Pharmacological mechanism of Liuwei Dihuang Decoction in treating Osteoporosis and Alzheimer’s disease based on Network Pharmacology

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Abstract

Objective: To predict the active components, targets of Liuwei Dihuang Decoction for the treatment of osteoporosis and Alzheimer’s disease with network pharmacology analysis. Methods: The components and targets of all the herbs of Liuwei Dihuang Decoction were obtained from ETCM database, Osteoporosis and Alzheimer’s disease targets were extracted from the GeneCards and DrugBank databases, selected based on STRING database for PPI network construction and build the network through the software of Cytoscape, Kyoto Encyclopedia of Genes and Genomes and Gene Ontology analyses were carried out by R software. Results: 181 active compounds and 762 disease-related common targets obtained from Venn Diagram. IL6, TNF, PPARA, PPARG, SRC and other 11 targets are the node protein of the whole network. In the multisystem biological reaction process such as neurotransmitter, glucose and lipid metabolism, calcium and phosphorus metabolism. Conclusion: Liuwei Dihuang pill may play a co-therapeutic role in Osteoporosis and Alzheimer’s disease by participating in the multi-system biological reaction process such as neurotransmitter, glucose and lipid metabolism, calcium and phosphorus metabolism.

Keywords: Liuwei Dihuang Decoction, Osteoporosis, Alzheimer’s Disease, Network pharmacology
Introduction

Treatment determination based on pattern differentiation is the basic principle of diagnosis and treatment of diseases. One disease may include several different syndromes, and conversely, different diseases may exhibit the same pattern in the course of their development. Thus, TCM may “treat the same diseases with different methods”; or it may “treat different disease with the same therapeutic method”. So, called “same treatment for different diseases” refers to the fact that, for different diseases, because there is the same path mechanism in their course of development, the same or similar treatment is given. In traditional Chinese medicine theory, osteoporosis is like the equivalent of “bone dysfunction” and “bone blight”; Alzheimer’s disease is like the equivalent of “senile dementia”. The deficiency of kidney essence is believed as the fundamental cause of the both diseases.

Osteoporosis (OP) and Alzheimer’s Disease (AD) are two common multifactorial progressive degenerative diseases. Due to the aging of the population and the extension of life expectancy, OP and AD constitute a worldwide significant social burden [1, 2]. Epidemiological investigations have shown that patients with osteoporosis have a higher risk of AD, and patients with AD have a higher risk of osteoporosis. Studies have shown that the two may influence each other in the pathogenesis [3, 4]. In recent years, bones are considered an endocrine organ, secreted proteins, cytokines and other substances can affect the function of the nervous system, and may play a certain role in the occurrence and development of Alzheimer’s disease. The central nervous system can also regulate bone mass by producing specific neurotransmitters and processing peripheral hormone signals that affect bone homeostasis [5]. The proposal of this theory provides a new research direction for the common pathogenesis and disease treatment of OP and AD.

Liuwei Dihuang Decoction was published in Qian Yi’s Xiao Er Yao Zheng Zhi Jue in the Song Dynasty. It mainly focuses on the pathological characteristics of children’s kidneys often have no substantial disease, but lack adjustment and nutrition. Mainly manifested as malnutrition, pale complexion, and so on, etc. [6]. Network pharmacology is based on network biology and multi-directional pharmacology, and is suitable for revealing the complex mechanism of action of Chinese medicine. This study uses network pharmacology methods to explore, and explain the potential comorbidity mechanism links between osteoporosis and Alzheimer’s disease.

Methods

Screening of active ingredients of drugs and screening of drug targets

Liuwei Dihuang Decoction includes Radix Rehmanniae preparata, Poria, Rhizoma Alismatis, Cortex Moutan, Fructus Corni and Rhizoma Dioscoreae. The active components and targets of herbal medicines were obtained by searching the ETCM database.

Disease target screening for osteoporosis and Alzheimer’s disease

Use the GeneCards database and enter the keywords “Osteoporosis” and “Alzheimer’s disease” to search for disease targets related to osteoporosis and Alzheimer’s disease, and use Venn to obtain drug targets and disease targets.

Analysis of the interaction between targets

Import the common targets of drugs and diseases into the STRING database to obtain the interaction relationship between potential targets, and use the CytoNCA plug-in to screen for important targets with a degree value greater than the median value.

Target function annotation analysis

Use the R language Cluster Profiler package to perform GO function enrichment analysis and KEGG pathway enrichment analysis for potential targets. P < 0.05 was regarded as the biological signal pathway with statistically significant difference, and the results were sorted according to the P value.

Results

Screen the active ingredients and predicted targets of Liuwei Dihuang Decoction

After screening, the 181 active ingredients of Liuwei Dihuang Decoction were obtained from the ETCM database, including 12 Radix Rehmanniae preparata, 55 Fructus Corni, 15 Cortex Moutan, 45 Rhizoma Dioscoreae, 33 Poria, 30 Rhizoma Alismatis, and repeated ingredients. Nine, such as diosgenin, cornoside, ursolic acid, retinol, paeonol, resveratrol, etc. are all related to OP and AD. 762 predicted disease targets were obtained from Liuwei Dihuang Decoction, and the components from Radix Rehmanniae preparata, Fructus Corni, Rhizoma Dioscoreae, and Poria correspond to a large number of disease targets. The main information of Liuwei Dihuang Decoction is shown in Table 1.

Table 1. The main information of Liuwei Dihuang Decoction

<table>
<thead>
<tr>
<th>Chinese Name</th>
<th>English Name</th>
<th>Latin Name</th>
<th>Abbreviation</th>
<th>Dosage (g)</th>
<th>Effect</th>
<th>Main ingredients</th>
<th>Main targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>熟地黄</td>
<td>Prepared Rehmannia Root</td>
<td>Radix Rehmanniae preparata</td>
<td>DH</td>
<td>24</td>
<td>Nourish yin</td>
<td>Fructose; Daucosterol; Cetylic Acid</td>
<td>TNF, PPARA, TLR4, INS</td>
</tr>
<tr>
<td>山茱萸</td>
<td>Asiatic Cornelian Cherry Fruit</td>
<td>Fructus Corni</td>
<td>SZY</td>
<td>12</td>
<td>Invigorate the kidney and stop emission</td>
<td>Ursolic Acid; Malic Acid; Cetylic Acid</td>
<td>PPARA, PPARG, SRC, PPARG</td>
</tr>
<tr>
<td>牡丹皮</td>
<td>Tree peony Bark</td>
<td>Cortex Moutan</td>
<td>MDP</td>
<td>9</td>
<td>Nourishing yin and blood nourishing</td>
<td>Saffruticosol A; Paconol; ESR1, CBRE1</td>
<td></td>
</tr>
<tr>
<td>山药</td>
<td>Common Rhizome</td>
<td>Yarn Rhizoma Dioscoreae</td>
<td>SY</td>
<td>12</td>
<td>Invigorate the kidney and stop emission</td>
<td>Sitosterol; Stigmasterol; Dioscin; Campesterol; Deltomin</td>
<td>PPARA, ERS1, IL6</td>
</tr>
<tr>
<td>茯苓</td>
<td>Indian Bread</td>
<td>Poria</td>
<td>FL</td>
<td>9</td>
<td>Eliminating dampness and resolving phlegm</td>
<td>Lauric Acid; Caprylic Acid; PPARA, PPARG, INS</td>
<td></td>
</tr>
<tr>
<td>泽泻</td>
<td>Oriental Waterplantain Rhizome</td>
<td>Rhizoma Alismatis</td>
<td>ZX</td>
<td>9</td>
<td>Eliminating dampness and resolving phlegm</td>
<td>Neoisolidol; Alisol A; Alisol A</td>
<td>TNF, IL1B, ESR1</td>
</tr>
</tbody>
</table>

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Screening disease targets
We obtained 2219 osteoporosis disease targets and 2795 Alzheimer’s disease targets through GeneCards database, and then intersected 762 Liuwei Dihuang Decoction prediction targets with the two disease targets, and obtained 144 common targets. The common targets are shown in Figure 1. And the main target of Liuwei Dihuang Decoction is in Figure 2.

Drug target network
Import the above-mentioned 144 intersection targets into the STRING software platform to obtain the protein-protein interaction data list and PPI network diagram, as shown in Figure 3. After screening according to the degree value greater than the median value, 17 top-ranked important targets such as INS, GAPDH, AKT1, IL6, TNF, VEGFA, MAPK3, SRC, IL1B were obtained (Figure 4). The 17 targets were imported into the BioGPS platform to obtain tissue distribution information. The results showed that the 17 targets were mainly distributed in the nervous system, liver, skeletal muscle, smooth muscle, fat cells, and other parts.

Enrichment analysis
Use the R Cluster Profiler package to perform GO and KEGG enrichment analysis on 144 targets of the potential effects of Liuwei Dihuang Decoction in the treatment of osteoporosis and Alzheimer’s disease. The main results are shown in Figures 5 and 6. GO function enrichment According to \( P \leq 0.05 \) and \( q \leq 0.05 \), 2433 items were obtained. It was found that Liuwei Dihuang Decoction play an important role in the treatment of osteoporosis and Alzheimer's disease. This study found 2433 GO terms, including 2184 biological process (BP) terms, 70 cellular component (CC) terms, and 148 molecular function (MF) terms. The top 10 most important terms of BP, CC, and MF are shown in Figure 3. BP terms mainly include response to drug, regulation of protein secretion, regulation of peptide secretion positive regulation of small molecule metabolic process response to peptide hormone regulation of small molecule metabolic process protein secretion. CC terms were mainly enriched in membrane raft, membrane microdomain, membrane region, secretory granule lumen. MF terms mainly involved nuclear receptor activity, ligand-activated transcription factor activity, carboxylic acid binding, steroid binding, organic acid binding, steroid hormone receptor activity, transcription coactivator binding, drug binding.
One hundred and seventy-seven pathways were acquired through the KEGG pathway enrichment analysis. The top 30 most important terms of the KEGG pathway enrichment analysis were chosen for the visual analysis. The potential targets of Liuwei Dihuang Decoction in treating OP and AD were principally enriched in pathways in HIF-1, Insulin resistance Human cytomegalovirus infection, Tuberculosis, AGE-RAGE signaling pathway in diabetic complications, Lipid and atherosclerosis and Prolactin signaling pathway.
Discussion

Deficiency of kidney essence affects the bones, and skeletal development delays can be seen, such as delayed fontanelle in children, soft bones or skeletal deformities; adults with weak waist and knees, unable to stand and walk for a long time; elderly people with low back and leg pain, easy to fracture, tooth loss, etc. Insufficient kidney essence, involving the brain, children with
developmental delay, low intelligence; adult fatigue, dizziness, tinnitus, memory loss; old people forgetful, slow reactions, and low intelligence. Clinical studies have found that patients in the OP group and AD group with deficiency of kidney essence syndrome have unresponsiveness, forgetfulness, soft bones, scorching/whitening/hair loss, tooth shaking/tooth loss, deafness, sluggish expression, dizziness, tinnitus, frequent urination. Frequent nocturia, less complexion and other symptoms [7], prove that osteoporosis and Alzheimer's "different disease" have "the same syndrome (deficiency of kidney essence)". The pathogenesis of osteoporosis and Alzheimer's disease is complex. Although a large number of biological targets with potential therapeutic effects have been identified, the current first-line drugs cannot meet the needs of multi-target therapy. The holistic treatment concept of Chinese medicine fits the characteristics of the two diseases, and has unique advantages in the prevention and treatment of the two diseases. Annotation to Jiangyueyuan Ancient Prescriptions: Those who lack the essence can make up for it. The bitter taste of Radix Rehmanniae preparata enters the kidneys, solidifies the root of the string, and the salty taste of Rhizoma Alismatis enters the bladder, opens the source of Qi and replenishes the essence of Shaoyi and Taiyang. The sour taste of Fructus Corni enters the liver, replenishing the extreme work, the pungent taste of Fructus Corni in the gall bladder, clearing the vitality of the middle and upright, both of them replenish the essence of Jueyi and Shaoyi. The sweet taste of Rhizoma Dioscoreae enters the spleen, which is the mechanism for invigorating and disappearing. The taste of Poria enters the stomach and facilitates the inflow and outflow. Both of them replenish the essence of Taiyi and Yangming [8]. It can be seen that Liuwei Dihuang Decoction nourishes the six meridians and cures the viscera, which can consolidate the root of the sting, replenish the vitality of the work, invigorate and eliminate the opportunity, and fill the kidney essence to nourish the bone marrow and the brain. The biologically active substances secreted by bones, such as osteomodulin, growth factors, adipokines, cytokines, active peptides and hormones, can regulate bone metabolism through autocrine and paracrine methods, and can also act on remote secretion. Target organs or tissues such as the pancreas, liver, kidney, skeletal muscle, and brain play a corresponding biological role and are regarded as endocrine organs with biological activity [9]. Combining the analysis results of this study and the current existing studies, Liuwei Dihuang Decoction may exert a therapeutic effect on OP and AD through IL6 and other substances secreted by bones. IL6 is a cytokine with multiple physiological functions, which can be secreted by osteoblasts, T cells, B cells, monocytes, macrophages, fibroblasts, stromal cells, etc., and act on the body through autocrine and paracrine methods. Participate in a variety of physiological processes [10, 11].

Clinical studies have shown that IL6 level changes are an important indicator of the degree of bone loss [12]. The increase in IL6 expression in the blood of OP patients is positively correlated with the decrease in bone density [13]. The results of zoology further reveal the role of IL6 in various bone loss. IL6 can regulate bone metabolism by regulating the development and function of osteoclasts and osteoblasts, and plays an important role in the pathogenesis of osteoporosis. IL6 has an inhibitory effect on the osteogenic function of osteoblasts. Studies have found that IL6 can affect the differentiation of osteoblasts through three signaling pathways: SHP2/MEK/ERK, JAK/STAT3, and SHP2/PI3K/AKT2 pathways. Among them, IL6 mainly inhibits osteoblast differentiation through SHP2/ ERK pathway and SHP2/PI3K/AKT2 pathway, but it can up-regulate osteoblast differentiation through JAK/ STAT3 pathway [14]. Under normal physiological conditions, IL6 prevents excessive bone resorption, but under pathological conditions, IL6 promotes the generation and differentiation of osteoclasts. When normal bone marrow cells are cultured in vitro, IL6 directly inhibits the differentiation of osteoclast progenitor cells into osteoclasts by inhibiting the nuclear factor-kB receptor activator (RANK) signaling pathway. After IL6 binds to soluble interleukin 6 receptor (sIL6R), it can induce the production of nuclear factor-kB receptor activator ligand (RANKL) distributed on the surface of osteoblasts. RANKL binds to RANK on the surface of osteoclasts, thereby Activate a series of downstream factors in osteoclasts to promote the proliferation and differentiation of osteoclasts. Inhibiting the expression of IL6R can reduce the differentiation and bone resorption of osteoclasts induced by monocytes in vitro, reduce the production of osteoclasts in the joints where inflammatory reactions occur in mice, and alleviate bone erosion [15, 16]. Similarly, AD patients also show increased IL6 expression [17]. Previous studies have shown that IL6 has a negative effect on the formation of memory. Blocking IL6 can enhance long-term memory and improve long-term memory in hippocampal-dependent tasks [18]. Amyloid plaques and tau protein can induce microglia and astrocytes to secrete IL6 and other cytokines, while IL6 can increase the formation of amyloid plaques and phosphorylated tau protein [19], speeding up the development of AD. Ferreira et al. [20] believed that the related systemic chronic inflammation caused by peripheral metabolic disorders may play a role in the pathogenesis of AD. Natalia M et al. [21] found that compared with cognitively healthy control subjects, AD patients have higher levels of IL6 and IFN-gamma, which may be signs of inflammation in the hippocampus. Autopsy pathological examination of AD hypothalamus showed the presence of hyperphosphorylated tau protein and tangled structures, as well as parenchymal and vascular amyloid deposits surrounded by astrocytes. The T2 hyperintensity on MRI is positively correlated with plasma IL-6, and both are negatively correlated with the cognitive ability and hypothalamic/hippocampal volume of AD patients. Increased IL-6 and Suppressor of Cytokine Signaling 3 (SOCS3) were observed in AD autopsy brain. In addition, activation of the IL-6 pathway was observed in the hypothalamus and hippocampus of AD mice. In the brains of AD mice, inhibition of IL-6 neutralization and signal transducer and activator of transcription 3 (STAT3) signals alleviated memory impairment and peripheral glucose intolerance, and normalized plasma IL-6 levels. The results indicate that IL-6 is the link between cognitive impairment and peripheral metabolic changes in AD, and targeting pro-inflammatory IL-6 signaling may be a strategy to alleviate memory impairment and metabolic changes in the disease.

The occurrence and development of osteoporosis and Alzheimer's disease are the result of the comprehensive effects of multiple systems such as nerve, endocrine, immunity, and musculoskeletal. This study also found that Liuwei Dihuang Decoction may also be distributed in the target points of the whole-body tissues through TNF, SRC, INS, NFKB1, HNF4A, etc., and participate in the regulation of neurotransmitters such as amino acid dopamine, glucose and lipid metabolism, calcium and phosphorus metabolism, circadian rhythm, and more. This approach exerts a co-treatment effect on osteoporosis and Alzheimer's disease. Bone is becoming a new and important brain function regulator and a potential regulatory system for AD disease progression. It may be a secondary brain regulatory system that coordinates various physiological processes. Further attention to these interactions may lead to the development of new Strategies for the treatment of AD and other neurological diseases. In summary, Liuwei Dihuang Decoction can not only participate in the metabolism of bone tissue and nerve tissue through IL6 and other skeletal secretions, but also play a therapeutic effect on the body's multi-system integrated intervention through other targets and pathways.

References

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