Exploration on Anti-Depression Mechanism of Baihe Zhimu Decoction Based on Network Pharmacology and Molecular Docking

Xianbin ZHANG¹, Caihong WANG²

¹Jinan University Zhuhai Campus, Zhuhai, Guangdong, 519000, China
²Jinan University Zhuhai Campus, Zhuhai, Guangdong, 519000, China

Abstract: Objective To analyze anti-depression mechanism of Baihe Zhimu decoction (BZD) based on network pharmacology method, which provides reference for the development of new drugs and the clinical application of classical prescriptions. Method The main chemical components and targets of Baihe and Zhimu were obtained through traditional Chinese medicine pharmacology system technology platform (TCMSP) database, and the active components of TCM were filtered according to ADME; Major targets for anti-depression were get through Gencards, OMIM and DRUGBANK databases; Protein interaction analysis was performed using the String platform; Build PPI networks and mine potential protein functional modules in the network; The Metascape platform was used to analyze the "drug-ingredients-target" and its involved biological processes and pathways; Finally, the molecular docking validation was performed by Systems Dock Web Site. Results The core active ingredients of BZD treating depression are kaempferol and Stigmasterol, The core targets are AKT1, TNF, TP53, PTGS2, and CASP3. The biological pathway of the anti-depression mainly acts on Lipid and atherosclerosis, Chemical carcinogenesis and receptor activation. Molecular docking results showed that AKT1, TNF and TP53 have good affinity with components kaempferol and Stigmasterol. Conclusion This study initially revealed the mechanism of multicomponent, multiple target and multiple pathway of anti-depression, which may be related to neuroactive ligand-receptor interaction, atherosclerotic, PI3K-Akt and TNF signaling pathway.

Keywords: Baihe Zhimu decoction, Molecular Docking, Depression, Data analysis, Network Pharmacology

1. Introduction

Depression is a common psychiatric condition that causes emotional and physical damage, with significant and persistent low state of mind as the main clinical features, and state of mind is not commensurate with its situation, serious suicidal thoughts and behaviors may occur in severe case. The main performances of depressive disorders are sad, loss of interest and pleasure, guilt and self-worth, appetite and sleep disturbances, etc.¹, belong to the category of Traditional Chinese medicines "depression syndrome".

¹ First Author: Xianbin ZHANG (E-mail: 15170627820@163.com)
² Corresponding Author: Caihong WANG, Jinan University Zhuhai Campus, Zhuhai, Guangdong, 519000, China; E-mail: wcaihong1996@163.com
It continue to be a global burden affecting approximately 350 million people worldwide[2]. At present, the treatment drugs for depression are mainly western medicine, but their therapeutic effects were not satisfying, poor compliance, and high recurrence rate after drug withdrawal[3]. Hence, the exploration and development of ideal antidepressant drugs from natural plants has attracted more and more international attention.

In China, Chinese herbal prescriptions have been popular for treating depression. Baihe Zhimu Decoction (BZD) is one of the Chinese herbal recipes. A famous Chinese herbal formula include Baihe and Zhimu. It was first mentioned in the famous prescription book "Jin Gui Yao Lue" compiled by the Chinese physician Zhang Zhong Jing. In clinical practice, it has been used to therapy "Bai He disease" since the Eastern Han Dynasty. Patients with "Lily's Disease" show symptoms similar to those of depression, including mental instability, limb dyskinesia, anorexia, and sensory disturbance.

Many studies suggested, research on the basis and mechanism of the use BZD for the treatment of depression are emerging. Liu Qi et al. demonstrated that BZD has a better antidepressant effect, in which its mechanism is related to increasing the serum and the level of monoamine neurotransmitters in the cerebral cortex[4]; Yuan Li et al. discovered that BZD ameliorates hippocampal neuronal damage in depressed rats and may be associated with increased expression of Cam, CMK II, and CREB in hippocampal signaling pathways.[5]. The cause of depression is complex, which is a pathological network formed by the disorder of polygene and multifunctional protein interaction. Therefore, how to comprehensively and systematically explain the antidepressant effect and mechanism in people has become a bottleneck restricting its development and utilization.

Network pharmacology is a multidisciplinary, systematic and comprehensive approach to the mechanisms of pharmacological intervention in disease networks. This coincides with the Comprehensive principles view, syndrome differentiation and prescription compatibility of traditional Chinese medicine, which helps to reveal the scientific connotation of Chinese traditional medicine compound, discover drug targets, and guide the optimization of old prescription and new compound compatibility[6].

The objectives of this study is to use network pharmacology to inquiry molecular mechanisms of antidepressant depression which based on the material basis of BZD and to provide an ensured theoretical basis for subsequent research.

2. Materials and methods

2.1. Gathering and selecting of relevant targets of BZD

The chemical components of Baihe and Zhimu were collected by the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). The objective ingredients of the two herbal medicines were initially selected based on their Oral Bioavailability (OB) ≥ 30% and Drug-Likeness (DL) ≥ 0.18 ADME attribute values to extraction of BZD active substances and their target proteins, and a known target containing unknown active substances based on previous literature reports. The targets obtained from the TCMSP database screening were blend with the known targets supplemented by literature reports, and the protein targets of the collected
compounds acting were then standardized, corrected and de-duplicated through the Uniprot protein database for standardized protein target information.

2.2. Depression-related target collection and screening

Using "Depression" as a keyword, we collected potential disease targets for depression from the GeneCards database and the OMIM database. This was supplemented by mining the DRUGBANK database for clinical frontline Western medicine target that modulate depression. The protein targets obtained were knock over into gene symbols through Uniprot database, and above retrieved targets integrated and de-duplicated to obtain the disease-related target database.

2.3. Building of PPI network of vigorous ingredient-depression disease target of BZD

In order to clear up the interaction among the herbal targets of BZD and Depression, the intersection between with two targets was handled and a Venn diagram was simplified by the Venny2.1. The intersected targets were also taken to STRING11.0 to draw a protein interaction network, setting the biological species as "Homo sapiens" and the minimal interaction threshold as “highest confidence” (>0.9). The PPI network was further tested by the MCODE module in CytoScape 3.8.0 to acquire the functional modules of the potential proteins and then analyze their involvement in biological processes and functions.

2.4. Functional and pathway analysis of BZD component expression targets.

The targets of BZD for Depression were recorded into the Metascape platform, set to \( P < 0.01 \). The results of our major biological analysis of the enrichment of the pathway of metabolism are being visualized in the context of Origin Labor 2018.

2.5. Composition of BZD component-depression disease target-pathway network map

Constructing of BZD component-depression disease target pathway network map through CytoScape 3.8.0. Network topology parameters such as Degree, Betweenness and Closeness of active ingredients and targets were analysed using the CytoScape 3.8.0 embedded tool to determine core objectives and key active ingredients that exerted medicinal effects.

2.6. Molecular docking validation

The first three targets in the degree value of BZD component-depression disease target-pathway network map were analyzed and their crystal structure of proteins were get from the RCSB PDB database. Protein crystal structures were dehydrated and hydrogenated using AutodockTools 4, and the receptor structures were prepared. The small molecule library was prepared using Open Babel and Autodock 4 programs for splitting and other preparations, and the Autodock 4 program was used for docking, and results were finally imported into Pymol for visualization of the docking results.
3. Results

3.1. Acquisition of active ingredient targets of BZD

In the TCMSP, "Baihe" and "Zhimu" were searched in turn, and the screening conditions were OB ≥ 30% and DL ≥ 0.18, and 22 active ingredients were obtained, among which the number of active ingredients of Baihe was 7 active ingredients and 67 targets, and 15 active ingredients and 146 targets of Zhimu. Aim to avoid the omission of some validated chemical components due to the non-compliance of parameters, the chemical components of these drugs were supplemented by searching the published literature, and then the total number of active components and target proteins of the drugs was 21 and 99 after correction and de-duplicate of the collected chemical components and target proteins by Uniprot database. (Table 1)

Table 1: Number of herbal drug components and targets of BZD for depression

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number of active ingredients</th>
<th>Target Points</th>
<th>Post-calibration target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baihe</td>
<td>7</td>
<td>67</td>
<td>31</td>
</tr>
<tr>
<td>Zhimu</td>
<td>15</td>
<td>146</td>
<td>68</td>
</tr>
</tbody>
</table>

3.2. Depression-related target collection and screening

Depression-related targets were obtained from various databases as follows: 3594 targets from Genecards and 37 targets from OMIM. The targets for depression geted from all databases were aggregated and de-duplicated to obtain 3537.

3.3. Construction of intersection target Venn diagram and PPI network

The intersection of 99 targets in the Chinese medicine target database of BZD and 3537 targets in the target database of Depression disease were intersected using Venny2.1 online tool and a Venn diagram was drawn, 76 targets were intersected, and the intersection of the two targets was shown (Figure 1), and the depression-Chinese medicine component-target diagram was also drawn using CytoScape 3.8.0 (Figure 2). Aim to understand the interrelationship of the intersecting targets more intuitively, the 76 intersecting targets were input into the STRING to Construct the PPI network (Figure 3).

![Figure 1. Venn diagram](image-url)
Figure 2. Depression-Chinese medicine composition-target map

Figure 3. PPI network
3.4. Functional and pathway enrichment analysis of BZD

Using Metascape platform to analyze the signal pathway of BZD in the therapy of depression, and using Origin Lab 2018 to do the visualization analysis, The results revealed that the function of multiple targets is proximity related to the development of depression. GO enrichment analysis is mainly carried out from the following three parts: Molecular Function, Biological Process, Cellular Component. Among these biological processes are Response to drug, Reactive oxygen species metabolic process, Response to metal ion, Response to steroid hormone, Response to nutrient levels (Figure 4). The pathways involved are Lipid and atherosclerosis, Chemical carcinogenesis - receptor activation, Kaposi sarcoma–associated herpesvirus infection, Pathways of neurodegeneration – multiple diseases. Related targets for depression are mainly refined in Nuclear receptor activity, Amide binding, Ligand-activated transcriptional activity, Peptide binding, drug binding, etc. (Figure 5).
3.5. Construction of component-depression disease target-pathway network map of BZD

CytoScape 3.8.0 was taken to build the Construction of BZD component-depression disease target-pathway network map (Figure 6).
The network topology parameters of BZD in the treatment of depression were resolved through cytoscape 3.8.0. The core components and the core targets were obtained (Table 2 and Table 3).

CytoScape network analysis confirmed that Kaempferol had connectivity of 24, a mediocrity of 0.461106656, and a tightness of 0.428571429, predicting Kaempferol as the key component of BZD for the treatment of depression, followed by Stigmasterol, Beta-sitosterol, Diosgenin, and 3-Demethylcolchicine.

AKT1 had a relevance of 48 in network, a mediocrity of 0.114927065, and a tightness of 0.718446602, predicting AKT1 to be the main target for the treatment of depression with BZD. TNF, TP53, PTGS2, CASP3, VEGFA, JUN, PPARG, ESR1, and CAT were also relatively important targets.

### Table 2

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Degree</th>
<th>Mediation</th>
<th>Tightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaempferol</td>
<td>24</td>
<td>0.461106656</td>
<td>0.428571429</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>17</td>
<td>0.335739107</td>
<td>0.400921659</td>
</tr>
<tr>
<td>Beta-sitosterol</td>
<td>13</td>
<td>0.258219727</td>
<td>0.386666667</td>
</tr>
<tr>
<td>Diosgenin</td>
<td>11</td>
<td>0.217856188</td>
<td>0.379912664</td>
</tr>
<tr>
<td>3-Demethylcolchicine</td>
<td>7</td>
<td>0.844693932</td>
<td>0.533742331</td>
</tr>
</tbody>
</table>
3.6. Molecular docking validation

The molecular docking website SystemsDock can analyze ligand selectivity and ligand actions through docking simulations and molecular pathway maps on the basis of complex networks, and then evaluate protein-ligand binding potential based on their combined characteristics. Molecular docking of Kaempferol and Stigmasterol with core targets AKT1, TNF, and TP53 was performed by AutodockTools4 software molecules, as shown in Table 4 below. Molecular docking binding kinetics <-1 kcal, mol-1 indicates connecting activity, and <-5 kcal.mol-1 indicates good connecting activity. The results showed that Kaempferol and Stigmasterol had better connecting activity to the core targets AKT1, TNF, and TP53.

<table>
<thead>
<tr>
<th>Target Points</th>
<th>Degree</th>
<th>Mediation</th>
<th>Tightness</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>48</td>
<td>0.114927065</td>
<td>0.718446602</td>
</tr>
<tr>
<td>TNF</td>
<td>43</td>
<td>0.054690213</td>
<td>0.666666667</td>
</tr>
<tr>
<td>TP53</td>
<td>39</td>
<td>0.021953476</td>
<td>0.627118644</td>
</tr>
<tr>
<td>PTGS2</td>
<td>38</td>
<td>0.019125298</td>
<td>0.621848739</td>
</tr>
<tr>
<td>CASP3</td>
<td>37</td>
<td>0.02967528</td>
<td>0.616666667</td>
</tr>
</tbody>
</table>

Table 4 Molecular docking results

<table>
<thead>
<tr>
<th>Binding energy (Kcal/mol)</th>
<th>Kaempferol</th>
<th>Stigmasterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>-6.83</td>
<td>-6.67</td>
</tr>
<tr>
<td>TP53</td>
<td>-6.68</td>
<td>-5.96</td>
</tr>
<tr>
<td>TNF</td>
<td>-6.88</td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

Depression is a serious mental illness that worsens mental and physical health. The use of BZD to treat depression is better than the clinical first-line medicine, especially has great potential in improving symptoms, for example, Liu Zhirui found that compared with fluoxetine, BZD can affect more metabolic pathways and exert antidepressant effects\(^7\). Studies have shown that the BZD is rich in saponins, flavonoids, anthraquinones, and alkaloids, which can improve cardiovascular activity, regulate endocrine, hypoglycemic, and anti-depression effects\(^8\). Pharmacological studies on BZD treating depression mainly focus on decoctions or effective parts, less on monomer components, and the specific pharmacodynamic substances and mechanisms are unclear\(^9\).

The study began with the analysis of the active ingredients of BZD as Kaempferol and Stigmasterol through network pharmacological methods. Kaempferol is a kind of flavonol compound, which has strong anti-inflammatory, anti-diabetes, anti-cancer and neuroprotective effects\(^10-13\), studies have shown that it can reduce the damage of hippocampus in depressive model rats by anti-inflammatory, antioxidant and reducing the rate of apoptosis of hippocampal neurons, thus alleviating depression in mice\(^14\).
Stigmasterol belongs to the steroidal active ingredients, which have a wide range of biological activities under the action of functional groups around their tetracyclic core. It has been reported that Stigmasterol showed significant antidepressant effects in mice forced swimming tests by shortening the rest time of mice, increasing the motor activity of mice and antagonizing the hypothermia of mice\(^{[15]}\).

The results of this study suggested that the anti-depression targets of BZD mainly focused on AKT1, TNF, TP53, PTGS2 and CASP3. AKT1, as a downstream product of PI3K pathway, is closely related to depression. Activation of PIK3CA-AKT1 signaling pathway plays an antidepressant-like role in depressed olfactory bulbectomy rat models\(^{[16]}\). It has been shown that Akt1 regulates the function of antidepressants and contributes to the formation of synaptic plasticity and neurotransmission\(^{[17]}\). TNF is an inflammatory factor. Studies have shown that in Depression, inflammatory response will activate the HPA axis, and stimulation of the HPA axis will relevant to the relevant of pro-inflammatory cytokines IL-1β, TNF-α, IL-6\(^{[18]}\). Köhler et al. conducted a statistical review and meta-analysis of cytokines and chemokines in depressed patients and healthy patients, and the outcomes exhibit that compared with healthy patients, the level of TNF-α in depressed patients was significantly add, indicating that inflammatory factors were closely related to depression\(^{[19]}\). AKT signaling is at the crossroads of "damage response genes" and plays an antidepressant role by regulating the synthesis of GSK-3 by its downstream effector glycogen\(^{[20]}\). TNF acts through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR to regulate the upstream signaling sites of cell apoptosis, thereby reducing depression levels\(^{[21]}\). In conclusion, it is speculated that Baihe Zhimu Decoction exerts anti-depression effect through multi-component and multi-target synergism.

5. Conclusion

This study predicted the feasibility of BZD in the treatment of depression, and further clarified its characteristics of multi-ingredient, multi-target and multi-pathway treatment. However, the regulation of key biological processes and key pathways still needs to be further studied, which should be verified in vitro cell experiments and in vivo animal experiments to ensure the scientific and correct prediction results.

References


