STUDY ON MECHANISM OF YINLAI DECOCTION’S ANTI-INFLAMMATORY EFFECTS WITH THE METHOD OF NETWORK PHARMACOLOGY

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Introduction: Yinlai decoction is an experiential prescription composed of Jin Yin Hua (honeysuckle flower), Lai Fu Zi (radish seed), Lian Qiao (weeping forsythia capsule), Huang Qin (scutellaria root), Yu Xing Cao (heartleaf houttuynia herb), Qian Hu (hogfennel root) and Gua Lou (snakegourd fruit). It is widely used with a significant effect in common cough, pneumonia and respiratory tract infections.(1)

In recent years, studies have prompted that the medical category of heat pattern has some connection with inflammation of modern medicine. Yinlai decoction is significantly effective in treating lung-stomach heat retention pattern. Pre-existing experimental study has showed that Yinlai decoction achieves associated effects by regulating Tumor Necrosis Factor-α (TNF-α), Interleukin-6 (IL-6), Interleukin-2 (IL-2), Interleukin-10 (IL-10), Secretory Immunoglobulin A (sIgA) and other inflammatory immune targets(2,3,4,5). However, because of these fragmented experimental indicators, the regulatory mechanism of Yinlai decoction in inflammatory processes still needs to testify.

Chinese herbal compound prescription is composed of many different compounds with various structures and functions. It has multi-level, multi-link and multi-target action characteristics(6).
Thus, we use the network pharmacological method, based on the theory of systems biology, from the review of holistic and systematic interactions of Chinese decoction, target points and disease, through the use of complex biological network model, to reveal the whole complex biological network relations of Chinese decoction, genes, target points and the disease, and based on that we will analyze and forecast pharmacological mechanisms of the Chinese decoction. We analyze the existing data, and then collate target points and their chemical molecules which are associated with inflammation in Yinlai decoction; through the analysis of the potential interaction between the various target points, we construct network of target-points relevant to Yinlai Decoction’s anti-inflammation, and then analyze associated pathological pathways, and summarize inflammation-related mechanism of Yinlai decoction.

Materials and methods

Establish database for inflammation-related target points of Yinlai decoction

Traditional Chinese Medicine System Platform (TCMSP) is a distinctive Chinese herbal pharmacological platform system, which combines pharmacodynamics, pharmacokinetics and network, genomics and system analysis to form a unified systematic model. We can obtain information about the relationship between herbal targets and disease through the platform. The platform consists of 499 Chinese herbs registered in “Pharmacopoeia of the People’s Republic”, 29,384 active components, 3311 target points and 837 related diseases. Among the platform, the systematic pharmacological database includes herbal components, action target points and associated diseases, which have been widely recognized by the industry.

Log in TCMSP’s home page (http://lsp.nwsuaf.edu.cn/tcmsp.php) and check the main chemical components of each herb in Yinlai decoction in “HERB”. Database of chemical components in Yinlai decoction is constructed; the action target point of each chemical components is searched and databases of action target point of Yinlai decoction is constructed; then each target point is searched in “Target name” to obtain the corresponding disease of each target point, forming components - target points - diseases network; finally, inflammation-related target points and their action molecules are screened out.

Draw relational graph of interaction between target points

String database is a database used to retrieve the interaction between known and predicted proteins in order to provide a critical evaluation and integration between protein interaction, including direct (physical) and indirect (functional) association. The new version STRING v10.0 covers 2031 kinds of creatures, including 9,643,763 proteins. It introduces many levels and structured orthologous annotation.

In various systems, the level of resolution will occur into the family of proteins, to achieve a forecasting process between proteins inferred from the co-expression of the associated data, and users can perform statistical analysis of enrichment-related testing in the database network.

Log in String Database’s Home page (http://string-db.org/), select “multiple names”, input relevant target point name, select “homo sapiens” in “organism” for the search, and after gain the corresponding protein graph, select “action view” to analyze and record the interaction types and scores between proteins.

Acquire inflammation-related pathways of Yinlai decoction

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database jointly developed by Kyoto University and University of Tokyo. It organically combines genomic information and high-level functional information. This database is composed of six databases, namely gene database, pathway database, ligand chemical reaction database, sequence similarity database, gene expression database and protein molecule interrelation database. The database has powerful graphic capabilities, expressing numerous metabolic pathways and the relations between them by use of graphics. KEGG can provide researchers with richer information by establishing a connection with the world’s other major bioinformatics databases.

Log in KEGG database’s homepage (http://www.genome.jp/kegg/), retrieve in the retrieving bar of “KEGG” according to the corresponding summarized target points of Yinlai decoction, select human as biological species, compile relevant data of pathways, and enrichment analysis of the obtained results of pathways are carried out.
Results

Database of inflammation-related target points of Yinlai decoction

A total of 20 inflammation-related target points of Yinlai decoction are obtained from retrieval, which include: inhibitor of nuclear factor kappa B kinase beta subunit (IKBKB), Peroxisome proliferator activated receptor delta (PPARD), Mitogen-activated protein kinase 8 (MAPK8), Mitogen-activated protein kinase 9 (MAPK9), Tumor necrosis factor (TNF), Mitogen-activated protein kinase 14 (MAPK14), Beta-2 adrenergic receptor (ADRB2), Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6), Nitric-oxide synthase, endothelial (NOS3), C-C motif chemokine 2 (CCL2), D(2) dopamine receptor (DRD2), Phospholipase A2 (PLA2G1B), Toll-like receptor 2 (TLR2), Prostaglandin G/H synthase 2 (PTGS2), Prostaglandin G/H synthase 1 (PTGS1), Leukotriene A-4 hydrolase (LTA4H), Arachidonate 5-lipoxygenase (ALOX5), 5-hydroxytryptamine 6 receptor (HTR6) and Neutrophil collagenase (MMP8).

These 20 inflammation-related target points in each herb are distributed as follows: 13 inflammation-related targets in Jin Yin Hua (honeysuckle flower); 8 in Lai Fu Zi (radish seed); 12 in Huang Qin (scutellaria root); 14 in Lian Qiao (weeping forsythia capsule); 16 in Qian Hu (hogfennel root); 14 in Gua Lou (snakegourd fruit); 12 in Yu Xing Cao (heartleaf houttuynia herb).

Correlations between the various target points

By using string database, we analyze possible relationships between the target points. A total of 71 correlations exist in 20 related target point network from analyzing interactive target points, shown in Figure 1.

Yinlai decoction regulating inflammation-related pathways

In order to more systematically analyze the biological function of the target points, we use sectional PATHWAY database of KEGG to carry out Pathway analysis for target points. Relevant 53 passages (P-Value <0.001), are obtained.

In these pathways, signaling pathways are most closely associated with inflammation, including TNF signaling pathway, NOD-like receptor signaling pathway and Toll-like receptor signaling pathway. These three important pathways jointly contain NF-kappa B signaling pathway and MAPK signaling pathway two Article important signal transduction pathways. Disease-related pathways include not only Chagas disease, Influenza A and other infectious diseases, but also inflammatory process of chronic diseases such as rheumatoid arthritis, inflammatory bowel disease (IBD), type 2 diabetes and cancer. All pathways establish linkages through key target points. By analyzing inflammation-related target points of Yinlai decoction in various pathways, the most relevant six core target points, TNF, IL-1B, IL-6, IKBKB, MAPK8 (JNK) and MAPK14 (P38) are obtained. KEGG pathway analyses are carried out for TNF signaling pathway, TLRs signaling pathway and NOD signaling pathway, and the results are shown in Figure 2-4:

Discussion

Inflammation is a complex defense reaction of living tissue with a vascular system to damage factors. There are multiple pathways functioning together in the inflammatory reaction process together. There are mainly 20 inflammation-related target points of Yinlai decoction, 53 inflammation-related pathways, six core anti-inflammatory target points: TNF, IL-1B, IL-6, IKBKB, MAPK8 (JNK) and MAPK14 (P38), and three key role pathways.

As an important cytokine, TNF can cause extensive signal transduction of pathway, which is closely related to inflammation and immunity. Activated TNF combines with corresponding receptors which include TNFR1 and TNFR2, among which TNFR1 is the major TNFα receptor that can activate the transcription factor NF-κB, regulate apoptosis and regulate inflammation. TRADD is formed after the combination of both TNFR1 and TNFR2 and further promote the activation of additional genes.
These genes are mainly influenced by two different pathways: one is influenced by NF-κB signaling pathway and MAPK cascade pathway releasing IL-1β, IL-6, TNFα and other inflammatory factors, and may also release PTGS2 forming inflammatory response; the other leads to apoptosis or necrosis\(^{(10,11)}\).

Based on this pathway, Yinlai decoction’s inhibiting mechanism of inflammatory factors may be through regulating NF-κB signaling pathway and the levels of IKK, MAPK8 and MAPK14 in MAPK cascade pathway, or through regulating TLR2 to cause MyD88 to generate a relevant role, thus to achieve its anti-inflammatory effect.

NLRs can sensitively detect multiple pathogens, and produce innate immune response. In NLRs family, NOD1 and NOD2 are two important types. Once the pathogen is found, it will drive the NF-κB signaling pathway and the activation of MAPK cascade pathway, but can also lead to production and apoptosis of cytokines. The pathway, on the other hand, exists a series of NLRs. These receptors can induce the activation of CASP1 through the aggregation of inflammasome, in the synergy of TRLs signaling pathway, they can further cause the release of pro-inflammatory factors and resulting in heat production of the bod, cell death and other inflammatory responses\(^{(14,15,16,17,18)}\). Based on this pathway, Yinlai decoction’s anti-inflammatory effects may block inflammatory processes through regulating NF-κB-based signaling pathway and the levels of IKK, MAPK8 and MAPK14 in MAPK cascade pathway, or acting on NOD and its downstream RIP2 factors. In addition, Yinlai decoction may achieve its anti-

**Figure 2:** KEGG analysis chart of inflammation-related target points of Yinlai decoction in TNF signaling pathways.

**Figure 3:** KEGG analysis chart of inflammation-related target points of Yinlai decoction in TLRs signaling pathways.

TLRs plays an important role in the body’s innate immune system. After TLRs recognizing pathogens, based on the level of the release of pro-inflammatory factors and upregulation of costimulatory molecules, it rapidly activates the innate immunity of the human body. The signaling pathway can be divided into two branches: MyD88-dependent pathway and MyD88-independent pathway. The former causes the production of pro-inflammatory factors by rapidly activating NF-κB signaling pathway and MAPK cascade pathway, and thereby enables the body to produce inflammatory response\(^{(12,13)}\). Based on this pathway, Yinlai decoction’s regulating mechanism of inflammatory factors may be through regulating NF-κB signaling pathway and the levels of IKK, MAPK8 and MAPK14 in MAPK cascade pathway, or through regulating TLR2 to cause MyD88 to generate a relevant role, thus to achieve its anti-inflammatory effect.
inflammatory effects by regulating the level of CASP1.

Thus, Yinlai decoction’s anti-inflammatory mechanisms of anti-inflammation on different pathways are different but also relevant. Its possible anti-inflammatory mechanisms can be summarized as follows:

- Directly inhibiting IL-1β, IL-6, TNFα and other inflammatory factors;
- Regulating the expression level of NF-κB in NF-κB signaling pathway and its upstream of IKK;
- Regulating the levels of MAPK8 and MAPK14 in MAPK cascade pathway expression;
- Inhibiting the binding of TNF and TNFR to reduce expression levels of TRADD and TRAF;
- Indirectly regulating expression levels of MyD88 through TLR2;
- Regulating the expression levels of NOD and its downstream RIP2;
- Regulating the expression level of CASP1.

Thus, it can be found that Yinlai decoction’s anti-inflammatory mechanism of action is not to regulate against a single pathway or some target point, but probably through the joint intervention of multiple target points and complicated pathways to exert effects.

Based on the target points, herbal composition of Yinlai decoction is further analyzed. The synergy of Jin Yin Hua (honeysuckle flower), Lian Qiao (weeping forsythia capsule), Qian Hu (hogfennel root) and Yu Xing Cao (heartleaf houttuynia herb) can regulate MAPK8. All herbs in the formula can act on IL-6, TNFα and PTGS2. Jin Yin Hua (honeysuckle flower) also can acts on TLR2. In addition, Gua Lou (snakegourd fruit) can act on MAPK14 and IKBKB, as the basic substance for anti-inflammation. The compatibility of all herbs in this formula can commonly strengthen effects, and the clinical treatment effect is significant.

This study declares the preliminary findings of Yinlai decoction is reliable from prospective of network pharmacology, but because of the single perspective of data, the present study has some shortcomings. The experimental study of anti-inflammatory mechanism of Yinlai decoction needs further verification, thus we can more systematically and scientifically explain that.

References


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Acknowledgement
This work was financially supported by the National Natural Science Foundation of China (81403328).

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