**The Mechanisms of Pei-yuan-Tong-nao Capsule as a Therapeutic Agent against Cerebrovascular Disease**

Yu Wei, Xiao-Jing Fan, Ming-Hui Zhang, Mai-Qiu Wu, Wei Li, Ping Wang, Wei Xiong, Jian-Ping Lin

Objective: Pei-Yuan-Tong-Nao (PYTN) capsule has been widely used for the treatment of cerebrovascular disease (CVD), including cerebral ischemia. However, due to the complexity of traditional Chinese Medicine (TCM) and the lack of proper inspection methods, the pharmacological mechanisms of the actions of PYTN capsule on CVD remain ambiguous. In this investigation, a network pharmacology method was used to investigate the mechanism of action of PYTN capsule in the treatment of CVD. **Methods:** In this method, a chemical similarity ensemble approach was employed to predict potential targets for chemicals derived from PYTN capsule. **Results:** Thus, a total of 351 compounds and 650 proteins were identified as potential active ingredients and putative CVD-related targets. In addition, two interaction networks were constructed, including a network between putative targets of PYTN capsule and known CVD-related targets, and a network between candidate active compounds and putative CVD-related targets. **Conclusion:** The mechanism of PYTN capsule in the treatment of CVD was found to be based on three functional modules, namely, antioxidation, anti-inflammation, and antiapoptosis modules through multiple signaling pathways, such as PI3K-Akt, mitogen-activated protein kinase, and TNF signaling pathways. We hope that this work can provide insight into the pharmacological mechanisms of TCMs in the treatment of CVD and provide a basis for further development and the discovery of more effective clinical strategies for the treatment of complicated diseases.

Keywords: Cerebrovascular disease, mechanism, network pharmacology, Pei-Yuan-Tong-Nao, traditional Chinese medicine

**INTRODUCTION**

Cerebrovascular disease (CVD) is the general term that includes multiple types of devastating disorders, such as ischemic stroke, hemorrhagic stroke, and cerebrovascular anomalies.[1] The incidence of ischemic stroke is much higher than that of hemorrhagic stroke.[2] The cause of CVD can be a variety of factors, for instance, damage to blood vessels in the brain, and can result in decreased or no blood supply to the brain. Brain damage is irreversible once no oxygen is being transported to the brain. The main pathophysiological mechanisms of ischemic stroke include excitotoxicity, acidotoxicity, ionic imbalance, oxidative/nitrative stress, inflammation, apoptosis, and depolarization around the infarct, ultimately leading to cell death and tissue necrosis. According to statistics, in 2017, globally, there were approximately 104 million individuals suffering from stroke and approximately 6.17 million who died from CVD.[3,4] Currently, there are multiple medications used for the treatment of specific types of CVD, including antiplatelets (for example, aspirin and clopidogrel), blood thinners (for example, heparin and warfarin), antihypertensives, and antidiabetic medications.[4]

Recently, traditional Chinese medicine (TCM) has been widely used in the treatment of CVD[5] and approved by China’s State Food and Drug Administration. Pei-Yuan-Tong-Nao...
Capsule (PYTN), a TCM that has been approved for stroke treatment in clinical uses, consists of *Polygoni Multiflori Radix* (zhiheshouwu), *Rehmanniae Radix Praeparata* (shudihuang), *Asparagus Radix* (tiandong), *Testudinis Carapaxet Plastrum* (gujia), *Cervi Cornu Pantotrichum* (lurong), *Cistanches Herba* (roucongrong), *Cinnamomi Cortex* (rougui), *Radix Paeonie Rubra* (chishao), *Scorpio* (quanxie), *Hirudo* (shuzhi), *Lumbricus* (qiyuin), *Crataegi Fructus* (shanzha), *Poria* (fuling) and *Glycyrrhizae Radixet Rhizoma* (zhigancao). However, the mechanisms of action of PYTN capsule on CVD are ambiguous and unclear. Therefore, there is a need to clarify and illustrate the function and action modes of PYTN capsule on targets involved in CVD.

Currently, network pharmacology has been used more frequently in drug discovery and clarification of the mechanisms of action of traditional medicine. Recent work by Zuo et al. elucidated the mechanisms of Qing-Luo-Yin in treating Hot Syndrome-related rheumatoid arthritis using network pharmacology and metabolomics methods. To better identify the active compounds in an herbal formula and illustrate the action mechanisms of that herbal formula, a TCM network platform that integrates multiple methods for TCM network pharmacology was constructed by Zhang et al. In addition, a network pharmacology approach has demonstrated its ability to determine the bioactive ingredients and pharmacological mechanisms of Ge-Gen-Qin-Lian decoction in the treatment of type 2 diabetes. Compared to conventional methods, network-oriented approaches focus on the comprehensive and systematic analysis of therapeutic mechanisms, which makes network pharmacology a preferable approach in the elucidation of the comprehensive mechanisms of complicated chemical compositions in TCM.

In this study, a network pharmacology approach was utilized to identify and demonstrate the active compounds, potential targets, and mechanisms of action of PYTN in CVD treatment [Figure 1]. The chemical similarity ensemble approach (SEA) was employed to predict putative targets for chemicals with an ADME requirement derived from PYTN. The interrelationship between predicted putative targets and CVD-related targets was explored by constructing PPI (protein–protein interaction) networks. The potential key targets of PYTN were used for an enrichment analysis of biological functions and KEGG pathways. Subsequently, we further demonstrated the underlying mechanisms of action of PYTN on CVD using the constructed network and signaling pathways analysis. Network target analysis showed that PYTN could regulate three functional modules (antioxidation, anti-inflammation, and antiapoptosis) in CVD development through multiple signaling pathways, such as PI3K-Akt, mitogen-activated protein kinase 1 (MAPK) and TNF signaling pathways. These results provide a basic understanding of the molecular mechanisms of PYTN in the treatment of CVD-related diseases and may be helpful in drug development.

Figure 1: Flowchart of the approach for uncovering the pharmacological mechanisms of Pei-Yuan-Tong-Nao actions on cerebrovascular disease

**Materials and Methods**

**Compound database**


The active compounds in herbal medicine formulae with potential biological effects often exhibit favorable pharmacokinetics. In this study, QikProp[15] was used to predict absorption, distribution, metabolism, and excretion (ADME) descriptors for the chemicals in 14 herbs in PYTN and filter out the compounds with poor pharmacokinetic properties. The active compounds were selected according to the following criteria: (1) the partition coefficient (QPlogPo/w) values ranged from -2.0 to 6.5; (2) the blood-brain coefficient QPlogBB values ranged from -3.0 to 1.2; (3) the percent human oral absorption was more than 25%; (4) the number of likely metabolic reactions was from 1 to 8; and (5) Lipinski’s rule of five was lower than 5.

**Target identification for Pei-Yuan-Tong-Nao**

Target identification of herbs plays a key role in investigating the pharmacological mechanisms of herbal compounds. In this study, the chemical SEA[11] was used to identify potential targets of chemicals in the PYTN formula. The
SEA method, which is based on the assumption that similar ligands tend to have similar targets, has been widely used for predicting drug side effects and new therapeutic targets. The multivoting scheme based on the four different fingerprints makes multivoting SEA models promising methods for target prediction. The ligand-target pair was considered if predictions with significance level $P < 0.05$ from the five SEA models.

Cerebrovascular disease-related targets

In this work, known CVD-related targets information was derived from the Therapeutic Target Database (TTD)[16] and Comparative Toxicogenomics Database (CTD).[17] We search for CVD-related targets using a keyword search with “cerebrovascular disorder”. Such targets were considered known targets for chemical-disease association marked as “marker/mechanism” or “therapeutic” in the CTD database. In total, we obtained 237 proteins associated with CVD from the TTD and CTD databases.

Protein–protein interaction data

Human protein–protein interaction data were derived from five PPI databases, including STRING,[18] Human Annotated and Predicted Protein Interactions database,[19] Molecular interaction Database,[20] InAct[21] and Human Protein Reference Database.[22]

Network construction

To elucidate the anti-CVD therapeutic mechanisms of the PYTN formula, as well as the roles of specific targets and compounds, we constructed a compound-target (C-T) network and target-function network. Cytoscape3.5.1 (https://cytoscape.org/, USA).[23] software was used to visualize and depict the biomolecular connectivity networks. The nodes and edges represent compounds/targets and the links between them, respectively. Three topological properties, “Degree,” “Node betweenness” and “Closeness,” were defined for assessing the topological importance of each node in the interaction network. “Degree” refers to the number of connections linking to the node by other nodes. “Node betweenness” refers to the number of the shortest paths that pass through the node. “Closeness” refers to the reciprocal of the distance, which is the sum of the length of the shortest paths between the node and all other nodes.

Pathway analysis and go enrichment analysis for Pei-Yuan-Tong-Nao-related targets

To explore the therapeutic mechanism of potential targets of PYTN, the Database for Annotation, Visualization and Integrated Discovery (DAVID)[24] was used to conduct Gene Ontology enrichment analysis. DAVID is a free online functional annotation tool that provides functional interpretation of genes. In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG)[25] was used to perform pathway enrichment analysis.

Molecular docking

The docking algorithm Glide[26] was used to explore the binding modes and affinity between the candidate compounds (MOL000481, MOL00098 and MOL002714) and potential protein targets (MAPK1, epidermal growth factor receptor [EGFR], Androgen receptor [AR] and RAC-alpha serine/threonine-protein kinase [AKT1]). The cocrystal structures of MAPK1 (PDB ID 1PME), EGFR (PDB ID 1M17), AR (PDB ID 1T65) and AKT1 (PDB ID 3MVH) were downloaded from the RCSB Protein Data Bank (http://www.rcsb.org/). The proteins were subsequently processed with the Protein Preparation Wizard implemented in Maestro[27] to add the hydrogen atoms and optimize their positions. All crystallographic water molecules were removed from the system. The resulting structure was refined using the default parameters of energy minimization for docking studies. Each compound structure was prepared using the LigPrep program[28] to add hydrogen atoms, generate stereoisomers, and convert 2D chemical structures to 3D structures. The protonation states were assigned for physiological pH using Epik.[29] The 3D structures were energy minimized using an OPLS_2005 force field. The default parameters were used to generate Glide energy grids and perform molecular docking.

Results and Discussion

Chemicals of Pei-Yuan-Tong-Nao

A total of 638 chemicals from 14 herbs in the PYTN formula were collected from the TCM Analyzer, TCMSP, and TCM Database@taiwan databases. By in silico ADME and Lipinski filtering, the number of compounds predicted to be potential active and contain appropriate pharmaceutical properties was 351. The numbers of predicted active compounds from Radix Paeoniae Rubra, Poria, Testudinis Carapax Plastrum, Polygoni Multiflori Radix, Cervi Cornu Pantomitchium, Lumbricus, Scorpio, Cistanches Herba, Cinnamomi Cortex, Crataegi Fructus, Rehmanniae Radix Praeparata, Hirudo, Asparagi Radix and Glycyrrhizae Radixet Rhizoma were 71, 24, 1, 19, 6, 5, 2, 45, 79, 28, 39, 4, 25 and 3, respectively. Among the 351 active compounds, one molecule was found in the herbs Cistanches Herba and Rehmanniae Radix Praeparata. This finding indicated that there are potential synergies among the herbs of the PYTN formula that might exert pharmacological effects. The structural information of the 351 potential active compounds is presented.

In an effort to analyze the drug-likeness of PYTN active compounds, six physicochemical properties including MW (molecular weight), nHAcc (number of hydrogen bond acceptors), nHDon (number of hydrogen bond donors), RBN (number of rotatable bonds), logP (octanol-water partition coefficient) and PSA (polar surface areas) were calculated for the PYTN herbal active compounds and 7797 molecules in DrugBank database.[30] The distribution diagrams of calculated physicochemical properties for the PYTN herbal active compounds and DrugBank drugs are shown in Figure 2. As seen in Figure 2, the tendency of six parameters from the PYTN herbal active compounds is quite similar to that of the DrugBank drugs, indicating that the active compounds of the PYTN formula are very likely to become drugs.
Targets prediction for chemicals of Pei-Yuan-Tong-Nao

In this study, the SEA algorithm was employed for in silico target prediction of 351 potentially active compounds. The calculated results showed that 650 putative targets were predicted for the 211 potential active compounds. Specifically, 52 (0.08%) predicted targets for 121 potential active compounds were identified as known CVD-related targets. Detailed information about the predicted drug targets for PYTN is described.

The putative target overlap among the 14 herbs of PYTN is listed in Table 1. More interestingly, Radix Paeoniae Rubra and Cistanches Herba shared the most common potential targets, totaling 229. The top five herbs with the most putative targets are Radix Paeoniae Rubra, Poria, Asparagi Radix, Cistanches Herba and Polygoni Multiflori Radix. As Figure 3 shows, the top five herbs cover a total of 570 putative targets. Among them, 110 are common target proteins that exist in each of these five herbs simultaneously. This indicates that these common targets make it possible to magnify the pharmacological effects of the PYTN formula. Among the 110 common target proteins, nine of them are known CVD-related targets, including EGFR, tumor protein p53 (TP53), arachidonate 5-lipoxygenase, prostanoid-endoperoxide synthases (PTGS1 and PTGS2), MAPK1, vascular endothelial growth factor receptor 2 (VEGFR2/KDR), xanthine dehydrogenase, and hypoxia-inducible factor 1-alpha. As reports of previous studies show, EGFR is an EGFR with TGF alpha that is an investigated anti-CVD drug, indicating that the inhibition of epidermal growth factor prevents APOE4 and amyloid-beta-induced cognitive and cerebrovascular deficits.[31] Among the putative targets of PYTN, TP53, or tumor protein p53, has been demonstrated to play an important role in cerebral ischemia.[32] Pifithrin-ɑ, a specific inhibitor of p53, could inhibit the upregulation of p53-upregulated modulator of apoptosis (PUMA) by the p53 transcriptional pathway, thus displaying neuroprotective effects against cerebral ischemia. In 2012, Singh et al. noted that regulation of HIF-1 activity and signaling through small molecules was likely to provide a new therapy for reducing the cellular damage caused by ischemic injury.[33] The HIF-1α subunit plays an important role in the biological activity of HIF-1, which is an essential transcriptional factor that participates in the regulation of various genes responsible for protective responses against CVD. The above discussion suggests the potential role of the PYTN formula in the treatment of CVD.

Investigating the therapeutic mechanism of Pei-Yuan-Tong-Nao for cerebrovascular disease

Network construction

For a better understanding of the pharmacological mechanisms of PYTN, we constructed the PPI interaction network to explore the functional relationship between putative targets and known CVD-related targets. Thus, 650 putative targets of PYTN and 237 known CVD-related targets formed a total of 11442 pairs of PPIs. According to the reports of Li et al.,[34] a node was identified as a hub if its degree is more than 2-fold of the median degree of all nodes.

Afterwards, three topological features, “Degree,” “Node between ness,” and “Closeness” (defined in the “Materials and methods” section), were calculated for each hub in the network to screen the major hubs. The median values of “Degree,” “Node betweenness” and “Closeness” were 41.7,
Wei, et al. Mechanisms of PYTN capsule against cerebrovascular disease

39.5, and 0.01, respectively. Thus, hubs with “Degree” > 41.7, “Node betweenness” > 39.5, and “Closeness” > 0.01 were identified as major hubs. Finally, a total of 21 major hubs were identified. As shown in Figure 4, the C-T network consists of 136 nodes (14 herbs, 108 compounds, and 14 putative targets) and 246 C-T interactions. Previous analysis indicates that several putative targets, such as EGFR and TP53, play a key role in the pathogenesis of CVD. Therefore, the PYTN formula may regulate the CVD disease network by targeting these proteins.

For potential targets, the top three ranked proteins are mitogen-activated protein kinase 1 (MAPK1), nuclear factor NF-κappa-B p105 subunit (NFKB1) and cellular tumor antigen p53 (TP53), interacting with 44, 41 and 35 compounds, respectively [Figure 4]. Among the candidate compounds, genistein (MOL000481), quercetin (MOL000098) and baicalein (MOL002714) are the top three, interacting with 11, 10 and 10 targets, respectively. These compounds have been found to possess a multitude of pharmacological activities. For example, MOL000481, as an isoflavonoid derivate, has broad bioactivities such as antioxidant, antiangiogenic and immunosuppressive effects.\[35\] MOL000481 not only increases the activities of the antioxidant enzymes but also inhibits superoxide anion generation. In addition, MOL000481 has been explored in clinical trials as a selective estrogen receptor modulator to help prevent cardiovascular disease. MOL000098 is a flavonoid derivative and has been found to exhibit antiproliferative effects by modulating EGFR or estrogen-receptor mediated signal transduction pathways.\[36\] Moreover, MOL000098 has anti-inflammatory effects by inhibiting the lipoxigenase and cyclooxygenase pathways, and then preventing the production of proinflammatory mediators. MOL002714 is a potent xanthine oxidase inhibitor confronting

---

**Table 1: Putative target overlap among fourteen herbs of Pei-Yuan-Tong-Nao**

<table>
<thead>
<tr>
<th>Herbs</th>
<th>ChiShao</th>
<th>FuLing</th>
<th>TanDong</th>
<th>RouCongRong</th>
<th>HeShouWu</th>
<th>RouGui</th>
<th>ShuDiHuang</th>
<th>ShanZha</th>
<th>ZhiGanCao</th>
<th>Lurong</th>
<th>ShuiZhi</th>
<th>GuiJia</th>
<th>QiuYin</th>
<th>QuanXie</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChiShao (349)</td>
<td>186</td>
<td>169</td>
<td>232</td>
<td>273</td>
<td>130</td>
<td>91</td>
<td>186</td>
<td>153</td>
<td>154</td>
<td>143</td>
<td>71</td>
<td>25</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>FuLing (313)</td>
<td>123</td>
<td>122</td>
<td>123</td>
<td>144</td>
<td>147</td>
<td>144</td>
<td>122</td>
<td>144</td>
<td>122</td>
<td>123</td>
<td>144</td>
<td>144</td>
<td>144</td>
<td>144</td>
</tr>
<tr>
<td>TanDong (291)</td>
<td>232</td>
<td>202</td>
<td>152</td>
<td>130</td>
<td>154</td>
<td>147</td>
<td>232</td>
<td>152</td>
<td>152</td>
<td>232</td>
<td>130</td>
<td>154</td>
<td>154</td>
<td>154</td>
</tr>
<tr>
<td>RouCongRong (273)</td>
<td>130</td>
<td>129</td>
<td>129</td>
<td>147</td>
<td>152</td>
<td>147</td>
<td>130</td>
<td>129</td>
<td>129</td>
<td>130</td>
<td>129</td>
<td>152</td>
<td>152</td>
<td>152</td>
</tr>
<tr>
<td>HeShouWu (156)</td>
<td>124</td>
<td>129</td>
<td>129</td>
<td>130</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
<td>124</td>
</tr>
<tr>
<td>RouGui (156)</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>15</td>
<td>71</td>
<td>71</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>ShuDiHuang (156)</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td>ShanZha (143)</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>ZhiGanCao (143)</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Lurong (130)</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
<td>106</td>
</tr>
<tr>
<td>GuiJia (25)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>QiuYin (20)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>QuanXie (3)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

---

**Figure 3:** Venn diagrams showing the putative target overlap among the top five herbs of Pei-Yuan-Tong-Nao
oxidative stress-induced cell injury for the treatment of cardiovascular diseases. MOL002714 also exerts antioxidant activity by eliminating the superoxide radical. Thus, PYTN formula exhibits its pharmacological activity through the multi-ingredient and multitarget cooperative mechanism.

**Pathway analysis**

The network of direct interactions among hub nodes was constructed (Figure 5). During ischemia, a large number of cytokines and chemokines are produced, such as IL-1, IL-6, TNF-α and CINC, MCP-1; these cytokines and chemokines aggravate stroke-related brain damage, mainly by inducing inflammation, aggravating edema and disrupting the function of blood-brain barrier (BBB). The inflammatory response is a complex process involving cellular inflammatory response, cytokine response, and response to Toll-like receptor activation. The cellular inflammatory response is mainly characterized by a large number of neutrophils accumulating in the ischemic brain, which can block microcirculation and prevent the complete recovery of cerebral blood flow after reperfusion. At the same time, these neutrophils release oxygen free radicals and proteolytic enzymes, leading to tissue damage. Neutrophil depletion, inhibition of neutrophil adhesion, and inhibition of neutrophil function have been demonstrated to reduce infarct volume and improve prognosis.

Ischemic brain injury first induces cell death through an apoptosis-inducing mechanism, and apoptotic cells predominate in the ischemic penumbra, eventually leading to cell death and tissue necrosis. The three main types of cell death include apoptosis, autophagic death and necrotic death. These three types of cell death mechanisms also affect each other. Autophagy mediates apoptosis, and molecules of the apoptotic pathway can also participate in autophagy regulation. At the same time, autophagy can promote and inhibit necrosis under different conditions. Oxidative and nitrative stress are caused by high levels of Ca²⁺, Na⁺ and ADP in the cells, causing harmful levels of reactive oxygen species in the mitochondria. Nitric

---

**Figure 4:** The compound-target network of candidate compounds in 14 herbs in the Pei-Yuan-Tong-Nao formula. Green circle: candidate compound; blue triangle: herb; pink square: target

**Figure 5:** Interaction network of hubs selected from network of putative targets (pink) of Pei-Yuan-Tong-Nao and known cerebrovascular disease-related targets (red)
oxide synthase (NOS) is activated after cerebral ischemia and increases the production of nitric oxide (NO), which combines with superoxide to produce peroxynitrite, a potent oxidant. After ischemia and reperfusion, the production of superoxide, NO and peroxynitrite increased sharply. A large number of oxygen free radicals activate MMPs, destroying the integrity of the vascular wall and increasing the permeability of the BBB.

Melusin transgenic mice overexpressed melusin, activated the AKT/ERK/GSK3β pathway, and expressed high levels of HSP90 in mouse myocardial injury induced by ischemia-reperfusion, protecting injured cardiomyocytes. Caesalpinia sappan ethanol extract inhibits the JAK-STAT signaling pathway by downregulating the phosphorylation of Jak2 and Stat3, resulting in the inhibition of neuroinflammation.

The hubs were enriched in KEGG pathways using the DAVID v6.8 pathway-enrichment analysis. Figure 6 shows that the PI3K-Akt signaling pathway played an important role in PYTN activity on cerebral hemorrhage. This could be due to the antioxidative and antiapoptotic effects of PI3K-Akt signaling pathway, through which PYTN can improve neuronal function in cerebral hemorrhage. PI3K/Akt is an important upstream molecule that regulates the activity and expression of endothelial NO synthase (eNOS). PI3K transfers inactive cytoplasmic Akt to the plasma membrane and activates Akt to dephosphorylate eNOS Thr49 residues that bind to calmodulin on the plasma membrane, thereby activating eNOS. NO regulated by cerebral blood flow is mainly produced by nitrogen and oxygen molecules at the end of L-arginine catalyzed by eNOS and neuronal NO synthase. Increasing eNOS catalytic activity and gene expression has become an important way of preventing and controlling cerebral vasospasm. Ca2+, Na+, and high levels of intracellular ADP cause mitochondria to produce deleterious levels of reactive oxygen species. Ischemia activates NO synthase and increases the generation of NO, which combines with superoxide to produce peroxynitrite, a potent oxidant. Following reperfusion, there is a surge in the production of superoxide, NO and peroxynitrate. The extra- and intracellular signaling of VEGF and MAPK systems are activated in focal cerebral ischemia. VEGF can promote the establishment of collateral circulation in hypoxic tissue, which is beneficial to restoring the integrity of vascular endothelial cells and rescuing neurons in the ischemic penumbra. The MAPK/ERK and PI3K/AKT signal pathways are stimulated by activating EGFR during brain ischemia/reperfusion to protect cells or potentiate cell injury.

The MAPK signaling pathway is involved in the process of transducing extracellular signals from the cell surface to the interior of the cell and is involved in the activation of downstream proinflammatory mediators and mediates apoptosis. Studies have found that CBSA-PEG-TIIA-NPs can inhibit the expression of p38MAPK, p-ERK1/2 and p-JNK and inhibit the inflammatory response by blocking the MAPK signaling pathway. It has also been found that TTR, a neurotrophic factor, activates the MAPK signaling pathway ERK1/2 and Akt through Src, stimulates neurite outgrowth in megalin-dependent manner, and promotes neuroprotection under ischemic conditions. These signaling pathways may be involved in the antioxidative and anti-inflammatory effects of PYTN against cerebral ischemia. The OPG/RANKL/RANK axis is a critical inflammatory signaling system in the ischemic brain, and PYTN may be involved in attenuating the inflammatory immune response and neuroprotection by blocking osteoclast differentiation.

The PI3K/Akt signaling pathway is one of the key pathways regulating cell survival, proliferation and metabolism. It has been found that melatonin can reduce apoptosis and reduce neuronal apoptosis through the PI3K/Akt pathway. It has also been found that ARIA regulates EPC angiogenesis and regulates neovascularization by modulating PI3K/Akt/eNOS signaling. TRPV4 antagonist can attenuate the phosphorylation of Akt protein in hippocampus cells and increase the level of P-p38 MAPK protein in MCAO mice after 48 h of reperfusion and protect injured nerve cells.

NF-κB is a key regulator of inflammation, platelet aggregation, and atherogenesis, and L5 + Aβ mediates platelet activation and aggregation through NF-κB signaling, which is a predisposing factor for thrombotic events in patients with low-density lipoprotein. At the same time, studies have shown that TRIM9 can inhibit the production of NF-κB-dependent proinflammatory mediators, indicating that NF-κB is an important inflammation-mediated pathway in the mechanism of cerebral ischemia.

In addition, PYTN could serve a neuroprotective function by stimulating antioxidant pathways, for instance, the TNF signaling pathway. Research indicates that drugs targeting the TNF signaling pathway could be beneficial in treating
stroke.[53] There is also evidence that drugs can alleviate the cognitive impairment induced by cerebral ischemia through an antiapoptotic mechanism in which the HIF-1 signaling pathway is involved.[54] HIF-1α is an intracellular oxygen homeostasis regulator that adapts to the body’s adaptive response under hypoxic conditions. In hypoxia, HIF-1α aggregates with HIF-1β into HIF-1 in the nucleus. HIF-1 binds to vascular endothelial factor to mediate transcriptional activation of VEGF, activates related ischemic and hypoxia-mediated transduction pathway, and promotes damaged brain tissue. In neonatal angiogenesis, HIF-1α may be involved in mediating neuronal apoptosis in craniocerebral injury. HIF-1α is a cellular transcription factor for detecting hypoxic environment. HIF-1α plays an important role in the development and progression of hypoxic-ischemic brain damage caused by craniocerebral injury. After cerebral ischemic injury, the expression of VEGF in the brain was significantly improved compared to before the injury occurred. A hypoxic environment promotes HIF-1 levels and targets downstream VEGF expression after ischemic stroke. VEGF induces BBB leakage and edema in the acute phase. It has been found that angiopoietin-like 4 (ANGPTL4) can regulate Src kinase and the PI3K-Akt signaling pathway to protect against VEGF-mediated BBB disruption and increased permeability.[55] The neurotrophin signaling pathway also plays an important role in regulating neuronal apoptosis.[56] According to their associated pathways, these hub nodes were mainly involved in the pathological processes of oxidation, apoptosis and inflammation during the CVD progression [Figure 5].

Validation of compound-target interactions by molecular docking

The potential interactions between three candidate compounds (MOL000481, MOL000098 and MOL002714) and their receptors (MAPK1, EGFR, AR and AKT1) were evaluated using the molecular docking method by the Glide SP module. To characterize the pharmacological profiles, the top binding pose [Figure 7] and docking scores [Table 2] between the three candidate compounds and cocrystallized ligands retrieved from their respective receptors were compared.

For MAPK1, the cocrystal ligand is a potent pyridinyl imidazole inhibitor (SB203580) exhibiting a Ki of 0.76 nM.[57] Figure 7a-c display the interaction modes of the MAPK1/MOL000481, MAPK1/MOL000098, and MAPK1/MOL002714 complexes. Comparison of MAPK1/MOL000481, MAPK1/MOL000098, MAPK1/MOL002714 and MAPK1/SB203580 identified M108 as an important residue for the binding of flavonoid compounds by forming an intermolecular hydrogen bond. The phenyl of MOL002714 inserts into hydrophobic region surrounded by residues I53, L75, I84, 186, L103, V104 and T105 and creates favorable hydrophobic interactions. Of note, the docking score of MOL002714 (-8.17 kcal/mol) was better than the docking scores of MOL000481 (-7.83 kcal/mol) and MOL000098 (-7.71 kcal/mol).

For MOL000481, the binding modes of EGFR/MOL000481, AR/MOL000481 and AKT1/MOL000481 are presented in Figure 7d-f. The docking score of cocrystal ligands (erlotinib for EGFR with a K_i of 0.1 nM, SRC2-2 for AR with a K_i of 0.2 nM, compound 42 for AKT1 with an IC_{50} of 12 nM)[58-60] are better than those of MOL000481. However, MOL000481 captures the binding characteristics of cocrystal complexes. For example, MOL000481 is hydrogen-bonded with residues M769 in EGFR, N705 and R752 in AR, and E228, A230 and D292 in AKT1, similar to the binding modes of cocrystal ligands and their respective receptors. A comparison of the interactions of MOL000481 with EGFR/AR/AKT1 to those between cocrystal ligands and receptors shows that the flavonoid compounds and cocrystal ligands bind to the proteins in a similar fashion.

Based on the above understanding, the interactions between these candidate compounds and potential targets could comprise the basis of PYTN’s biological activities.

Conclusions

In this investigation, a network pharmacology strategy including ADME property filtering, target prediction, interaction network construction, and pathway analysis was employed to explore the pharmacological mechanisms of PYTN capsule acting on CVD. The results suggest that 351 out of 638 compounds in 14 herbs and 650 proteins are potentially active compounds and putative CVD-related targets, respectively. These active compounds and putative targets may provide a basis for pharmacological profiles of PYTN therapy in CVD. In addition, three functional modules including antioxidation, anti-inflammation and antiapoptosis may play a key role in the action of PYTN capsule in treating CVD. Moreover, multiple signaling pathways are involved in the implementation of these functions, such as the PI3K-Akt signaling pathway, MAPK signaling pathway and TNF signaling pathway. In a word, PYTN capsule utilizes multiple factors, multicomponent and multitarget, to exert pharmacological and therapeutic effects in the treatment of CVD. This investigation may facilitate the systematic understanding of the mechanisms of action of PYTN for the treatment of CVD and provide insight into the development of TCMs and clinical strategies for complex diseases.

Acknowledgments

This study was supported by the National Key R and D Program of China (No. 2017YFC1104400).
Figure 7: The potential binding modes of three candidate compounds (MOL000481, MOL000098 and MOL002714) in their predicted binding sites. (a) The interaction mode of MOL000481 with MAPK1 in 1PME. (b) The interaction mode of MOL000098 with MAPK1 in 1PME. (c) The interaction mode of MOL002714 with MAPK1 in 1PME. (d) The interaction mode of MOL000481 with EGFR in 1M17. (e) The interaction mode of MOL000481 with AR in 1T65. (f) The interaction mode of MOL000481 with AKT1 in 3MVH. (The cocrystallized ligands are shown as lines, whereas the three candidate compounds modeled into the receptors are shown as sticks. The intermolecular hydrogen bond interactions are represented using red dotted lines. Receptors are shown as a cartoon, and residues in active sites are shown as sticks.)

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES
Wei, et al. Mechanisms of PYTN capsule against cerebrovascular disease


43. Lennym F. Signal Transduction in Focal Cerebral Ischemia: Experimental Studies on VEGF, MAPK and Src Family Kinases; Uppsala University; 2002.


