Potential Pharmacodynamic Mechanism of the Main ingredients in Licorice for Chronic Obstructive Pulmonary Disease

Cai Chen^{1&}, Jianpeng An^{2&}, Guodong Shen¹, Yang Shen^{3#}

¹Shandong Institute of Advanced Technology Chinese Academy of Sciences, Jinan, 250000, China.

²Integrated Traditional Chinese and Western Medicine Hospital of Tongzhou District, Beijing, China

³Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, 430030, Hubei, China

&These authors contributed equally to this work.

*Corresponding author: YangShen

Ministry of Environmental Protection, School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, 430030, Hubei, China.

Abstract

Purpose: This study aimed to investigate the effect of essential ingredients of licorice on the chronic obstructive pulmonary disease (COPD). Method: The ingredients information were obtained from *PubChem* (<https://pubchem.ncbi.nlm.nih.gov/>), related genes about COPD was collected from *geneCards* (<http://www.genecards.org/>). Network pharmacology was utilized in this study. Result: The intersection data set contains 20 molecular targets between COPD and liquorice. Protein-protein interaction network showed that there are a total of 58 nodes and 137 edges involved. The link number of AKT1 in PPI network was 39, which is the highest level of interaction. MAPK1 is an important target of Licorice on COPD. Conclusion: MAPK signaling pathway could be the important key target of main ingredients of licorice on COPD.

Keyword: COPD, licorice, Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, network pharmacology

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37 **I. Introduction**

38 Chronic obstructive pulmonary disease (COPD) is one of the common preventable
39 and treatable disease, with the emblematic symptom of persistent airflow restriction,
40 which is associated with an increased chronic inflammatory response of the airways
41 and lungs to toxic particles or gases[1-4]. According to the World Health Organization,
42 approximately 3 million people in the world dead as a consequence of COPD each
43 year. One meta-analysis of global prevalence of COPD, which purposed to examine
44 the global prevalence of COPD in men and women, revealed that prevalence rate of
45 COPD was 9.23% (95% CI:8.16%-10.36%) among male group and 6.16% (95%
46 CI:5.41%-6.95%) among female group[5]. Moreover, there were some complications
47 of COPD, such as cardiovascular disease, anxiety and depression, lung cancer [6-8],
48 which leads to the lower living quality and much more burden of patient's life[9, 10].

49

50 Liquorice, widely distributed among Asia, Europe, America, etc., has been used
51 as medicinal material for long years[11-13]. Some researches have proved that
52 liquorice has the function of anti-inflammation, preventing coughing, antianaphylaxis
53 and so on[14, 15]. It was reported that the essential ingredients of liquorice are
54 glycyrrhizic acid, glycyrrhizin, quercetin, and formononetin[16, 17]. Generally,
55 liquorice, the whole plant is utilized to made as pills or decoction. Consequently, the
56 mechanism and molecular pathways for every component was not clear. Up to now,
57 much researches concentrated on the biological effect of liquorice as a whole, while
58 the molecular effect of its every component hasn't been figured out. Considering its
59 bargain price, widespread distribution and unique biological effect, this research
60 purposed to investigate the mechanism of essential component's signaling pathways
61 (formononetin, liquiritin, glycyrrhizic acid, glabridin, quercetin and isoliquiritigenin)
62 based on network pharmacology.

63

64 II. Method and Materials

65 The ingredients of liquorice involved in this research were formononetin,
66 liquiritin, glycyrrhizic acid, glabridin, quercetin and isoliquiritigenin, respectively.
67 Their two-demension and three-demension struction were from public database,
68 *PubChem* (<https://pubchem.ncbi.nlm.nih.gov/>). Pharmacokinetic information of these
69 compound was obtained from *Traditional Chinese Medicine Systems Pharmacology*
70 (*TCMSP*), including drug-likeness (DL), oral bioavailability (OB), intestinal epithelial
71 permeability (Caco-2) and number of H-bond donor/acceptor (Hdon/Hacc).

72

73 The target molecule of the Liquorice ingredients were attained from public
74 database *Swisstargetprediction* (<http://www.swisstargetprediction.ch/>). The potential
75 molecular target was selected when the probability was above zero. The related genes
76 about COPD was collected from *geneCards* (<http://www.genecards.org/>), and the
77 genes were used in this research when *relevance score* was more than 30.

78

79 Construction and analysis of component-action target network was based on
80 Cytoscape software (version 3.7.2). Six compound and common target between
81 COPD and Liquorice was imported to Cytoscape, after position adjustment network
82 was finished.

83

84 The String database (<https://string-db.org/>) is one database containing known and
85 predicted protein-protein interactions, in which a large number of protein-protein
86 interactions were collected, involving a total of 9643763 proteins and 138838440
87 interactions, including data detected experimentally and predicted by bio-information
88 methods. The common targets between disease and Liquorice were imported into the
89 String database to define human species and obtain the protein interaction relationship.

The results were saved in TSV format. The node1, node2 and combined score information in the file was retained and imported into Cytoscape software to draw the interaction network. The core of protein-protein interaction network was calculated in R software. Then GO enrichment analysis and KEGG pathway annotation analysis were finished in *Rstudio* with the p-value <0.05 as well.

III. Result

Figure 1 and **Figure 2** showed the two and three dimension structure of formononetin, liquiritin, glycyrrhizin, glabridin, quercetin and isoliquiritigenin, respectively. Pharmacokinetic information was illustrated in **Table 1**. The number of H-bond donor/acceptor for formononetin, liquiritin, glycyrrhizin, glabridin, quercetin and isoliquiritigenin were 1/4, 8/16, 5/9, 2/4, 5/7 and 6/9, respectively. The oral bioavailability of formononetin, liquiritin, glycyrrhizin, glabridin, quercetin and isoliquiritigenin were 69.67, 9.06, 65.69, 53.25, 46.43 and 8.61, respectively.

There were 417 target molecule for COPD according to relevance score mentioned above and 64 target molecule for Liquorice (**Figure 3**). The intersection data set contains 20 molecular targets between COPD and liquorice. The information of the six active ingredients and molecular targets of Licorice was introduced into Cytoscape to construct the network, as shown in **Figure 4**. There are a total of 58 nodes and 137 edges involved. The black type indicates the six ingredients of Licorice, blue ellipse represented the potential target. It can be seen from the **Figure 4** that the same target could be corresponding to different active ingredients or the same active ingredient, which fully reflects the multi-component and multi-target action characteristics of Liquorice.

Figure 5 was the result after the process of String database. Color ‘green’ is the gene neighborhood, ‘black’ represents the co-expression, ‘blue’ is gene co-occurrence,

‘red’ is the gene fusions. As shown in **Figure 6**, the link number of AKT1 was 39, which is the highest level of interaction. **Figure 7** presented the top ten key targets. GO enrichment analysis is a finite acyclic graph that counts the number or composition of proteins or genes at a functional level. The generatio of endopeptidase activity and phosphatase were greater than the others. Nuclear receptor activity and transcription factor activity, direct legend regulated sequence specific DNA binding were least (**Figure 8**, **Table 2**). The results of KEGG analysis are shown in **Figure 9**. The counts of proteoglycans in cancer, endocrine resistance, MAPK signaling pathway, EGFR tyrosine kinase inhibitor resistance, relaxin signaling pathway, Rap1 signaling pathway was 14,12, 12,10,10 and 10, respectively.

IV. Discussion

COPD is a chronic bronchitis and/or emphysema characterized by airflow obstruction that could progress to pulmonary-heart disease and respiratory failure as a common chronic disease[2, 4, 18]. It has been formed a broad consensus that COPD is associated with abnormal inflammatory reactions with high morbidity and mortality[19]. Licorice has been internationally utilized for medicinal herb, while its mechanism of some main ingredients for COPD still kept unclear. The purpose of the present study was to investigate the effect of main ingredients on COPD according to network pharmacology.

In this study, it revealed that Akt1 is the common target between COPD and licorice. AKT1, also named as protein kinase B, is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulates many processes including metabolism, proliferation, cell survival, growth and angiogenesis[20-22]. It appeared as one key node in PI3K-AKT signaling, protects against acute lung injury[23]. Qu’s experiment indicated that glycyrrhizic acid inhibited the production of inflammatory factors in LPS-induced ALI by

regulating the PI3K/AKT/mTOR pathway related autophagy[24]. Vito Lorusso and Ilaria Marech summarized that isoliquiritigenin could suppress HIF-1 α level, VEGF expression and secretion, cell migration and to reduce the expression and secretion of MMP-9/-2 and these effects might be mediated through inhibition of p38, PI3K/Akt and NF- κ B signaling pathways[25].

As shown in **Figure 8**, MAPK1 was one of critical targets, which is an important transmitter of signals from the cell surface to the inside of the nucleus. The result of KEGG enrichment analysis also illustrated that licorice relieves COPD symptoms via MAPK signaling pathway. It has been reported that MAPK pathway is one of the common intersection pathways of signal transduction pathways including stress, inflammation, cell proliferation, differentiation, functional synchronization, transformation, apoptosis and so on. Previous studies demonstrated that MAPK signal pathway was involved in the inflammation reaction and oxidative stress[26, 27]. Some researchers also found that the ingredients of licorice has the function to mediate the expression of MAPK signaling pathway[28, 29].

The limitation of the present study was that this study was performed abstractly, in the future study, we will operate animal experiment to prove it with ethical approval.

V. Conclusion

MAPK signaling pathway could be the important key target of main ingredients of licorice on COPD.

VI. Availability of data and materials

The ingredients of liquorice involved were attained from *PubChem* (<https://pubchem.ncbi.nlm.nih.gov/>). Pharmacokinetic information of these compound was obtained from *Traditional Chinese Medicine Systems Pharmacology (TCMSP)*.

The target molecule of the Liquorice ingredients were attained from public database *Swisstargetprediction* (<http://www.swisstargetprediction.ch/>). The related genes about COPD was collected from *geneCards* (<http://www.genecards.org/>).

VII. Consent to Publish

All author consent to publish this article in this article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1 Pharmacokinetic information about six compounds

	Hdon	Hacc	OB (%)	Caco-2	DL
Formononetin	1	4	69.67	0.78	0.21
Liquiritin	8	16	9.06	-2.23	0.11
Glycyrrhizin	5	9	65.69	-1.06	0.74
Glabridin	2	4	53.25	0.97	0.47
Quercetin	5	7	46.43	0.05	0.28
Isoliquiritigenin	6	9	8.61	-1.36	0.6

Table 2 GO enrichment analysis result

ID	Description	pvalue	Count
GO:0019902	phosphatase binding	2.12E-09	9
GO:0004713	protein tyrosine kinase activity	3.60E-09	8
GO:0019903	protein phosphatase binding	5.09E-09	8
GO:0004252	serine-type endopeptidase activity	1.46E-08	8
GO:0004714	transmembrane receptor protein tyrosine kinase activity	2.03E-08	6
GO:0008236	serine-type peptidase activity	3.98E-08	8
GO:0017171	serine hydrolase activity	4.72E-08	8
GO:0019199	transmembrane receptor protein kinase activity	8.87E-08	6
GO:0004175	endopeptidase activity	2.61E-07	10
GO:0004222	metalloendopeptidase activity	4.33E-07	6

Figure 1 The two and three demonsion structure compounds (A: formononetin; B: liquiritin; C: glycyrrhizin)

Figure 2 The two and three demonsion structure of compounds

Figure 3 The target molecule of COPD and liquorice

Figure 4 The interaction network COPD, liquorice and molecular targets

Figure 5 The structure of protain-protain interaction network

Figure 6 PPI network link number

Figure 7 The top ten targets in the PPI network

Figure 8 The result of GO enrichment analysis

Figure 9 The result of KEGG enrichment analysis

Figure 1 The two and three demonion structure compounds (A: formononetin; B: liquiritin; C: glycyrrhizin)

Figure 2 The two and three demethoxylation structure of compounds (A: glabridin; B: quercetin; C: isoliquiritigenin)

Figure 3 The target molecule of COPD and liquorice

Figure 4 The interaction network COPD, liquorice and molecular targets

Figure 6 PPI network link number

Figure 7 The top ten targets in the PPI network

A

B

Figure 8 The result of GO enrichment analysis

Figure 9 The result of KEGG enrichment analysis