Computational systems pharmacology analysis of Tong-Jing-Yi formula in the treatment of dysmenorrhea

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Methods: The components of TJY formula were identified by ultra performance liquid chromatography–quadrupole-time of flight/mass spectrometry. SwissTargetPrediction database was used to predict the targets of TJY formula, and targets associated with primary dysmenorrhea were also collected through other databases. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were conducted. Results: A total of 91 compounds with identified structures were screened, including 3 groups of isomers. The results predicted 854 TJY formula–related targets and 363 disease-related targets. GO and KEGG analysis showed that the top 5 target genes were PIK3CA, AKT1, EGFR, AKT2 and CYP19A1. PI3K-Akt signaling, chemokine signaling, focal adhesion, and Rap1 signaling were ranked in the top 15 pathways. Conclusion: TJY formula might play roles in the treatment of dysmenorrhea underlying mechanisms relating to the involvement of TNF-α, interleukin and PI3K-Akt signaling pathway. Potential pathways have been identified that need mechanistic confirmation in a laboratory setting in the future.

Keywords: Tong-Jing-Yi formula; Dysmenorrhea; Network pharmacology; PI3K-Akt signaling pathway; Component identification; Bioinformatic analysis

1. Introduction

Dysmenorrhea refers to abdominal pain during menstruation that leads to lumbosacral pain, nausea, loss of appetite, headache, restlessness, insomnia, fatigue, diarrhea, and even syncope [1,2]. Dysmenorrhea can be classified into two main categories: primary dysmenorrhea (PD) and acquired dysmenorrhea (AD) [3]. PD is defined as pain during the menstrual cycle in the absence of an identifiable cause [3]. AD refers to dysmenorrhea caused by pelvic organic diseases such as endometriosis, adenomyosis, chronic pelvic inflammation, uterine malformation and so on [4]. The incidence of PD in adolescent women ranges from 16% to 93% [5]. PD is related to an increase of endometrial prostaglandin (PG) and interleukin (IL) during menstruation. Prostaglandins (PGF2-α) stimulates the contraction of uterus and reduces the amount of blood perfusion through myometrial compression of the blood vessels. Then the uterus cramps by lack of oxygen and the body feels abdominal pain [2]. Both PG synthase inhibitors such as nonsteroidal anti-inflammatory drugs [6] and oral contraceptives are prescribed for dysmenorrhea. PG synthetase inhibitors suppress PG production by inhibiting the activity of PG synthetase, which prevents excessive uterine contraction and spasm and consequently alleviates or eliminates dysmenorrhea [3]. Oral contraceptives reduce the PG content in menstrual blood by inhibiting ovulation [7]. Vitamins (V₁, V₂, V₃, V₄, V₅, V₆, V₇, V₈, V₉, V₁₀, V₁₁, V₁₂), calcium, magnesium, olive oil, fennel, dietary fiber, zinc, Omega-3 and Omega-6 Fatty Acids are also used as adjuvant therapy for dysmenorrhea [2,8]. However, these treatments show limited clinical efficacy as well as various side effects, and thus more effective treatments for dysmenorrhea are required.

Traditional Chinese medicine has been widely used for the treatment of dysmenorrhea in China [9,10]. Tong-Jing-Yi (TJY) formula, consisting of Leonurus, fried Toosendan and processed Cyperus, etc. The therapeutic effect of TJY on dysmenorrhea has been clinically validated, but the underlying mechanism remains unclear. The present study aimed to explore the possible molecular targets of TJY and the potential mechanisms.
ish Yin. Hawthorn, bergamot and green peel are as the adjuvant, and assist the minister medicine to soothe the liver, promote Qi and relieve pain. Achyranthes bidentata is used to induce all kinds of drugs to go down. The whole prescription is used together to warm the uterus and disperse cold. It regulates Qi, nourishes blood, and relieves pain. Although the efficacy of TJY formula on dysmenorrhea and menstrual disorders has been clinically verified, both clinical studies and exploration of the pharmacological mechanisms of TJY in dysmenorrhea are limited.

In the present study, a network pharmacology-based analysis of TJY formula was performed to explore the underlying mechanism of TJY formula in the treatment of dysmenorrhea.

2. Methods

2.1 Preparation of TJY formula solution

To prepare TJY, 15 g Leonurus, 12 g fried toosendan, 12 g processed cyperus, 6 g bergamot, 24 g raw hawthorn, 12 g vinegar corydalis, 12 g honey bran roasted green peel, 6 g Artemisia argyi leaf, 12 g raw radix paoniae alba, 12 g Achyranthes bidentata, 12 g fried mustard seed, and 6 g processed Evodia rutaecarpa were weighed and placed in a dish with 1000 mL deionized water (3 cm above the medicinal material). After soaking for 1 h, medicinal materials were decocted on low fire for 30 min and then filtered with a 1000 mesh gauze while hot. The filtrate was collected and 700 mL of water was added to the residue. Medicinal materials were decocted on low fire for 30 min after the water was boiled and then the samples were filtered with 1000 mesh gauze while hot. The filtrate was collected and mixed with previously collected samples. The filtrate was cooled to room temperature and centrifuged (12,000 rpm, 5 min). The supernatant was filtered using a 0.22 μm membrane. The filtrate was concentrated and lyophilized.

2.2 Component identification

The components of TJY formula were analyzed by ultra performance liquid chromatography-quadrupole-time of flight/mass spectrometry (UPLC-Q-TOF/MS) [11–14]. Briefly, Chromatographic column: Waters ACQUITY UPLC HSS T3 (2.1 × 100 mm, 1.8 μm); Batch No.: 0200372571; Column temperature: 25 °C; Injection volume: 1 μL; Detection wavelength: 280 nm. Mobile phase ratio and flow rate: 0.1% formic acid aqueous solution for phase A, 0.1% formic acid acetonitrile for phase B. Mass spectrometric detection was performed in Negative/Positive ion mode.

2.3 Bioinformatic analysis

The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to confirm the molecular structures. The SwissTargetPrediction database (http://www.swisstargetprediction.ch/) was used to predict the target of selected compounds from TJY formula. Targets related to PD were collected through a series of databases by performing keyword search of “dysmenorrhea” and the species was set as “human”, including GeneCards (https://www.gene_cards.org/), TTD (http://db.idrblab.net/tdt/), DiGSeE (http://www.disgenet.org/), Drugbank (https://www.drugbank.ca/) and OMIM (https://omim.org/). The PPI network diagram was depicted based on the targets of candidate components of the TJY formula and PD listed in the STRING database (http://string-db.org/). GO and KEGG pathway enrichment analysis of core targets were performed via the DAVID database (FDR <0.05).

3. Results

3.1 Identification of the components of the TJY formula

A total of 96 compounds were identified from TJY formula according to the multi-level mass spectrometry information of samples and the database of high-resolution mass spectrometry of natural products. Among the 96 compounds, 5 compounds with unidentified structures were removed. A total of 91 compounds were selected (Supplementary Table 1), including 3 group isomers. We used PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to confirm the molecular structures. The results showed that 12 compounds were from Artemisia argyi leaf (Monarch drug) and 1 compound was from processed Cyperus (Monarch drug). A total of 13 compounds were detected from Corydalis vinegar (Minister drug), 7 compounds were detected from processed Evodia rutaecarpa (Minister drug), 6 compounds were detected from fried Toosendan (Minister drug), 4 compounds were detected from fried Mustard Seed (Minister drug), and 3 compounds were detected from Leonurus (Minister drug). In addition, 9 compounds were detected from raw Radix Paeoniae Alba (Assistant drug), 8 compounds were detected from green peel (Assistant drug), 7 compounds were detected from raw Hawthorn (Assistant drug), and 6 compounds were detected from Bergamot (Assistant drug). Eight compounds were detected from Achyranthes bidentata (Guide drug). The sources of the remaining compounds were unclear. The corresponding structures of Canonical SMILES are shown in Supplementary Table 1. The result in UPLC-HRMS basic peak ion diagram-negative ion mode is shown in Fig. 1A, in UPLC-HRMS basic peak ion current diagram-positive ion mode is shown in Fig. 1B, and in UPLC-UV chromatogram-UV 280 nm is shown in Fig. 1C.

3.2 Target prediction of the components of TJY formula

We used the SwissTargetPrediction database (http://www.swisstargetprediction.ch/) to predict the targets of selected compounds from TJY formula. A total of 4701 targets were collected, and 854 targets were obtained after de-duplication and imported into STRING database (http://STRING-db.org/cgi/input.pl). Protein-protein interaction (PPI) network analysis was conducted (Fig. 2).
Fig. 1. Identification of the components of TJY formula. (A) UPLC-HRMS basic peak ion diagram-negative ion mode. (B) UPLC-HRMS basic peak ion current diagram-positive ion mode. (C) UPLC-UV chromatogram-UV 280 nm.
3.3 Disease-related targets query

Targets related to PD were collected through a series of databases by performing keyword searches of “dysmenorrhea” and the species set as “human”, including in Genecards (https://www.genecards.org/), TTD (http://db.idrblab.net/ttd/), DiGSeE (http://www.disgenet.org/), Drugbank (https://www.drugbank.ca/) and OMIM (https://omim.org/). We collected 545 targets from Genecards (n = 287), TTD (n = 12), DiGSeE (n = 9) and Drugbank (n = 237). All targets were normalized to genesymbol by BioDBnet database, and 363 disease-related targets were obtained after de-duplication. Based on the PD-related targets, the data were retrieved in the STRING database (https://string-db.org/). The PPI network was established as the background network of gene mapping (Fig. 3). The PPI relationship with a confidence score ≥ 0.9 was selected. The PPI of component-related targets were “merged” with those of disease-related targets via Cytoscape 3.6.1 software (https://cytoscape.org/) and the overlapping targets were obtained. Three topological properties were calculated, including “degree”, “closeness centrality” and “betweenness centrality”. The predicted target was selected only when three parameters were greater than the median.

3.4 Core targets screening

To further explore the function and mechanism of candidate targets related to components and disease, the corresponding PPI network diagram was depicted based on the targets of candidate components of TJY formula and of PD listed in the STRING database (http://string-db.org/). The diagrams show a disease-target PPI (Fig. 2) and component-target PPI (Fig. 3). The PPI network of the core target consists of 56 nodes and 211 edges. The core target information is shown in Fig. 4. The top five targets are PIK3CA, AKT1, EGFR, AKT2 and CYP19A1, followed by HRAS, PTGS2, and MMP9. The top 15 targets are shown in Table 1.

3.5 GO and KEGG analysis of core targets

The GO and KEGG pathway enrichment analysis of 56 core targets were performed using the DAVID database (FDR <0.05). GO analysis revealed 36 items related to biological process, 13 items related to molecular function and 3 items related to cellular component. A total of 27 signaling pathways were involved according to KEGG pathway enrichment analysis. GO (Fig. 5) and KEGG analysis (Fig. 5B) results are shown in Fig. 5. Based on the results of enrichment analysis, the “target pathway” network diagram of the TJY formula in the treatment of PD was estab-
Fig. 3. PPI network analysis of dysmenorrhea.

The size and color of the nodes in the graph positively correlated with the degree value.

The top 15 signaling pathways and targets are shown in Table 2. The PI3K-Akt signaling pathway also ranked in the top 15 signaling pathways, which was consistent with the predicted core target genes. GO and KEGG analysis of core targets suggested that the TJY formula might play an anti-inflammatory role in the treatment of dysmenorrhea by affecting the expressions of core target genes.

Table 1. The top 15 central targets in the PPI network.

<table>
<thead>
<tr>
<th>Top 15 targets</th>
<th>Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIK3CA</td>
<td>45</td>
</tr>
<tr>
<td>AKT1</td>
<td>38</td>
</tr>
<tr>
<td>EGFR</td>
<td>37</td>
</tr>
<tr>
<td>AKT2</td>
<td>32</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>30</td>
</tr>
<tr>
<td>HRAS</td>
<td>29</td>
</tr>
<tr>
<td>PTGS2</td>
<td>29</td>
</tr>
<tr>
<td>MMP9</td>
<td>29</td>
</tr>
<tr>
<td>CCND1</td>
<td>23</td>
</tr>
<tr>
<td>PDPK1</td>
<td>23</td>
</tr>
<tr>
<td>ESR1</td>
<td>22</td>
</tr>
<tr>
<td>MMP3</td>
<td>22</td>
</tr>
<tr>
<td>VEGFA</td>
<td>21</td>
</tr>
<tr>
<td>PTK2</td>
<td>19</td>
</tr>
<tr>
<td>ERBB2</td>
<td>19</td>
</tr>
</tbody>
</table>

4. Discussion

The etiology of dysmenorrhea is not precisely understood, but is associated with altered prostaglandin synthesis by the uterus (PGF2α) [15]. Excessive release of PG from disintegrating cells during endometrial sloughing causes myometrial hypercontractility, resulting in ischemia and hypoxia of the uterine muscle as well as pain [16,17]. PGs are ubiquitously distributed intracellular substances that are derived from long-chain polyunsaturated fatty acids, such as arachidonic acid, a common component of cell membrane phospholipids [18]. PGs have a range of biological
effects on a wide variety of physiological and pathological activities including pain, inflammation, body temperature, and sleep regulation [16]. In the theory of traditional Chinese Medicine, dysmenorrhea is caused by blocking of Qi and blood. For the treatment of dysmenorrhea, there are multiple effective and clinically validated formulas of traditional Chinese medicine, such as Wenjing Tang, Shaofu Zhuyu Tang, and Danggui Sini Tang [19–21]. TJY formula also has been demonstrated to possess good clinical efficacy against dysmenorrhea in our hospital, which is worth further study.

In the present study, the TJY formula was analyzed and 96 compounds were detected. Among them, 91 compounds were identified while 5 compounds with unidentified structure were not identified. Among the 91 compounds, 13 compounds were from monarch drugs, 33 compounds were from minister drugs, 30 compounds were from assistant drugs, and 8 were compounds from guide drugs. The monarch drug Artemisia argyi leaf can be used as a therapeutic agent for abdominal pain and dysmenorrhea as well as eczema and itching. The anti-inflammatory effect of Artemisia argyi leaf is closely related to the inhibition of inflammatory mediators released from macrophages and inflammatory cytokine production in inflamed tissues [22]. As the most commonly used single herbs, Corydalis and Cyperus were found in 33.1% and 29.2% of all prescrip-
Table 2. The top 15 enriched KEGG pathways of core targets.

<table>
<thead>
<tr>
<th>NO.</th>
<th>KEGG_ID</th>
<th>Name</th>
<th>Pathway class</th>
<th>Degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>hsa05200</td>
<td>Pathways in cancer</td>
<td>Cancer: overview</td>
<td>36</td>
</tr>
<tr>
<td>2</td>
<td>hsa05205</td>
<td>Proteoglycans in cancer</td>
<td>Cancer: overview</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>hsa04151</td>
<td>PI3K-Akt signaling pathway</td>
<td>Signal transduction</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>hsa05161</td>
<td>Hepatitis B</td>
<td>Infecious disease: viral</td>
<td>27</td>
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<tr>
<td>5</td>
<td>hsa04062</td>
<td>Chemokine signaling pathway</td>
<td>Immune system</td>
<td>24</td>
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<tr>
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<td>hsa04510</td>
<td>Focal adhesion</td>
<td>Cellular community - eukaryotes</td>
<td>24</td>
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<tr>
<td>7</td>
<td>hsa04015</td>
<td>Rap1 signaling pathway</td>
<td>Signal transduction</td>
<td>24</td>
</tr>
<tr>
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<td>Cancer: specific types</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>hsa05142</td>
<td>Chagas disease (American trypanosomiasis)</td>
<td>Infectious disease: parasitic</td>
<td>22</td>
</tr>
<tr>
<td>10</td>
<td>hsa05203</td>
<td>Viral carcinogenesis</td>
<td>Cancer: overview</td>
<td>22</td>
</tr>
<tr>
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<td>hsa05215</td>
<td>Prostate cancer</td>
<td>Cancer: specific types</td>
<td>21</td>
</tr>
<tr>
<td>12</td>
<td>hsa04066</td>
<td>HIF-1 signaling pathway</td>
<td>Signal transduction</td>
<td>21</td>
</tr>
<tr>
<td>13</td>
<td>hsa05166</td>
<td>HTLV-I infection</td>
<td>Infectious disease: viral</td>
<td>21</td>
</tr>
<tr>
<td>14</td>
<td>hsa04668</td>
<td>TNF signaling pathway</td>
<td>Signal transduction</td>
<td>20</td>
</tr>
<tr>
<td>15</td>
<td>hsa04014</td>
<td>Ras signaling pathway</td>
<td>Signal transduction</td>
<td>20</td>
</tr>
</tbody>
</table>

tions in a study of women aged from 13 to 25 years with a single diagnosis of primary dysmenorrhea in Taiwan [23]. The composition of TJY formula is consistent with that previously described [22,23], which indicates that its composition is rational and effective.

The PPI network, GO and KEGG analyses showed that the top target and signaling pathways were the PIK3CA and PI3K-Akt signaling pathways, respectively. PIK3CA, AKT1 and AKT2 are members of the PI3K-Akt pathway. PIK3CA is a family of lipid kinases consisting of a regulatory subunit (p85) and a catalytic subunit (P110). The binding of ligand to the membrane receptor activates p85 and recruits P110, which catalyzes the formation of PI3P from PIP2 on the intracellular surface. As a second messenger, PI3P further activates PDK1 and Akt pathways. PI3K-Akt signaling activation promotes the proliferation of cell stimuli or toxic damage, and it mainly regulates basic cell functions such as transcription, translation, proliferation and growth [37]. Previous studies have shown that PI3K-Akt signaling activation promotes the proliferation of endometrial cells and also increases the contractility of human endometriosis stromal cells [38], which might be associated to dysmenorrhea in endometriosis [39].

There are some limitations of this study. Although the components of TJY formula were identified followed by network pharmacology analysis, the specific relationships among compounds, targets and signaling pathways remain unclear. Experiments in cells and animal models need to...
be supplemented to explore the mechanisms and verify our speculation.

5. Conclusions

TJY formula might play roles in the treatment of dysmenorrhea underlying mechanisms relating to the involvement of IL-8, L-33, IL-1β and TNF-α as well as the PI3K-Akt signaling pathway. Potential pathways have been identified that need mechanistic confirmation in a laboratory setting in the future.

Abbreviations

TJY, Tong-Jing-Yi; PD, primary dysmenorrhea; AD, acquired dysmenorrhea; PG, prostaglandin; IL, interleukin; UPLC-Q-TOF/MS, ultra performance liquid chromatography-quadrupole-time of flight/mass spectrometry; PI3K, phosphoinositide 3-kinase; PI3P, phosphatidylinositol 3-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PDK1, phosphoinosine dependent kinase 1; PKB, protein kinase B; EGFR, epidermal growth factor receptor; HIF-1α, hypoxia inducible factor-1α.

Author contributions

YYL—analysis of data, manuscript revision; JLZ—extraction and drafting of the manuscript; JT—design and revision and provide funding support. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Obstetrics and Gynecology Hospital of Fudan University (approval number: 2021-84).

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.ceog4904099.

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