Mechanism underpinning effects of Shichangpu (Rhizoma Acori Tatarinowii) on attention deficit hyperactivity disorder

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Abstract

OBJECTIVE: To investigate the mechanism underpinning the effects of Shichangpu (Rhizoma Acori Tatarinowii) on attention deficit hyperactivity disorder (ADHD).

METHODS: A network pharmacology approach integrating ingredients of Shichangpu (Rhizoma Acori Tatarinowii) and target with ADHD, network construction, molecular function interactions and pathway analysis was used.

RESULTS: This approach successfully helped to identify 7 active ingredients of CN, interacting with 21 key targets (ADRA1A, ADRA1B, ADRA2A, ADRA2B, ADRA2C, ADRB1, ADRB2, CHRM1, CHRM2, CHRM3, PTGS1, SLC6A2, SLC6A3, SLC6A4, DRD1, DRD5, HTR2A, ADRA1D, MAOB, GRIA2, HTR1A). The molecular function interactions among candidate targets mainly consisted of four groups: G-protein coupled amine receptor activity, catecholamine binding, monoamine transmembrane transporter activity and neurotransmitter receptor activity. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis indicated that Shichangpu (Rhizoma Acori Tatarinowii)-regulated pathways were mainly classified into signal transduction and monoamine neurotransmitters.

CONCLUSION: Our investigation revealed that Shichangpu (Rhizoma Acori Tatarinowii) could improve the symptoms of ADHD by regulating neurotransmitter, in multiple types of compounds-target-pathway, which may be implicated in the major pathological processes of ADHD.

Keywords: Attention deficit disorder with hyperactivity; Shichangpu (Rhizoma Acori Tatarinowii); Plants, medicinal; Pharmacology

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) as a neurodevelopmental disorder is more common in children and adolescents. It manifests with hyperactivity, inattention, or impulsivity and can persist into adulthood. A survey of WHO showed that ADHD was prevalent, seriously impairing and highly comorbidities but vastly under-recognized and undertreated across countries and cultures. Although current pharmacotherapies, such as stimulants and anti-depressants, are able to improve ADHD symptoms, there is still about 20%-40% of patients with ADHD who do not benefit
MATERIALS AND METHODS

Ingredients database construction of Shichangpu (Rhizoma Acori Tatarinowii)

All of the known ingredients of Shichangpu (Rhizoma Acori Tatarinowii) were manually collected from related phytochemical databases: Traditional Chinese Medicine Systems Pharmacology Database (TCMSP, http://ibs.hkbu.edu.hk/LSP/tcmsp.php, version: 2.3) and TCM Database@Taiwan (http://tc.mcu.edu.tw/, updated on Mar 25th, 2014). The ingredients were retrieved from the above databases with "Shichangpu (Rhizoma Acori Tatarinowii)".

Absorption, distribution, metabolism, excretion (ADME) screening of Shichangpu (Rhizoma Acori Tatarinowii) ingredients

ADME system was used to select the ingredients with favorable pharmacy-kinetics properties in this study including oral bioavailability (OB) and BBB (blood brain barrier). OB represents the efficiency percentage of the drug delivery to the systemic circulation, and reveals the convergence of the ADME process. In addition, high oral bioavailability often reflects a key indicator to determine the drug-like property of bioactive molecules as therapeutic agents. In our study, a reliable OB value of the constituents in Shichangpu (Rhizoma Acori Tatarinowii) was greater than or equal to 30%. Moreover, another problem for treatment of ADHD is whether the drugs can penetrate BBB, which determines the rate and extent of drug concentration and ultimately affects its bioavailability. Thus, BBB value, greater than or equal to 0.3, was used to predict the ability of biological activity molecule to permeate BBB. There were 63 screened molecules based on criterion above. It is worth noting that the OB value of elmericin and gamma-asarone are lower than 30%, but both of them had been widely reported as the active molecules in Shichangpu (Rhizoma Acori Tatarinowii). Thus, these two molecules should be also regarded as bioactive molecules for further analysis.

Identification targets for potential ADHD

DisGeNET (http://www.disgenet.org) is a search platform containing the largest publicly available collections of genes and variants associated to human diseases. The current version of DisGeNET (v5.0) contains 561 119 gene-disease associations (GDAs), between 17 074 genes and 20 370 diseases. ADHDgene database (http://adhdb.psych.ac.cn/, updated on Feb 14th, 2014) is the first genetic database for ADHD. It includes 359 multi-type genetic factors related with ADHD including SNPs, CNVs, VNTR, microsatellites, genes, chromosomal regions, and biological pathways based on comprehensive search in PubMed for genetic susceptibility studies of ADHD. Then, to better define the targets of ADHD, Therapeutic Target Data-
of Shichangpu (Rhizoma Acori Tatarinowii) and AD
database (Table S). We picked 25 targets from the TCMSP and TCM Da
Shichangpu (Rhizoma Acori Tatarinowii), and their cor
We recognized a total of potential active com
the matching targets because they lack appropriate
showed 21 potential targets (yellow ellipse), 7 can-
degree of potential treatment of ADHD targets connec-
followed by gamma-asarone (degree = 18), azaron
(degree = 17), beta-asarone (degree = 17), and elemicin
(degree = 17). For the 21 potential targets of ADHD
treatment, the network analysis showed ADRA1A, ADRA2B, ADRA2C, ADRB1,
ADRB2, CHRM1, CHRM2, CHRM3, PTGS1, SLC6A2, SLC6A3 and SLC6A4 had the all number of
compound-target interactions, followed by DRD1, DRD5 (6), and HTR2A and ADRA1D (5). The re-
remaining 3 targets showed interactions with only 1 to 3

RESULTS

Potential targets of Shichangpu (Rhizoma Acori Tatarinowii) on ADHD predicted by network pharmacological analysis
The compounds and targets interaction analysis results illustrated that a total of 25 proteins were inferred to interact with the 52 chemical ingredients of Shichangpu (Rhizoma Acori Tatarinowii) (Figure 1). The data of 165 targets which are affinitive with ADHD can be found in Table S1, where all the information were manually collected and integrated from the DisGeNET (score > 0.2), DrugBank, TTD and ADHDgene database (number of Studies ≥ 2). TCMs, such as Shichangpu (Rhizoma Acori Tatarinowii), contain multiple-chemical substances identified as fail in reaching the matching targets because they lack appropriate pharmaceutical properties. Thus, these compounds demonstrate limited efficacy that should be neglected. We recognized a total of 105 potentially active compounds in Shichangpu (Rhizoma Acori Tatarinowii) (Table S2). To further elaborate the potential relationship between the compounds and targets, we acquired 65 effective compounds, in the known 105 compounds of Shichangpu (Rhizoma Acori Tatarinowii), and their corresponding 84 targets from the TCMSP and TCM Database@Taiwan database (Table S3). We picked 25 identical targets from comparative analysis the targets of Shichangpu (Rhizoma Acori Tatarinowii) and AD-

Network construction and analysis
To further explore the relationships between the com-
mechanism of Shichangpu (Rhizoma Acori Tatarinowii)-ADHD was pictured by average degree. Ultimately, our study analyzed how one node interacts with others in common pathways. All the properties of the network were described by Network Analysis plugin.

Biological functional analysis network in screened targets
In total, 21 screened targets were obtained with our study strategy and verified in the UniProt database, and then used as input in the ClueGO+Cluepedia app for creating the biological functions analysis network. The complete data of the analysis is available in Figure 3 depicts the network and diagram obtained from ClueGO+Cluepedia. As shown in Figure 3, the results of molecular function interactions among candidate targets, were exhibited with 23 functions (Figure S1), and mainly consisted of four groups: G-protein coupled amine receptor activity, catecholamine binding, monoamine transmembrane transporter activity and neurotransmitter receptor activity.

Pathway-target network in screened targets
As a widely used Cytoscape plugin, ClueGO+Cluepe-
dia was used to further identify the Kyoto Encyclo-
dia of Genes and Genomes (KEGG) biological pathways in biological networks. A target-pathway-network was constructed with 21 potential targets and the 5 enriching KEGG biological pathways which consist of 9 KEGG pathways (Figure S2), deciphered the significant targets and potential mechanism of ADHD. Five
KEGG pathways including cocaine addiction, neuroactive ligand-receptor interaction (NLI), regulation of lipolysis in adipocytes, serotonergic synapse and cAMP signaling pathway showed extremely significant P value \((P < 0.05)\). As shown in the Figure 4, target-pathway network contains 29 nodes (9 pathways and 20 potential targets) and 69 edges.

**DISCUSSION**

**Analysis of potential targets of Shichangpu (Rhizoma Acori Tatarinowii)**

Our analysis results showed that various candidate compounds in Shichangpu (Rhizoma Acori Tatarinowii) were linked to multiple targets, which might represent potent therapeutic mechanism of ADHD. Some of the 7 screened compounds had been certified by experimental researches, for example, methyl isoeugenol can relieve the symptoms of ADHD by enhancing the concentration of 5-hydroxytryptamine (5-HT) which is one of the closely neurotransmitters associated with ADHD in Synapse gap of nerve cells. A recent study showed that methyleugenol could bind gamma-aminobutyric acid type A receptor and inhibited its activity, then leaded to gamma-aminobutyric acid (GABA) content increased. GABA is the most primary inhibitory neurotransmitter for the normal function of human brain and the decrease of GABA concentration is associated with ADHD. β-asarone can improving neuronal apoptosis and behavioral defects by reducing the expression of JNK and Beclin and improve the expression of anti-apoptotic protein Bcl-2 in the hydroxy dopamine model rats. By suppressing the pre synaptic membrane of the neurons to reuptake of dopamine (DA), another important neurotransmitter is bound up with ADHD, and β-asarone promotes the synaptic transmission function and improves the hyperactivity index of the children with ADHD. In addition, α-asarone as an indispensably active ingredient of

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Table 1: Information of 25 identical targets through comparative analysis the targets of Shichangpu (Rhizoma Acori Tatarinowii) and Attention deficit hyperactivity disorder.
Shichangpu (Rhizoma Acori Tatarinowii), belongs to the same compound with β-asarone in TCMS and it also has been widely studied by previous researches. These researches suggested that α-asarone could attenuates cognitive deficit via the decrease in NF-κB activation.  

24 α-asarone also exhibit remarkable regulative capability of maintaining the balance between excitatory and inhibitory transmissions, and reducing neuronal hyper excitability of excitatory neurons in the basolateral amygdala.  

25 A recent study demonstrated that α-asarone can directly inhibit acetylcholinesterase activity and up-regulation acetylcholine (Ach) levels in the striatum. It might improve comprehension of the neurobiological mechanism responsible for hyperactivity.

26 The result in another research indicated that inhibition of microglial activation and pro-inflammatory cytokines in the hippocampus might contribute to the ameliorating effect of α-asarone on memory deficits. Furthermore, α-asarone has been revealed excitatory-inhibitory bidirectional regulation effects on CNS in both noradrenergic and 5-HT systems.

**Biological functional analysis in screened targets**

As shown in Figure 3, enrichment molecular functions indicated that most potential targets were closely related to neurotransmitter receptors activity and transportation. G-protein-coupled amine receptors (GPCR) are indispensable mediators in monoamine neurotransmitter signal transduction, and the mutations in GPCR-encoding genes are deemed to be important contributing factors to the pathogenicity of the ADHD. From the perspective of GPCR signaling in the CNS, polymorphisms in GPCR play particularly important roles owning to their enrichment in prefrontal cortex and striatum and demonstrate roles in the control of hyperactivity and impaired learning mediated by down-regulation reuptake of catecholamine and 5-HT.

28 Several experiments revealed that the functions of selected targets were associated with catecholamine neurotransmitters (DA, noradrenaline and adrenergic), 5-HT, amino acids, monoamine oxidase, neurotransmitter transporter and Ach which were hot etiologies of ADHD.

**Pathway-target network analysis in screened targets**

As shown in the Figure 4, cAMP signaling pathway, serotonergic synapse, amphetamine addiction, calcium signaling pathway, cGMP-PKG signaling pathway and
NLI contribute to ADHD, and their degree were 8, 5, 4, 11, 8 and 16. Amphetamine addiction makes people addictive to amphetamine and methylphenidate, ADHD medications, similarly as cocaine addiction. They overly suppressive the dopamine transporter (DAT) and lead to addiction because of the accumulated DA on synaptic gap. Previous experimental results showed that β-asarone could enhance the MAOB and SLC6A3 levels and might reduce the expression level of DRD1 and GRIA2 to cut down drug addiction of ADHD. NLI and serotonergic synapse demonstrated various interactions between the neurotransmitter ligands and receptor and the monoamine neurotransmitter related genes expression altering among ADHD patients were related to NLI and Serotonergic synapse as reported in completed studies. cAMP signaling pathway affects the human cognition with modulatory neurotransmitters (DA, noradrenaline and 5-HT) which influence AC-cAMP-PKA signal transduction. A rat experiment was designed to prove cAMP-dependent protein kinase (PKA), an intracellular target of dopamine receptor stimulation, also affects attention and indicated that inhibited PKA produces hyperactivity and inattention. Calcium signaling pathway regulates Ca$^{2+}$ that enters the cell from the outside as a principal source of signal Ca$^{2+}$. Cells use signals of the external Ca$^{2+}$ to activate various entry channels devote to learning and memory. Positive associations had been tested for the D (1A) dopamine receptor (DRD1) and D (1B) dopamine receptor (DRD5), and suggested that other genes involved in DRD1/DRD5 signal might also contribute to ADHD. Moreover, a review discussed the interaction and crosstalk between the Ca$^{2+}$ and cAMP signaling which had been confirmed at multiple levels to control and tune the activity of each other. cGMP-PKG signaling pathway influences the intracellular second messenger, cyclic GMP (cGMP) that mediates the action of nitric oxide (NO), and regulates broad array of physiologic processes. A primary action of elevated cGMP levels is the stimulation of cGMP-PKG, the major intracellular receptor protein for cGMP, which phosphorylates substrate proteins to exert its actions. It
has become increasingly reported that PKG mediates some of the neuronal effects of cGMP, but how is not yet clear. cGMP-PKG signaling pathway has been reported in nerve terminals of striatum, where NO mediates phosphorylation of the cAMP-regulated phosphoprotein by cGMP-PKG. Another survey revealed cGMP-PKG as the key molecular sites which regulated protein phosphatases, intracellular calcium levels, and neurotransmitter receptors to exert neural function.

In conclusion, this study suggested that the selected compounds of Shichangpu (Rhizoma Acori Tatarinowii) may mediate monoamine neurotransmitters by regulating the screened targets implicated into the pathogenesis of ADHD. These results not only provided a new insight for deeper understandings of therapeutics mechanism for ADHD with Shichangpu (Rhizoma Acori Tatarinowii), but also demonstrated a promising technique for exploring the potential action mechanism of other TCMs. Further confirmation is still required for the predictive therapeutics mechanism.

SUPPLEMENTARY MATERIALS

Supplementary Materials are available online. Table S1: 165 targets about ADHD; Table S2: 105 potentially active compounds in Shichangpu (Rhizoma Acori Tatarinowii); Table S3: 65 effective compounds in Shichangpu (Rhizoma Acori Tatarinowii) and their corresponding 84 targets; Figure S1: 23 molecular function interactions among candidate targets; Figure S2: 9 KEGG pathways based on 21 potential targets.

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REFERENCES


