In silico search for multi-target therapies for osteoarthritis based on 10 common Huoxue Huayu herbs and potential applications to other diseases

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Abstract. Huoxue Huayu (HXHY) has been widely used in traditional Chinese medicine (TCM) as a key therapeutic principle for osteoarthritis (OA), and related herbs have been widely prescribed to treat OA in the clinic. The aims of the present study were to explore a multi-target therapy for OA using 10 common HXHY herbs and to investigate their potential applications for treatment of other diseases. A novel computational simulation approach that integrates chemical structure, ligand clusters, chemical space and drug-likeness evaluations, as well as docking and network analysis, was used to investigate the properties and effects of the herbs. The compounds contained in the studied HXHY herbs were divided into 10 clusters. Comparison of the chemical properties of these compounds to those of other compounds described in the DrugBank database indicated that the properties of the former are more diverse than those of the latter and that most of the HXHY-derived compounds do not violate the 'Lipinski's rule of five'. Docking analysis allowed for the identification of 39 potential bioactive compounds from HXHY herbs and 11 potential targets for these compounds. The identified targets were closely associated with 49 diseases, including neoplasms, musculoskeletal, nervous system and cardiovascular diseases. Ligand-target (L-T) and ligand-target-disease (L-T-D) networks were constructed in order to further elucidate the pharmacological effects of the herbs. Our findings suggest that a number of compounds from HXHY herbs are promising candidates for mult-target therapeutic application in OA and may exert diverse pharmacological effects, affecting additional diseases besides OA.

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Abbreviations: TCM, traditional Chinese medicine; HXHY, Huoxue Huayu; OA, osteoarthritis; ADAMTS-5, aggrecanase-2; MMP, matrix metalloproteinase; TNF-α, tumor necrosis factor-α; iNOS, inducible nitric oxide synthase; PPARγ, peroxisome proliferator activated receptor γ; PDE, phosphodiesterase; L-T-D network, ligand-target-disease network; TTD, therapeutic target database

Key words: osteoarthritis, multi-target therapy, Huoxue Huayu herbs, computational simulation

Introduction

Osteoarthritis (OA) is a degenerative joint disease, which causes chronic pain and functional restrictions in the affected joints (1). At present, there is no effective treatment for reversing or preventing its onset. Non-steroidal anti-inflammatory drugs (NSAIDs) are mainly used for treating OA, particularly in the early stages of the disease, but these drugs are often associated with clinically adverse effects (2). Furthermore, the social and economic cost related to OA remains particularly high (3). Therefore, ongoing research attempts to develop improved therapeutic strategies for OA.

Several lines of evidence suggest that treatment that aims at a number of targets at once may be more effective against complex diseases such as OA (4,5). On the other hand, traditional Chinese medicine (TCM), a complex system employing multiple components and targets, has been recognized in Western countries as a popular complementary and alternative medicinal approach. TCM has been used in the treatment of OA, and often has fewer side-effects than those reported for NSAIDs (6-8). Those reports indicate that TCM may provide a novel promising strategy for treatment of OA.

Huoxue Huayu (HXHY) has been widely used in TCM as a key therapeutical principle for OA in China (9,10). Ten common HXHY herbs, *Ligusticum chuanxiong*, *Salvia miltiorrhiza*, *Strychnos nux-vomica*, *Persicae semen*, *Corydalis yanhusuo*, *Drynaria fortunei*, *Commiphora myrrha*, *Carthamus tinctorius*, *Boswellia carterii* and *Achyranthes bidentata*, have been reported to play an important role in the treatment of OA (9-13). However, the mechanisms underlying the effects of these herbs are poorly studied. Therefore, in the present study, we investigated the pharmacology and effectiveness of

these herbs using an integrative model, combining chemical structure, ligand clusters, chemical space and drug-likeness evaluations, as well as docking screening and network analysis. Our study aimed to contribute in the elucidation of the mechanisms underlying HXHY herb effects and in the long term, in developing strategies for OA treatment.

Materials and methods

Chemical structures and clustering. The chemical structures of compounds contained in the 10 aforementioned HXHY herbs were downloaded from the Chinese Herbal Drug Database (14). Following duplicate exclusion, a total of 208 chemical structures were retained. They were converted into three-dimensional structures and energy optimizations were performed using the Discovery Studio 2.0 (DS 2.0) software (Accelrys, Inc., San Diego, CA, USA) based on the Merck Molecular Force Field (MMFF). The Cluster Ligands protocol was then used to cluster the compounds from HXHY herbs. In addition, 96 drug/drug-like compounds in association with OA disease were collected from the DrugBank database (15) and were optimized based on the MMFF.

Chemical space mapping and drug-likeness prediction. Chemical space was estimated by calculating a given set of descriptors for each molecule and using these values as coordinates in the multi-dimensional space (16). In the present study, 34 common descriptors were used to estimate the chemical space for compounds from HXHY herbs and DrugBank using the Calculate Molecular Properties protocol available in the Quantitative Structure-Activity Relationship module of DS 2.0 (17). Then, principal component analysis (PCA) was performed to map the distribution of compounds in the chemical space. In addition, calculations of the 'Lipinski's rule of five' and of chemical space were used to evaluate whether the tested herbal compounds were drug-like (18).

Docking simulations. The crystal structures of the protein-ligand complexes of 11 protein targets related to OA (Table I) were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB; www.rcsb.org) (19-21). Crystallographic water molecules were removed and hydrogen atoms were added in the file. The inhibitor reported on the PDB file was used to define the active site. The compounds from HXHY herbs were docked to these targets using the LigandFit module of DS 2.0. The docking was performed by generating sets of different compound conformations using a Monte Carlo algorithm, and matching these conformations to the binding site partitions (22). All 208 docked structures were thus sorted according to their DockScore. The DockScore of each target and its original inhibitor was used as the cut-off value, so that the targets and compounds with the higher DockScore were selected as potential targets and bioactive compounds.

Network construction and analysis. The procedure for network construction was the following: First, the 'ligand-target network' (L-T network) was established by connecting the predicted targets and bioactive compounds. Then, the disease list associated with the 11 targets was obtained from the Therapeutic Target Database (23) and the diseases were also classified into different groups using the medical subject headings terms (http://www.nlm.nih.gov/). A ligand-target-disease (L-T-D) network was constructed by connecting previously mentioned proteins to any associated diseases, and the diseases to different groups based on the L-T network. Cytoscape 2.8.3 analysis was carried out to construct these networks (24). In the networks, the compounds, targets and diseases are represented as nodes, and the edges between nodes represent intermolecular interactions. All data were analyzed using Cytoscape plugins (25).

Table I. Eleven protein targets related to osteoarthritis.

Protein	Full name	PDB code
MMP-12	Matrix metalloproteinase-12	3EHX
MMP-8	Matrix metalloproteinase-8	1ZP5
MMP-9	Matrix metalloproteinase-9	1GKC
MMP-3	Matrix metalloproteinase-3	1HY7
MMP-2	Matrix metalloproteinase-2	1HOV
ADAMTS-5	Aggrecanase-2	2RJQ
PPARγ	Peroxisome proliferator-activated receptor γ	2VSR
PDE-4a	Phosphodiesterase-4a	3I8V
PDE-4d	Phosphodiesterase-4d	3AIK
iNOS	Inducible nitric oxide synthase	2ORO
TNF-α	Tumor necrosis factor-α	2AZ5

PDB, Protein Data Bank (Research Collaboratory for Structural Bioinformatics member).

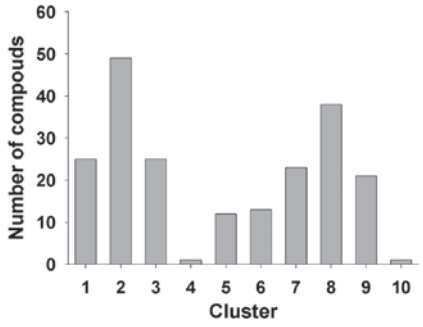

Figure 1. Distribution of compounds from Huoxue Huayu herbs based on clustering analysis.

Results

Chemical diversity and drug-likeness prediction. Compounds from HXHY herbs were subdivided into 10 clusters by employing the default settings of the Cluster Ligands protocol (Fig. 1). PCA revealed considerable dispersion in the chemical space distribution of the compounds from HXHY herbs (Fig. 2A). Some of compounds from HXHY herbs occupied similar chemical space with compounds from

Table II. The mean, minimum (Min) and maximum (Max) values of molecular descriptors for the compounds from 10 Huoxue Huayu herbs.

Name	Mean	Min	Max
C count	18.66	3	39
H count	23.05	6	60
O count	4.06	0	16
ALogP	3	-7.46	13.6
Apol	12,174.84	2,113.82	27,958.8
Molecular weight	319.15	59.11	742.92
No. of atoms	23.2	4	53
No. of rotatable bonds	3.79	0	26
No. of rings	3.27	0	7
No. of H acceptors	4.22	0	16
No. of H donors	1.69	0	15
Wiener	1,497.58	9	11,322
Zagreb	126.63	12	274
Molecular volume	220.79	55.56	444.52
Molecular surface area	312.02	91.4	692.23

Table III. Parameters of the ligand-target (L-T) network.

Parameter	HXHY L-T network value
No. of nodes	50
No. of edges	138
Network density	0.113
Network heterogeneity	0.99
Isolated nodes	0
No. of self-loops	0
Multi-edge node pairs	0
Network centralization	0.435
No. of shortest paths (%)	2,450 (100)
Characteristic path length	2.52
Average no. of neighbors	5.52

DrugBank (Fig. 2B). Furthermore, evaluation of the drug-like properties (Table II) showed that 95.19% of the compounds had a molecular weight <500, 92.79% had <10 H-bond acceptors, 90.38% had <5 H-bond donors and 82.69% had ALogP <5. These results demonstrated that the compounds from HXHY herbs possess chemical diversity and drug-likeness.

Prediction of potential targets and bioactive compounds. A virtual screening approach was adopted for compounds with the potential to inhibit protein targets related to OA. Among the 39 compounds screened, 28 were predicted to interact with more than one target.

The ligand-target space prediction. Potential ligand-target interactions were described in the HXHY L-T network (Fig. 3). Table III lists the values for a few simple parameters of the L-T network. The L-T network contains 50 nodes (39 ligands and 11 potential targets) and 138 edges. The network centralization and network heterogeneity were estimated at 0.435 and 0.99, respectively. This indicates that a few nodes are more central than others in the network. The analysis of the degree of connec-

Table IV. Top 10 compounds exhibiting the highest degrees of connectivity in the ligand-target network.

Index	Known	Chemical name	Degree
89	No	Salvianolic acid C	9
124	Yes	Safflor yellow A	8
78	No	Monomethyl lithospermate	8
113	No	6-Hydroxykaempferol-7-O-glucoside	7
88	No	Salvianolic acid A	7
103	Yes	Naringin	7
187	Yes	Coptisine	6
26	No	Folic acid	6
55	Yes	Danshensuan B	6
86	Yes	Rosmarinic acid	5

The term index represents individual compounds from the Huoxue Huayu herbs.

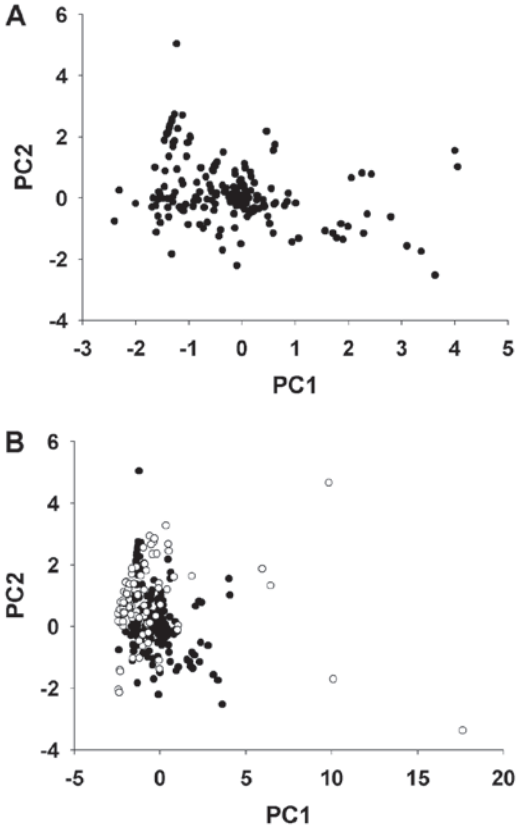

Figure 2. Plot of the first two principal components of compounds from Huoxue Huayu herbs (black circles) and the DrugBank (white circles). (A) Considerable dispersion in the chemical space distribution of the compounds from HXHY herbs. (B) Some of the compounds from HXHY herbs occupied similar chemical space with compounds from DrugBank. PC1, first principal component; PC2, second principal component.

Table V. The 49 diseases related to the 11 targets.

Index	Disease
D1	Abscess
D2	Adrenocorticotrophic hormone-secreting pituitary tumors
D3	Advanced lung cancer
D4	Asthma
D5	Atherosclerosis
D6	Atopic dermatitis
D7	Autoimmune diseases
D8	Behcet's disease
D9	Bladder cancer
D10	Brain Cancer
D11	Breast cancer
D12	Chronic fatigue syndrome
D13	Congestive heart failure
D14	Crohn's disease
D15	Diabetes mellitus
D16	Duchenne muscular dystrophy
D17	Emphysema
D18	Guillain-Barre syndrome
D19	Hepatocellular carcinoma
D20	Hormone-refractory prostate cancer
D21	Hyperimmunoglobulinemia D
D22	Inflammation
D23	Inflammatory bowel disease
D24	Insulin resistance
D25	Ischemia reperfusion injuries
D26	Ischemic heart disease
D27	Kaposi's sarcoma
D28	Lung cancer
D29	Multiple sclerosis
D30	Muscle atrophy
D31	Myocardial infarction
D32	Noninsulin-dependent diabetes mellitus
D33	Non-small cell lung cancer
D34	Obesity
D35	Ulcerative colitis
D36	Osteoarthritis
D37	Osteoporosis
D38	Ovarian cancer
D39	Pancreatic cancer
D40	Prostate cancer
D41	Psoriasis
D42	Renal cell carcinoma
D43	Renal interstitial fibrosis
D44	Restenosis
D45	Rheumatic diseases
D46	Rheumatoid arthritis
D47	Smooth muscle hyperplasia
D48	Testicular cancer
D49	Thyroid follicular carcinoma

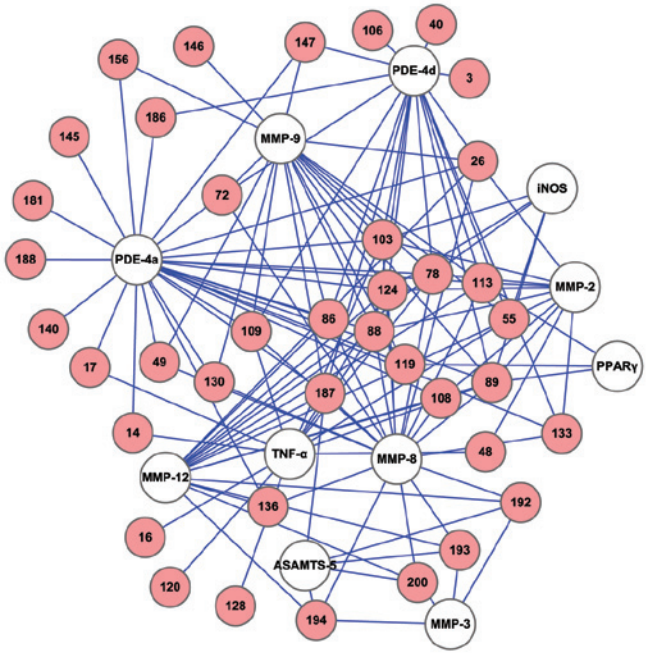

Figure 3. Huoxue Huayu (HXHY) ligand-target network. The pink and white nodes (circles) represent compounds (ligands) from HXHY herbs and the targets related to osteoarthritis, respectively. The edges represent interactions between nodes.

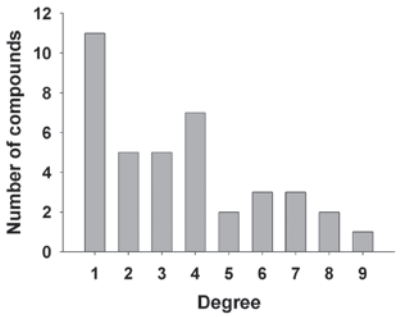

Figure 4. Distribution of compounds for different degrees of connectivity in the Huoxue Huayu ligand-target network.

tivity for the compounds in the HXHY L-T network is presented in Fig. 4 and the chemical names of the top 10 compounds with regards to the degree of connectivity are shown in Table IV.

Associations of HXHY herb compounds with other diseases. The predicted targets were associated with a total of 49 diseases (Table V). According to the Medical Subject Headings controlled vocabulary (http://www.nlm.nih.gov/), these diseases are classified into 19 groups. The L-T-D network was also constructed to explore the interactions and identify potential roles for the compounds of HXHY herbs (Fig. 5). Overall, these results indicate that HXHY herbs may act beneficially in a range of distinct diseases.

Discussion

The pathogenesis of OA appears to be the result of multiple abnormalities including those in protease and cytokine activities (26). A single drug is most probably insufficient for OA

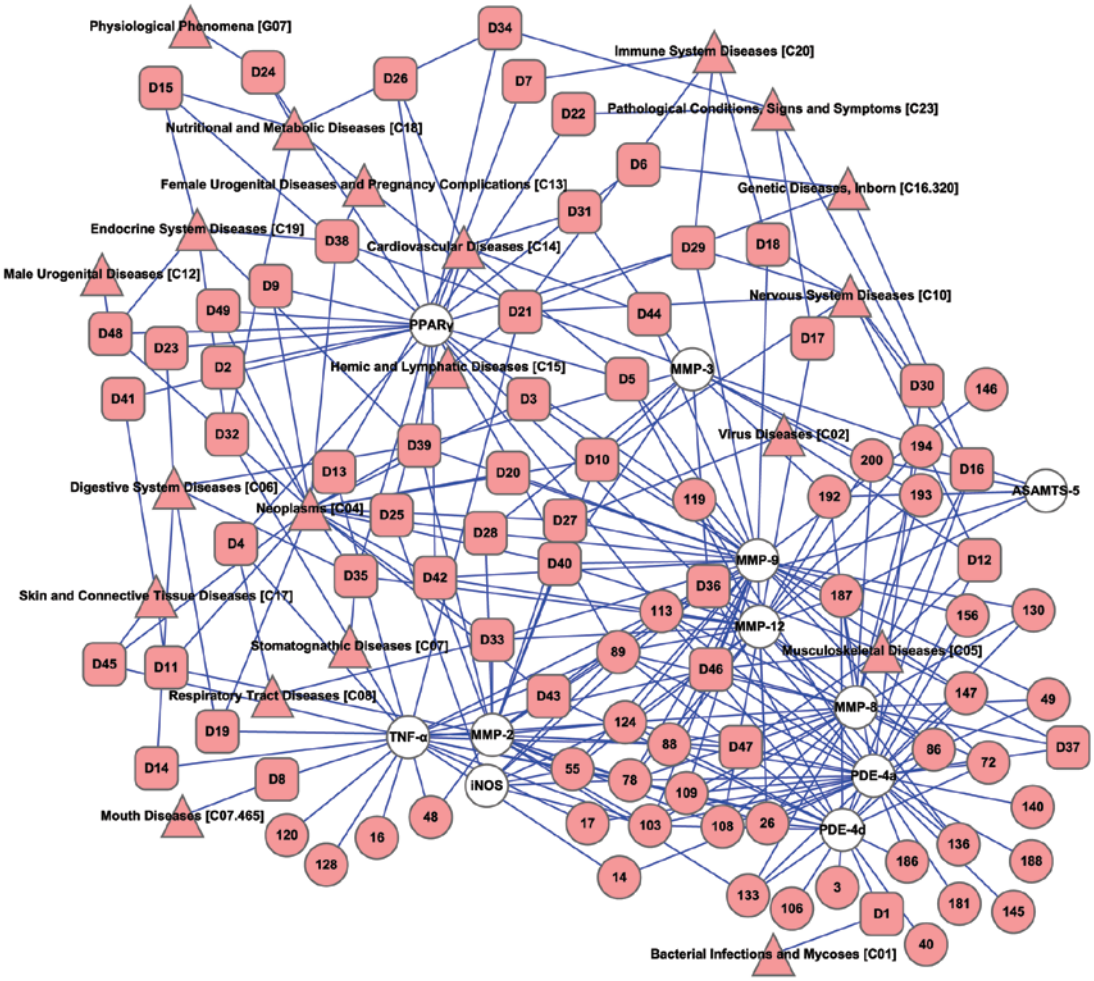

Figure 5. The ligand-target-disease network. The white and pink nodes (circles) represent the targets and the ligands (compounds) from Huoxue Huayu herbs, respectively, the rectangles represent the diseases related to the 11 targets and the triangles represent the disease classifications according to the Medical Subject Headings classification system.

therapy. It was previously hypothesized that a complex disease with multi-factorial pathophysiology can be more effectively treated through the use of a multi-drug mixture as compared to a single drug (27). Therefore, multi-target drug treatment is a promising approach for clinical OA therapy.

In the present study, we used docking simulations to identify the protein targets of HXHY herbs, based on the chemical diversity (Fig. 1) and drug-likeness (Fig. 2) of compounds (ligands) contained in these herbs. Twenty-eight compounds were predicted to bind to more than one protein. Evidence suggests that such compounds, also known as promiscuous drugs, may present several benefits (28). It is foreseeable that a 'one-drug-multiple-targets' therapeutic strategy is to be adopted for treatment of OA in the future. In addition, we identified 11 compounds predicted to bind to only one target. These compounds could be considerably potent when combined, with different combinations selectively targeting different multiple targets. These findings suggest that the studied herbs may be useful sources of both promiscuous drugs and combination drugs that can be used in combination therapies.

To elucidate the relationships between the active compounds and their targets, the L-T network was constructed by connecting ligands to the corresponding targets (Fig. 3). The average number of potential targets per compound was 3.5. Generally, the compounds with higher degree of connectivity are more potent pharmacologically (29). A few of the compounds identified here as having a high-degree of connectivity (Table IV) have been reported in the literature (30-32). These results overall suggest that HXHY herbs may simultaneously target multiple proteins.

Considering the distinct effects and applications of HXHY herbs in the clinic (33-35), we constructed the L-T-D network (Fig. 5) to link the 11 targets and the related diseases to gain a global understanding of diseases associated with the compounds of the L-T network. It is believed that compounds targeting the same protein that is associated with different diseases may be beneficial in different diseases (36). A total of 49 diseases showed associations with the compounds of HXHY herbs (Table V). This finding suggests that HXHY herbs demonstrate considerable potency for musculoskeletal diseases, as well as neoplasms, the nervous system, cardiovascular, nutritional and metabolic diseases. For instance, matrix metalloproteinase-12, which was predicted as the target of coptisine (Table IV), is associated with diverse diseases, including musculoskeletal, cardiovascular and digestive system diseases (23). Previous studies have provided evidence that coptisine selectively prevents vascular smooth muscle cell proliferation at low concentrations and exerts a cardioprotective effect through its antioxidative properties and inhibition of the RhoA/Rho

kinase pathway in rats with isoproterenol-induced myocardial infarction (37,38). This compound may thus prevent cardiovascular diseases. Therefore, HXHY herbs containing multiple compounds that target multiple proteins are expected to exhibit polypharmacological therapeutic effects, further allowing the prediction of new targets and applications for these herbs. These hypotheses are consistent with the principle of TCM, whereby diverse diseases are treated with the same herb.

In summary, findings from our study indicate that the compounds of HXHY herbs target multiple proteins associated with OA, which provides a molecular basis for the clinical application of HXHY herbs in multi-target therapeutic treatment of OA. Our findings also suggest that HXHY herbs may be used for the treatment of additional diseases besides OA. Moreover, the *in silico* approach adopted herein provides new insights on the molecular mechanisms underlying the beneficial effects of herbs used in TCM, thus promoting discovery of new drugs.

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