptor interaction and the cGMP–PKG, JAK–STAT, and cAMP signaling pathways. Molecular docking showed that the active ingredients' small molecules bond strongly to target proteins. The therapeutic effect of Pujin oral liquid on PWMI is multifaceted, involving multiple targets and pathways. Its clinical application in treating preterm white matter injuries is promising.

Abbreviations: BP = biological processes, CC = cellular components, ERK = extracellular regulated protein kinase, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, LPS = lipopolysaccharide, MF = molecular functions, NF-κB = nuclear factor kappa B, OL = oligodendrocyte, OPC = oligodendrocyte precursor cells, PWMI = Preterm white matter injury, TCMSP = Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform, TrkB = Tyrosine kinase receptor B.

Keywords: molecular docking, network pharmacology, preterm white matter injury, Pujin oral liquid, traditional Chinese medicine

1. Introduction

Prematurity is considered a global public health problem, with 1 in 10 births (gestational age < 37 weeks) occurring worldwide yearly.[1,2] As perinatal neonatal care improves, the survival rate in premature babies increases. However, premature infants face a higher risk of brain damage.[3] According to relevant statistics, approximately 25% to 50% of preterm babies have developmental impairments in the motor, visual, and cognitive areas.[4] The main type of brain injury in preterm infants is preterm white matter injury (PWMI); approximately 42.5% of children with cerebral palsy experience PWMI.[5] Furthermore, cerebral palsy is the most common neurological sequela of brain injury in preterm infants, with clinical manifestations such as impaired postural development and restricted movement, usually accompanied by perceptual abnormalities and cognitive impairment.[6] Therefore, it is necessary to explore early intervention drugs for treating preterm brain injury to reduce neurological damage at the earliest opportunity.

After systematically reviewing and studying traditional Chinese medicine literature on pediatric brain injury, we

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval is not required because individual patient data and privacy were not involved in this study.

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propose that the main pathological mechanism of premature brain injury according to traditional Chinese medicine is the theory of "phlegm and blood stasis obstructing the orifices and meridians." The mother's exposure to toxic pathogens at the beginning of pregnancy; injuries to the newborn during delivery such as intracranial hemorrhage, postnatal asphyxia, hypoxic-ischemic encephalopathy, nuclear jaundice, exposure to various pathogens, or improper medication; and congenital deficiency, can all lead to phlegm and blood stasis obstructing the orifices and meridians. Therefore, it is believed that "phlegm" and "blood stasis" are the main pathological factors that play an important role in neonatal brain injury and its neurological sequelae. Pujin oral liquid is composed of 4 Chinese herbs: Acori Tatarinowii Rhizoma, Curcumae Radix, Radix Salviae, and Carthami Flos. Furthermore, Pujin oral liquid can improve the lesioned structure of brain tissue and restore the thickness of the myelin sheath.[7,8] Furthermore, Pujin oral liquid is proven to have multiple functions, wherein it includes an anti-free radical effect, decreases the levels of inflammatory cytokines, reduces the levels of myelin-associated inhibitor factors mRNA expression, improves the apoptosis of oligodendrocytes (OL) and their precursors, and promotes neurological recovery and reorganization.[9–12] However, the effective components and mechanism of Pujin oral liquid in treating PWMI remain unclear. Therefore, this study used network pharmacology aiming to predict the potential targets and signaling pathways of Pujin oral liquid in treating PWMI. Furthermore, this study aimed to verify the identified targets through molecular docking, providing a basis for the development of drugs to treat PWMI.

2. Materials and methods

2.1. Screening of active ingredients and potential targets of Pujin oral liquid

The potential effective ingredients of Pujin oral liquid were screened and obtained using the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, https://www.tcmsp-e.com/#/database) using the keywords Acori Tatarinowii Rhizoma, Curcumae Radix, Radix Salviae, and Carthami Flos. The screening conditions were as follows: oral bioavailability ≥ 30%, drug-like ≥ 0.18. The targets of the active compounds were predicted, and data was collected using the TCMSP database. The protein names of the predicted targets were corrected to gene symbols using the UniProt database (https://www.uniprot.org). Some effective compounds in the TCMSP database failed to retrieve the targets; therefore, we captured the corresponding 2D structure of these compounds from PubChem (https://pubchem.ncbi.nlm.nih.gov) and uploaded the data to the SwissTargetPrediction database (http://swisstargetprediction.ch/) to obtain the targets and the corresponding gene symbol. Aim-listed probability > 0 was the standard screening target.

2.2. Screening of PWMI-associated targets

The PWMI-associated genes were collected by exploring "preterm white matter injury" and "periventricular leukomalacia" in the databases of GeneCard (https://www.genecards.org), Online Mendelian Inheritance in Man (https://www.omim.org/), DisGeNet (https://www.disgenet.org), Pharmgkb (https://www.pharmgkb.org), and CTD (http://ctdbase.org/). The disease targets of PWMI and PVL were obtained by merging all the collected data and removing duplicates.

2.3. Construction of active ingredient–intersection target network

To explore the interaction between the active ingredients of Pujin oral liquid and disease targets, Venny 2.1.0 was used to plot the Venny maps of "ingredient targets" and "disease targets." The intersection of the 2 targets was used to determine common targets of the active ingredients and disease targets. The selected intersection targets were imported into the Cytoscape 3.7.2 software to construct the visualization network of the "active ingredient–intersection target."

2.4. Construction and analysis of the protein–protein interaction (PPI) network

The drug–disease common targets were uploaded to the STRING database (https://string-db.org/), and the analysis mode was set to "Multiple proteins" and "Homo sapiens" to generate a PPI network of common target genes. To hide the wandering nodes in the network, we set the minimum required interaction score to the highest confidence (0.900). The tsv file containing the functional node relationships was downloaded and imported into the Cytoscape 3.9.1 software for visualization. The Network Analyzer and CytoNCA plug-ins in Cytoscape were used to analyze the topological parameters between the degree and betweenness of nodes and to screen the core targets with a degree greater than twice the average value as the criterion.

2.5. Gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

The Metascape database (https://metascape.org/) was used to determine the biological functions of the potential data. The GO functional analysis can clarify the role of the target proteins of herbal ingredients in gene function, including biological processes (BP), cellular components (CC), and molecular functions (MF). The KEGG enrichment analysis was used to identify enriched signaling pathways in the drug and disease targets. The top 20 biological functions and related signaling pathways were selected for visualization using bioinformatics (https://www.bioinformatics.com.cn/).

2.6. Molecular docking

The top 11 core targets were subjected to molecular docking with 6 key active ingredients, and the docking binding energy was calculated. The mol2 format of the 3D structure of the small-molecule ligands was downloaded from the TCMSP database. The pdb file for the 3D structure of the hub target protein was downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/). Pymol was used to remove the water molecules and small-molecule ligands from the macromolecules. The treated macromolecules were hydrogenated using AutoDock Tools, and the macromolecular charge was calculated and converted into pdbqt format. The binding pocket coordinates were generated using the proto ligand of the crystal structure as the center. Finally, molecular docking was performed using AutoDock Vina, and binding energies were calculated. The docking results were visualized using the PyMol software. It is generally acknowledged that binding energies of less than –4.25 kcal/mol, –5.0 kcal/mol, and –7.0 kcal/mol correspond to certain, good, and strong binding activities, respectively, between the ligand and the receptors.[13]

3. Results

3.1. The active ingredients and associated targets of Pujin oral liquid

According to the standard of oral bioavailability ≥30% and drug-like ≥0.18 to screen in the TCMSP database, there were

4 active ingredients in Acori Tatarinowii Rhizoma, 15 in Curcumae Radix, 64 in Radix Salviae, and 22 in Carthami Flos. After deduplication, 101 active components were identified. Among these, 5 were shared among the drugs. Moreover, these 101 active ingredients were matched with the UniProt protein and SwissTarget Prediction databases to obtain the drug–gene targets. After removing duplicates, 470 effective active targets were identified.

3.2. Disease-associated target genes and intersection target genes

After standardization and duplicate elimination, 13,290 potential targets were retrieved from the Genecards, Online Mendelian Inheritance in Man, DisGeNet, Pharmgkb, and CTD databases. A Venn diagram was plotted between the targets of drug and disease, which showed 407 intersecting targets (Fig. 1).

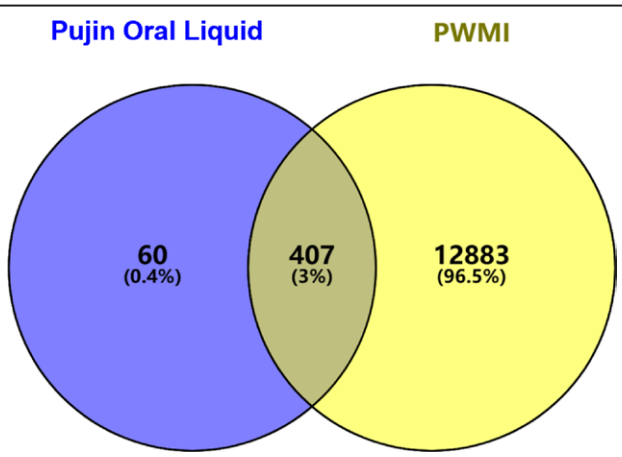

Figure 1. Venn diagram of Pujin oral liquid–PWMI intersection targets. PWMI = preterm white matter injury.

3.3. Active ingredients–intersection targets network graph construction and analysis

The active ingredients–intersection targets network of Pujin oral liquid was constructed using the Cytoscape 3.9.1 software (Fig. 2) with 492 nodes and 1447 edges. The diamonds represent traditional Chinese medicine, circles represent active ingredients, and squares represent the targets. By applying Network Analyzer analysis, the top 10 key active ingredients were (E)-1,7-Diphenyl-3-hydroxy-1-hepten-5-one, 6-hydroxynaringenin, kaempferol, α-amyrin, NSC 122421, tanshinone IIa, (E)-5-Hydroxy-7-(4-hydroxyphenyl)-1-phenyl-1-heptene, salviolone, beta-sitosterol, and dihydrotanshinlactone.

3.4. Construction of the PPI network and hub targets screening

The 407 intersection targets of Pujin oral liquid in the treatment of PWMI were imported into the STRING database to analyze the protein interaction of the potential targets of Pujin oral liquid in the treatment of PWMI. The PPI network consisted of 314 nodes and 3632 edges. The results were imported into Cytoscape 3.9.1 for visualization (Fig. 3). The upper median values of degree centrality, betweenness centrality, and closeness centrality were filtered, and after screening for values greater than the quartiles, core targets were obtained: SRC, MAPK3, MAPK1, TP53, STAT3, AKT1, PIK3R1, JUN, RELA, CTNNB1, and ESR1. Figure 4 illustrates the PPI network and screening process for the core targets.

3.5. The GO and KEGG pathway enrichment analyses

The potential targets of Pujin oral liquid for PWMI treatment were uploaded to the Metascape platform for GO function analysis (Fig. 4) and KEGG pathway enrichment analyses (Fig. 5).

The GO enrichment analysis showed that treating PWMI with Pujin oral liquid mainly regulated the response to inorganic substances, cellular response to organic cyclic compounds, response to xenobiotic stimuli, the system processes,

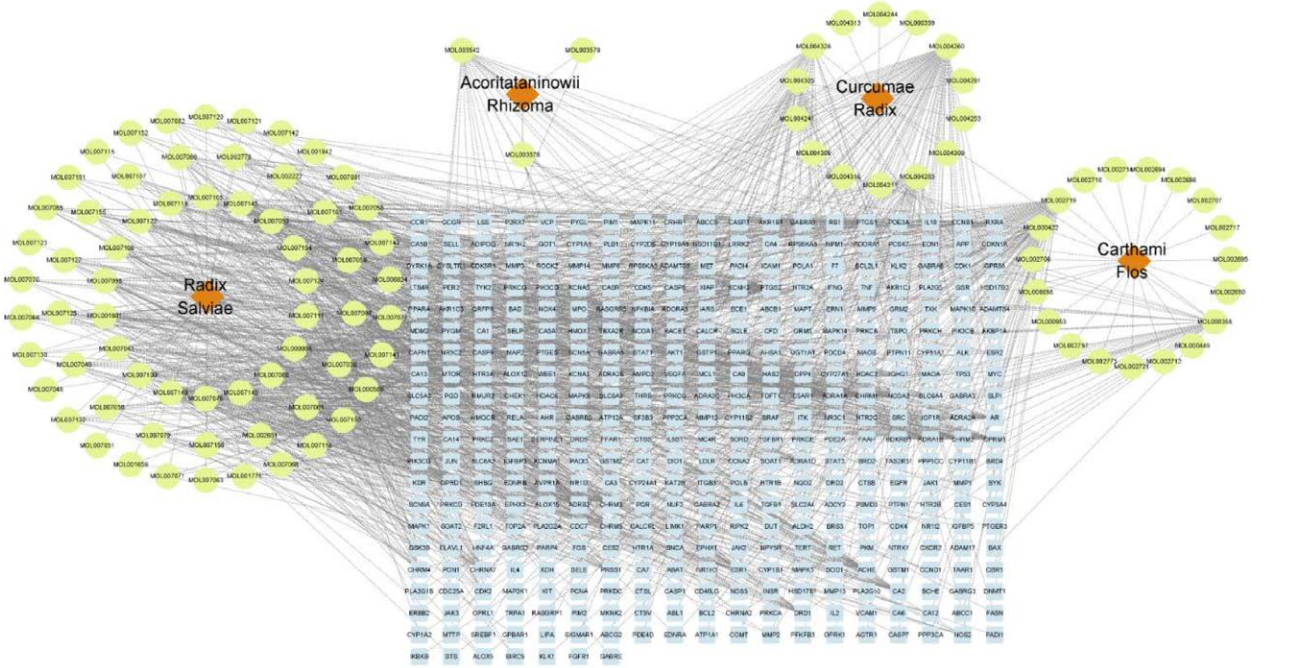

Figure 2. Network diagram of active ingredient–intersection targets.

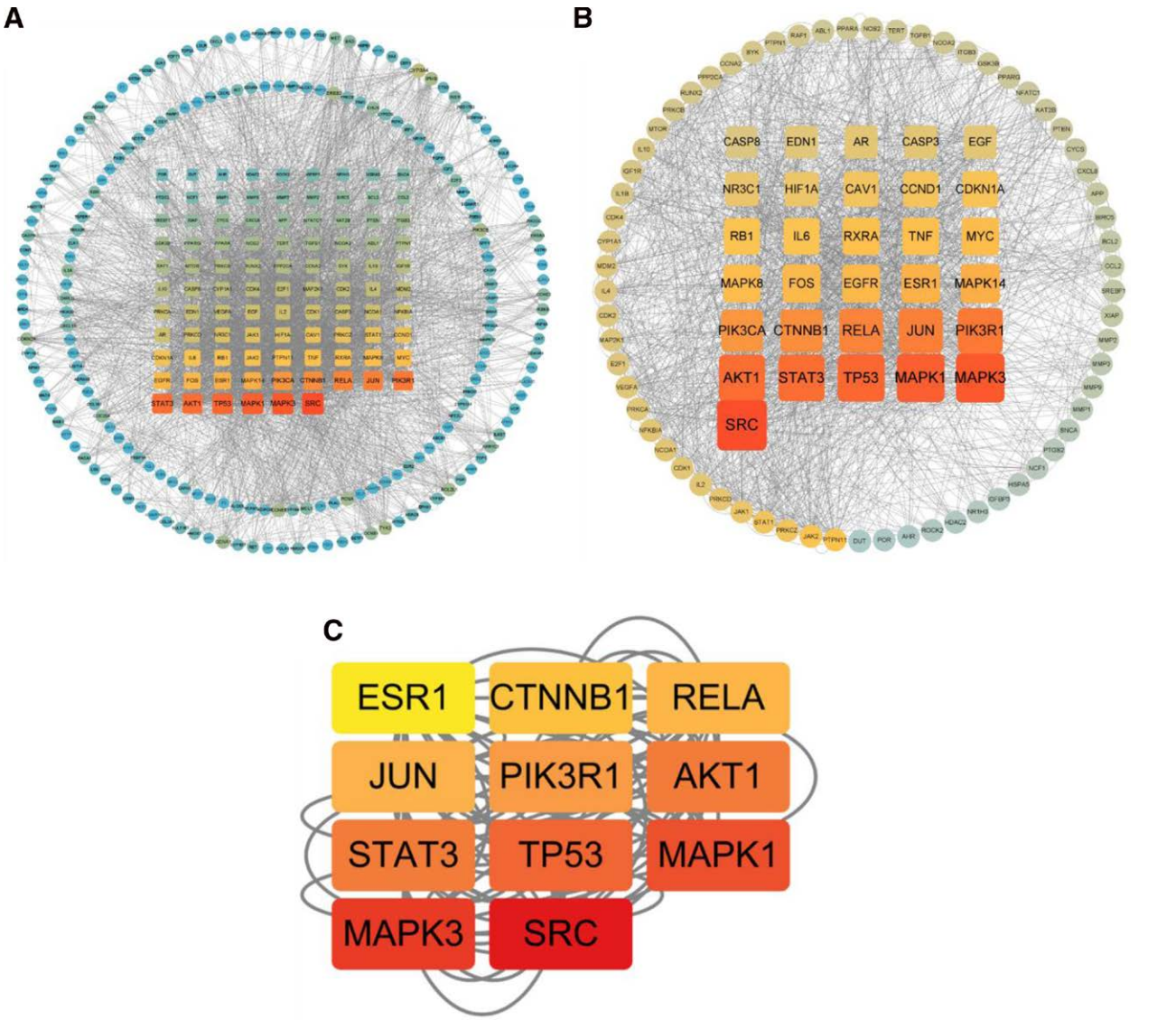

Figure 3. The target PPI network of Pujin oral liquid against PWMI. The size of the shape represents the degree of the gene in the network. The larger the degree value, the bigger the graph. PWMI = preterm white matter injury.

and protein phosphorylation. Regarding MF, Pujin oral liquid mainly regulated protein kinase activity, kinase binding, transcription factor binding, oxidoreductase activity, and protein homodimerization activity. At the CC level, Pujin oral liquid intervened with structures such as membrane rafts, dendrites, receptor complexes, perinuclear regions of the cytoplasm, and lytic vacuoles.

The KEGG pathway enrichment analysis of potential targets and bubble plots identified the following pathways, genes, and conditions closely related to PWMI: cancer, neuroactive ligand–receptor interaction, AGE–RAGE signaling pathway in diabetic complications, Alzheimer disease, prostate cancer, cAMP signaling pathway, insulin resistance, cGMP–PKG signaling pathway, JAK–STAT signaling pathway, and serotonergic synapses.

3.6. Molecular docking

According to the results of network pharmacology, we screened the top 6 key active ingredients, including (E)-1,7-Diphenyl-3-hydroxy-1-hepten-5-one, 6-hydroxy naringenin, kaempferol, luteolin, NSC 122421, tanshinone IIa, and naringenin. These 6 active ingredients were docked with the top 11 core targets: SRC, MAPK3, MAPK1, TP53, STAT3, AKT1, PIK3R1, JUN, RELA, CTNNB1, and ESR1. The results indicated that the active ingredients showed good binding to the core target protein, as shown in the heat map (Fig. 6). If the binding energy is <0, the ligand and receptor can spontaneously bind, and the smaller the value, the higher the binding activity. The best docking affinity of the 6 pairs was selected for visualization (Fig. 7).

4. Discussion

Preterm infants often have several complications contributing to high child mortality rates and neurodevelopmental disorders.[14,15] White matter injury is the most common brain injury in preterm infants, with a prevalence of up to 50% in very-low-birth-weight infants.[5] It is thought to be caused by myelin formation failure during white matter development.[16,17] Additionally, PVL is a unique and severe brain injury observed in preterm infants.

Acori Tatarinowii Rhizoma can improve cognitive function, relieve phlegm accumulation, increase alertness, and improve intelligence.[18] Additionally, its volatile oil can stimulate the central nervous system and protect nerves.[19] Moreover, Curcumae

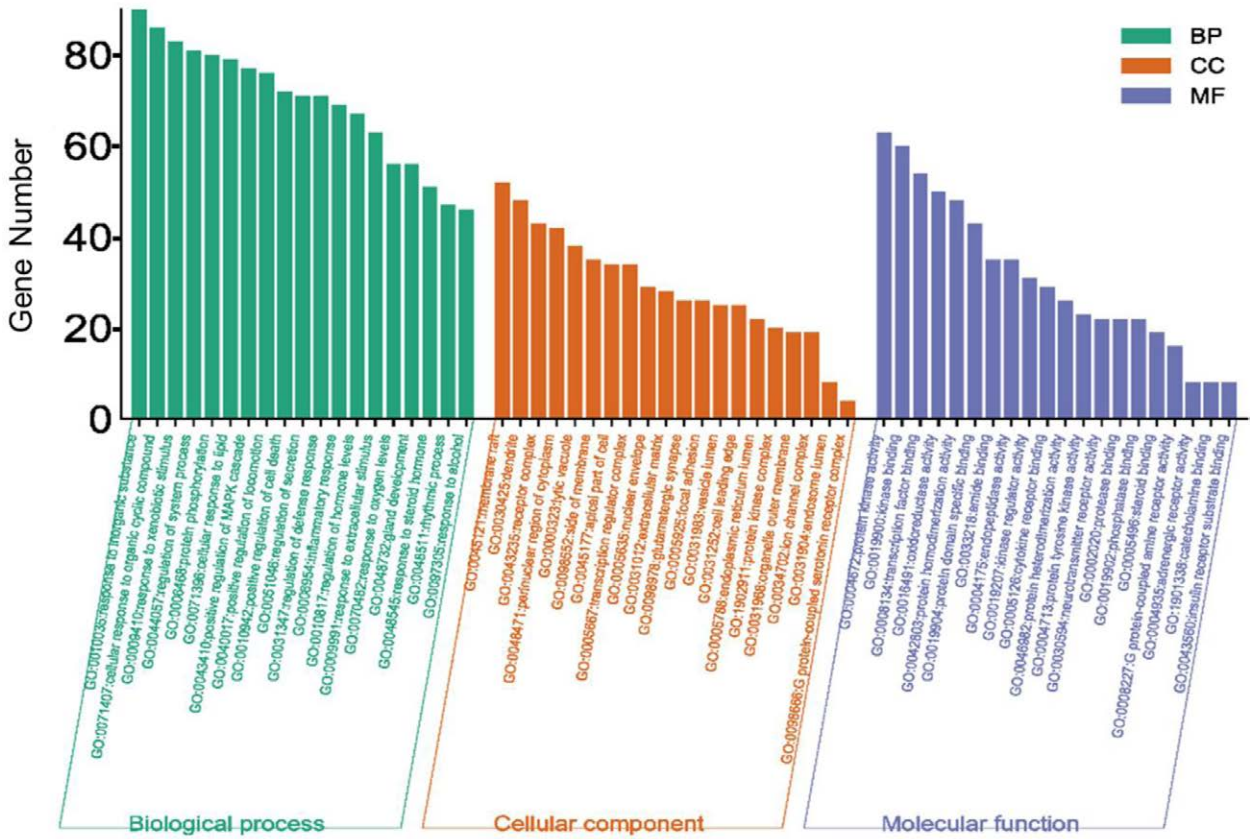

Figure 4. Gene ontology function enrichment analysis. GO = gene ontology, BP = biological process, CC = cell composition, MF = molecular function.

Radix can improve blood circulation and gallbladder function while relieving pain and jaundice.[20] In addition, its components can inhibit the inflammation caused by TNF.[21] Radix Salviae is rich in tanshinone and phenolic acid compounds, which have antibacterial and anti-inflammatory properties.[22] Carthami Flos can promote blood circulation and dissipate stasis, whereas Carthami Flos glycosides can improve cellular hypoxia tolerance.[23] Moreover, Carthami Flos can inhibit apoptosis and improve ischemic brain edema.[24]

Our study revealed the key ingredients in Pujin oral liquid for the treatment of PWMI and their potential molecular mechanisms. In this study, we built a subnetwork with 221 nodes and 1554 edges in PPI. The GO analysis found 2410 BP, 228 MF, and 106 CC, whereas the KEGG analysis identified 181 enriched pathways. Molecular docking of the 11 targets and 6 active ingredients provided an in-depth analysis of the genes and drug components.

(E)-1,7-Diphenyl-3-hydroxy-1-hepten-5-one,6-hydroxynaringenin, kaempferol, luteolin, NSC 122421, tanshinone IIa, and naringenin were the main pharmacodynamic components docking with the targets and have been shown to have neuroprotective and neurotrophic effects. Naringin inhibits the activation of astrocytes and microglia and suppresses neuroinflammation, thereby reducing neuronal damage.[25] Luteolin exhibits neuroprotective effects through various mechanisms, triggering overall changes in the microglia transcriptome and inhibiting the expression of microglia pro-inflammatory cytokines, resulting in a unique anti-inflammatory and neuroprotective phenotype.[26] Tanshinone IIa is the most abundant fat-soluble component of *Salvia miltiorrhiza*. Tanshinone IIa and its derivative, Tanshinone IIa sulfonate, have been proven to have various functions, including anti-inflammatory, antioxidant, and anti-fibrotic properties, and have been widely used clinically.[27] Kaempferol has anti-inflammatory and antioxidant effects in lipopolysaccharide (LPS)-induced inflammation and microglial activation, and its neuroprotective effects may be related to its anti-inflammatory and antioxidant effects, as well as the inhibition of neuronal apoptosis.[28–31] Beta-sitosterol can eliminate pro-inflammatory factors such as nitric oxide, TNF-α, cyclooxygenase-2, and inducible nitric oxide synthase, as well as inhibit the phosphorylation of nuclear factor kappa B (NF-κB) and extracellular regulated protein kinase (ERK) by suppressing the phosphorylation and degradation of NF-κB inhibitors.[32,33] Furthermore, ERK regulates various cytokines in the inflammatory pathway. In in vivo and in vitro experiments, baicalein can effectively improve neuroinflammation, increase the secretion of brain-derived neurotrophic factor, and activate tyrosine kinase receptor B and cyclic adenosine phosphate element binding protein.[34,35] In addition, baicalein can inhibit the activation of BV-2 cells, thereby inhibiting the inflammatory response caused by LPS. Its mechanism of action may lie in the ability of baicalein to regulate the TLR4/MyD88/NF-κB signaling pathway, reduce the expression of inflammation-related proteins, reduce the inflammatory response, and play a neuroprotective role.[36,37] The network pharmacology results highlight the potential clinical application of Pujin oral liquid and its effectiveness in treating PWMI.

The PPI network analysis showed that the core targets of Pujin oral liquid when treating PWMI included SRC, MAPK3, MAPK2, TP53, STAT3, AKT1, PIK3R1, JUN, RELA, CTNNB1, and ESR1. These targets are involved in BP, such as regulating the cellular response to nitrogen compounds, inorganic substances, organic cyclic compounds, xenobiotic stimuli, system processes,

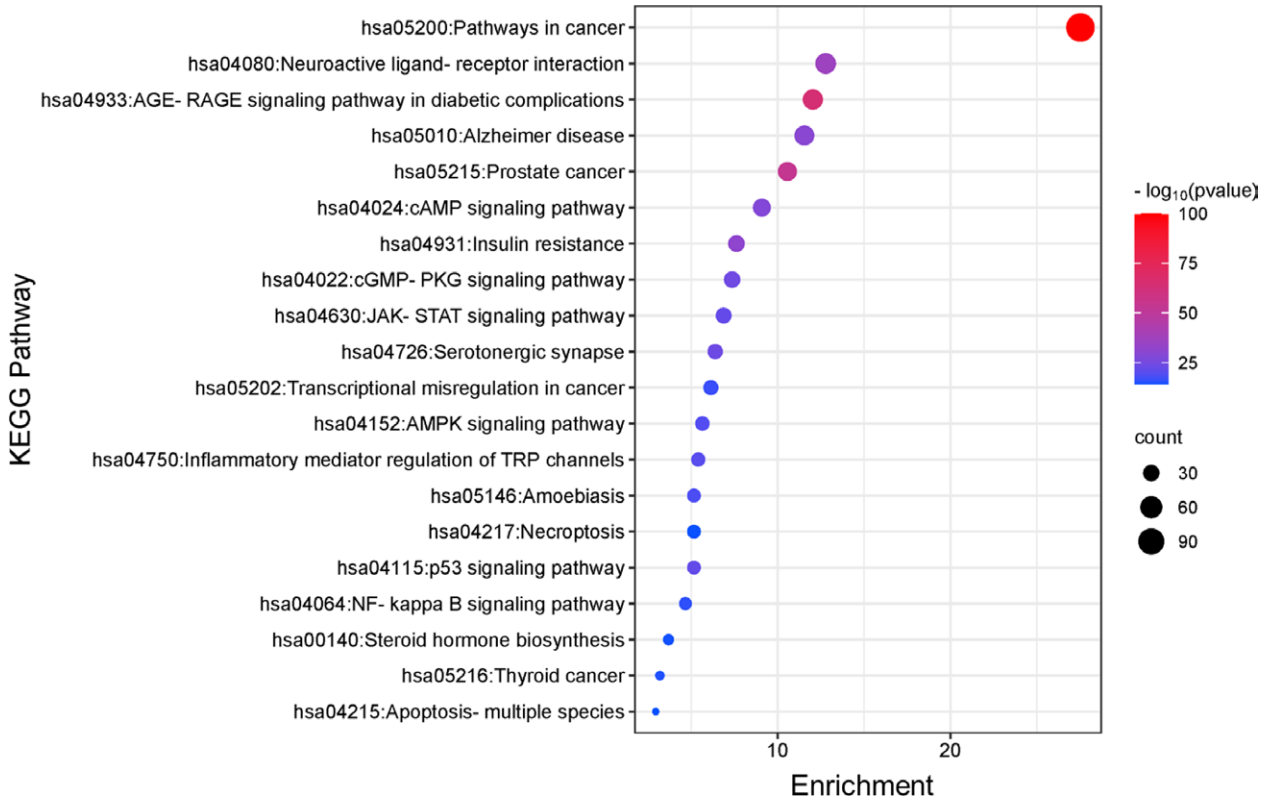

Figure 5. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. KEGG = Kyoto Encyclopedia of Genes and Genomes.

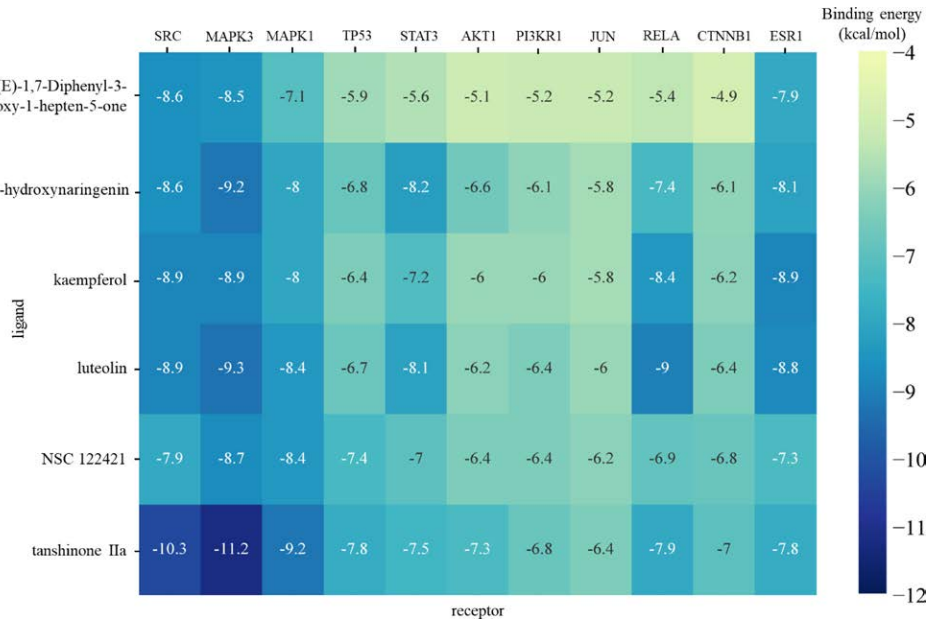

Figure 6. Binding energy heat map of the core active ingredients and targets.

protein phosphorylation, lipids, MAPK cascade, locomotion, cell death, secretion, defense response, inflammatory response, hormone levels, extracellular stimulus, and oxygen levels.

The main upstream initiating injury in PWMI is hypoxia-ischemia and/or inflammation, and oligodendrocyte precursor cells (OPC) are the primary affected target cells.[38] Previous studies have concluded that OPC are rich in iron and lipids, have a high oxidative metabolism rate, and are highly susceptible to damage, such as hypoxia-ischemia and inflammation.[39] After injury, regenerated OPC differentiate into mature OL; however, their ability to form a myelin sheath is compromised, leading to reduced myelin sheath formation and rapid conduction along the axon.[40] The maturation and myelination of OL are regulated by various transcription factors. Src kinases help in cell proliferation, neuronal differentiation, and axonal growth.[41,42] Src and Fyn have also been detected in developing brains.[43] Activation of Src kinase in the brain is closely related to myelin formation during early postnatal development. Previous studies have found that a lack of Src kinase or its activity can lead to defects in myelin formation in mice.[44,45] A previous study

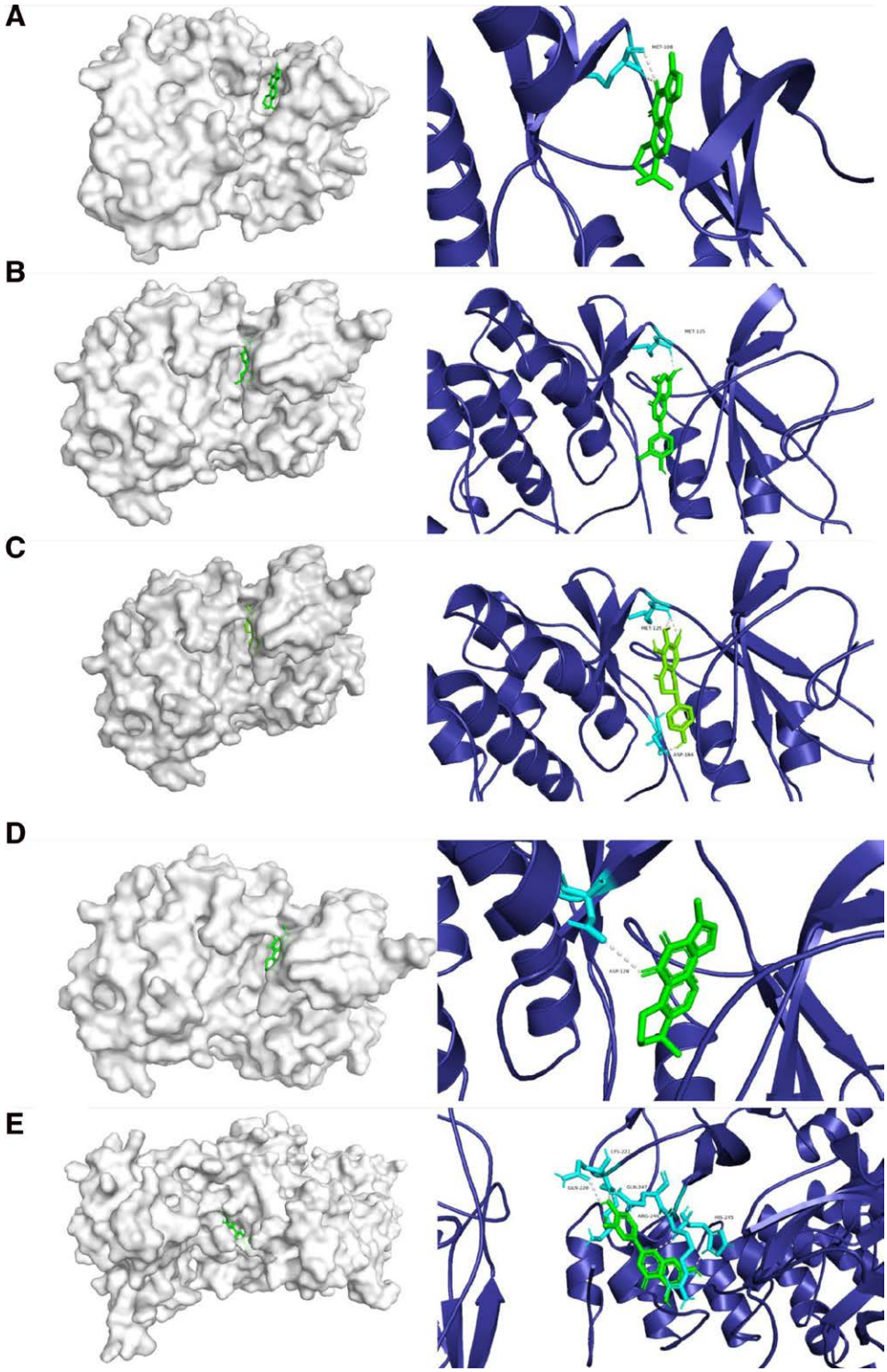

Figure 7. Molecular docking diagrams with the best 6 docking affinity. (A) Tanshinone IIa to MAPK1; (B) luteolin to MAPK3; (C) 6-hydroxynaringenin to MAPK3; (D) tanshinone IIa to MAPK3; and (E) luteolin to RELA.

suggested that phosphorylated Src kinase-mediated brain-derived neurotrophic factors promote OL myelination by activating Erk1/2 phosphorylation.[46] Another study showed that Src kinases were involved in the GABA regulation of myelin formation.[47] Moreover, ERK1/2, p38MAPK, and JNK involved in the MAPK signaling pathway regulate OL differentiation and myelin regeneration.[48,49] Furthermore, signal transduction and transcription activator transcription 3 (Stat3)-mediated activation of astrocytes can inhibit the release of transforming growth factor beta1 from microglia. In turn, this promotes the maturation of OL precursor cells, thereby playing a beneficial role in treating perinatal white matter injury lesions.[50]

According to the KEGG enrichment analysis, the important pathways for the treatment of PWMI by Pujin oral liquid included pathways involved in cancer, neuroactive ligand–receptor interaction, AGE–RAGE signaling pathway in diabetic complications, Alzheimer disease, prostate cancer, cAMP signaling pathway, insulin resistance, cGMP–PKG signaling pathway,

JAK–STAT signaling pathway, serotonergic synapse, transcriptional misregulation in cancer, AMPK signaling pathway, inflammatory mediator regulation of TRP channels, and necroptosis.

Additionally, PVL is mainly characterized by the activation of microglia and astrocytes, as well as the low myelination of axons.[51] Astrocytes and microglia are immune cells of the nervous system and the main immune cells involved in neuroinflammatory responses.[52] Furthermore, microglia are the main source of acute inflammatory factors in the early stages of the neuroinflammatory response, whereas astrocytes play an important role in the later stages of the chronic inflammatory response.[53] During brain injury, astrocytes undergo drastic transformation into reactive astrocytes. Activated astrocytes release inflammatory factors, lose their normal neurotrophic function, and affect the normal microenvironment of other peripheral nerve cells, affecting their development and leading to the formation of abnormal neural structures and functions.[54] JAK2–STAT3 is mainly expressed in the brain tissue. It is thought to affect the apoptosis, proliferation, and differentiation of neurons, glial cells, and vascular endothelial cells, thereby protecting neurons, reducing inflammatory reactions, promoting neural function remodeling, and repairing damaged brain tissues. Studies have confirmed that the downregulation of phosphorylation in the JAK2–STAT3 pathway can significantly reduce the secretion of pro-inflammatory factor TNF by microglia in the M1 state (α, IL-1 β) and promote the polarization of microglia into the M2 type state, thereby reducing the neuroinflammatory response.[55] The MAPK signaling pathway regulates the transformation of the microglial phenotype to the M1 type, which promotes inflammatory response.[56] Previous studies have shown the interaction between inflammation and the AMPK pathway. Inflammatory reactions inhibit AMPK phosphorylation under conditions of sufficient nutrition.[17] The activated AMPK pathway effectively inhibits inflammatory responses,[18] participates in regulating myelin regeneration, and reduces myelin loss.

We also conducted molecular docking analysis to verify whether the key active ingredients of Pujin oral liquid had strong binding activity with the core targets of PWMI. When the binding energy is <0, small-molecule ligands can spontaneously bind to protein receptors with lower binding energies, and the conformation of the complex after binding may be more stable. The molecular docking results showed that virtually all the binding energy of the simulated docking results was less than −5.0 kcal·mol^{-1}, indicating that the active ingredients of Pujin oral liquid had good affinity with the targets. Luteolin and tanshinone IIa had the lowest total binding energy with the core targets, and the lowest binding energy was observed in the interaction between tanshinone IIa and MAPK3: −11.2 kcal·mol^{-1}. Furthermore, tanshinone IIa can activate the PI3K/AKT pathway, reduce inflammation in the brain, and inhibit the excessive activation of microglia and astrocytes.[57] Luteolin can alter the M1/M2 polarization of macrophages and exert anti-inflammatory effects by downregulating p-STAT3.[58] Targeted inhibition of the STAT3 signaling pathway can also weaken LPS-induced astrocyte activation and cytokine production, exerting neuroprotective effects.[59] These combinations may be important in treating PWMI using Pujin oral liquid.

There are also some limitations in our study. We used only bioinformatics methods to explore the effects of Pujin oral liquid in the treatment of PWMI by using network pharmacology and molecular docking. Firstly, data from online databases are based on reviewed and predicted data, so the depth of basic research and the accuracy and real-time updating of the datum in each database are particularly essential. Secondly, although tanshinone IIa, luteolin, and 6-hydroxynaringenin were identified as important ingredients, this does not represent the entirety of Pujin oral liquid, and therefore pharmacodynamic and molecular biology experiments need to be considered to delve deeper into our findings. Thirdly, despite identifying core ingredients, quantitative studies remain incomplete, and future studies should be done on the specific concentrations of the components. Therefore, the potential mechanism of action of Pujin oral liquid for the treatment of PWMI has not yet been explained and confirmed, and we believe that this topic has great research potential and application value.

5. Conclusion

In this study, we used network pharmacology and molecular docking methods to explore the potential molecular mechanisms underlying the effects of Pujin oral liquid in the treatment of PWMI. The results revealed that tanshinone IIa, luteolin, and 6-hydroxynaringenin were the key active ingredients of Pujin oral liquid against PWMI. These components are thought to act on multiple signaling pathways, such as the PI3K/Akt, MAPK, and AMPK pathways, by regulating SRC, MAPK2, MAPK1, TP53, STAT3, AKT1, and other targets. These findings suggest that Pujin oral liquid plays a neuroprotective role by reducing apoptosis, alleviating inflammation, and limiting oxidative stress through the multi-ingredient, multi-target, and multi-pathway interactions involved in PWMI treatment. Results of molecular docking suggested that the interactions between SRC–tanshinone IIa, MAPK1–tanshinone IIa, MAPK3–tanshinone IIa, MAPK3–luteolin, RELA–luteolin, and MAPK3–6-hydroxynaringenin should provide important links in the mechanism of white matter injury recovery. This study theoretically elucidated the pharmacological mechanism of Pujin oral liquid in PWMI, providing directions for the future development of clinical treatments for PWMI.

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Writing – original draft: Xing-Ruo Gong.
Writing – review & editing: Bing-Xiang Ma.

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