Potential Mechanism of Analgesic Effect of Active Components of Rhizoma Typhonii based on Network Pharmacology

Jie Zhao[1], Hongjie Gao[2,*]

[1]SDepartment of Tendering and Procurement, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, 410103, China;

[2]The Key Laboratory of Biomedical Information Engineering, Ministry of Education, Mitochondrial Biomedical Research Institute, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, 710049, China.

*532136292@qq.com

Abstract

Through data mining and bioinformatics analysis platform, the pain signal pathway of Rhizoma typhonii extract was constructed and its potential molecular mechanism was predicted. TCMSP, PharmMapper, STITCH and other databases were used to obtain the chemical components and corresponding targets of Rhizoma typhonii. NCBI-GENE, GeneCards, OMIM and other databases were used to screen pain related targets. the "compound target" interaction network and protein interaction (PPI) network were constructed by using Cytoscape software and string database to screen the key components and key targets of therapeutic effect. Go and KEGG pathway enrichment analysis were used to identify the potential mechanism. The results showed that there were 43 active components and 900 targets in the analgesic effect of Rhizoma typhonii, including 3 key components and 12 key targets. The GO and KEGG Pathway analysis showed that the extract of Rhizoma typhonii was associated with the P53/NF-kB pathway in pain treatment. the extract of Rhizoma typhonii plays an important role in the treatment of pain through "multi-component, multi-target and multi-channel". This study provides the basis for the study of the material basis and mechanism of analgesic effect of Rhizoma typhonii.

Keywords

Rhizoma Typhonii; Active Ingredients; Analgesia; Beta-sitosterol; Signal Pathway.

1. Introduction

Pain is the first disease that human beings feel and recognize, that is, after the body receives the stimulation of internal and external environment, the pain causing substances are produced and released from the tissue, the pain receptor is sensitized, the pain information is transmitted, the sensory center perceives, and finally enters the consciousness stage, which leads to pain[1]. The pain causing substances include bradykinin, histamine, 5-HT and prostaglandin, which can excite or sensitize nociceptors when they receive various stimuli[2]. At present, western medicine is widely used in the treatment of various kinds of pain, which has the effect of addiction and tolerance, which limits the clinical application and reduces the therapeutic effect[3][4]. Yubaifu (Aconitum carmichaeli) is a dry tuber of the Araceae plant, which is pungent, sweet, warm, poisonous, and has been used to treat pain for thousands of years. Pharmacology has the function of analgesia and sedation, that is, calming convulsion, interpreting Sanjie and relieving pain. The main functions include stroke, phlegm stagnation, mouth and eye vortex, astringent language, phlegm headache, partial headache, laryngeal obstruction, sore throat and tetanus[5]. For external use, it can be used to treat scrofula, phlegm, and snakebite. The main components are beta-sitosterol, inactive inositol, p-sitosterol-d-glucoside and so on. Modern pharmacology has shown that yubaifu has sedative, analgesic, anticonvulsant, anti-inflammatory, anti-tumor activities. At present, processed products are mostly used as medicine. At present, the effective components and analgesic mechanism of yubaifu

are not clear[6]. In this paper, the effective chemical components and corresponding targets were obtained by TCMSP, STITCH and other databases; the pain related targets were screened by PubMed, CTD, TTD, GeneCards, OMIM, drugbank and other databases; the "compound target" interaction network and protein interaction (PPI) network were constructed by using Cytoscape software and string database to screen the key components and key targets that play a therapeutic role. GO and KEGG pathway enrichment analysis were carried out on the key targets to learn the potential mechanism of the extract[7].

2. Materials and methods

2.1 Collection and screening of chemical constituents from Rhizoma typhonii

This study was conducted through TCMSP. All the active components of Rhizoma typhonii were searched and the chemical index database was used to match the small molecular compounds. The main active components were further screened according to the oral availability (OB) ≥ 30% and drug like (DL) ≥ 0.18. [8] OB and DL are the key indicators to evaluate the effective utilization of drugs. Generally speaking, the active components with OB ≥ 30% and DL ≥ 0.18 can be regarded as the main active components of drugs.

2.2 Prediction of potential targets

The protein targets of compounds were predicted by using PharmMapper and STITCH, and the retrieval function of UniProtKB in UniProt database was used. By inputting the protein name and limiting the species to human, all target gene names were corrected to official gene symbol, and the active components without target were eliminated. Obtain the information of active components and related targets.

2.3 Pain target prediction

According to NCBI-GENE, GeneCards and OMIM databases, the key words "pain" were used to search and screen the related targets, remove the duplicate genes, and summarize the potential targets of pain.

2.4 Network construction and analysis

The protein-protein interaction (PPI) network of potential targets and disease targets was constructed by using Cytoscape 3.6.1, and the correlation function in the software was used to fuse and extract the intersection network of the two network graphs, that is, the direct and indirect target regulatory network of yubaifu for pain relief and treatment was obtained.

2.5 Enrichment analysis of biological process and pathway

In order to explain the potential targets of active ingredients in yubaifu, and the role of pain targets in gene function and signal pathway, this study used R language programming to analyze GO and KEGG pathway signal pathway, set threshold $P<0.05$, and predicted the possible mechanism of analgesic effect of yubaifu by gene enrichment analysis.

3. Results

3.1 Screening of chemical constituents from Rhizoma typhonii

According to TCMSP database, 43 kinds of active ingredients were obtained from Rhizoma typhonii, 27 kinds of active ingredients were obtained from chemical index database, and CAS number was listed, as shown in Table1; according to the principle of OB ≥ 30%, DL ≥ 0.18, three main active components of Rhizoma typhonii were obtained: sitosterol, beta-sitosterol, Mandenol.

More than 100 kinds of pain related diseases, including pain, arthritis, cardiovascular disease, dysmenorrhea, bone pain, migraine and cancer-related pain, were retrieved from TCMSP database.

3.2 Target prediction of compounds

Through pharmmapper and STITCH, 299 targets of sitosterol, beta-sitosterol and Mandenol were found. We obtained 519 target compounds by taking and union operations. Because the search results

of the target compounds are not gene symbol, 501 target compounds were obtained by gene name conversion. As shown in figure1 and figure2.

Table 1. The effective chemical constituents of Rhizoma typhonii were searched by TCMSP and CID database

Mol ID	Molecule Name	CAS	OB%	DL
MOL005483	2-Methylnaphthalene	91-57-6	33.69	0.04
MOL012628	2,3,6-Trimethylnaphthalene	829-26-5	20.27	0.06
MOL010766	3-METHYLPHENANTHRENE	832-71-3	17.11	0.11
MOL000358	beta-Sitosterol	83-46-5	36.91	0.75
MOL001894	Bicetyl	544-85-4	8.03	0.46
MOL000303	caprylic acid	124-07-2	16.4	0.02
MOL000394	choline	62-49-7	0.47	0.01
MOL002295	cinnamic acid	621-82-9	19.68	0.03
MOL007197	DFA	122-39-4	31.13	0.05
MOL001838	Dipalmitin	26657-95-4	21.16	0.44
MOL008119	Glyceryl trilinoleate	537-40-6	34.91	0.14
MOL005501	Green Oil	120-12-7	17.74	0.1
MOL002229	HEPTACOSANE	593-49-7	8.18	0.36
MOL000714	Hyacinthin	122-78-1	38.65	0.02
MOL001732	IFP	56-81-5	72.87	0.01
MOL005570	inositol	87-89-8	15.55	0.05
MOL001098	m-xylene	108-38-3	47.43	0.01
MOL001494	Mandenol	544-35-4	42	0.19
MOL000421	nicotinic acid	59-67-6	47.65	0.02
MOL000675	oleic acid	112-80-1	33.13	0.14
MOL000069	palmitic acid	1957/10/3	19.3	0.1
MOL005521	phytane	638-36-8	13.86	0.11
MOL000357	Sitogluside	474-58-8	20.63	0.62
MOL000359	sitosterol	64997-52-0	36.91	0.75
MOL000346	succinic acid	110-15-6	29.62	0.01
MOL006293	Thapsic acid	505-54-4	20.72	0.16
MOL006077	Thiamine	70-16-6	19.87	0.11

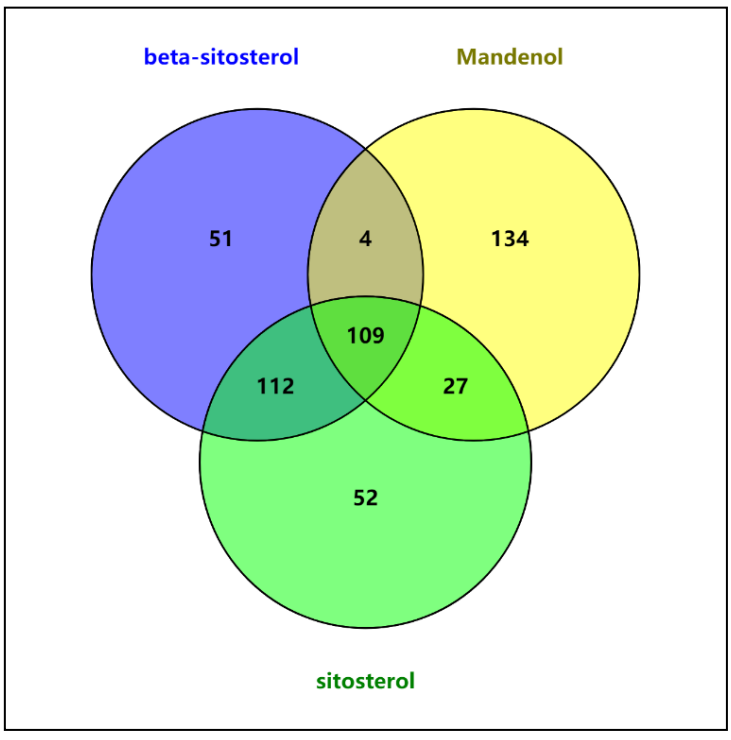

Figure 1. Venn diagram of potential targets of three active compounds

3.3 Retrieval and analysis of pain targets

8585 pain related targets were retrieved from GeneCards database, 92 pain related targets were retrieved from OMIM database, and 405 pain related targets were retrieved from NCBI-GENE database. these 8610 targets can be regarded as potential targets for pain.

3.4 Construction and analysis of drug target disease interaction network

The active components and potential targets of the drug were input into Cytoscape to establish a close network relationship among each node, as well as the Venn diagram of effective extract of Rhizoma typhonii and pain targets, as shown in Fig. 3 and Fig. 4. there are 91 targets in the PPI network graph, and each node represents a target. The connecting line between targets is edge, which indicates that there is interaction between connected targets. The number of edges between each target and other targets is the degree of the target. The higher the degree, the more core the target occupies in the network graph.

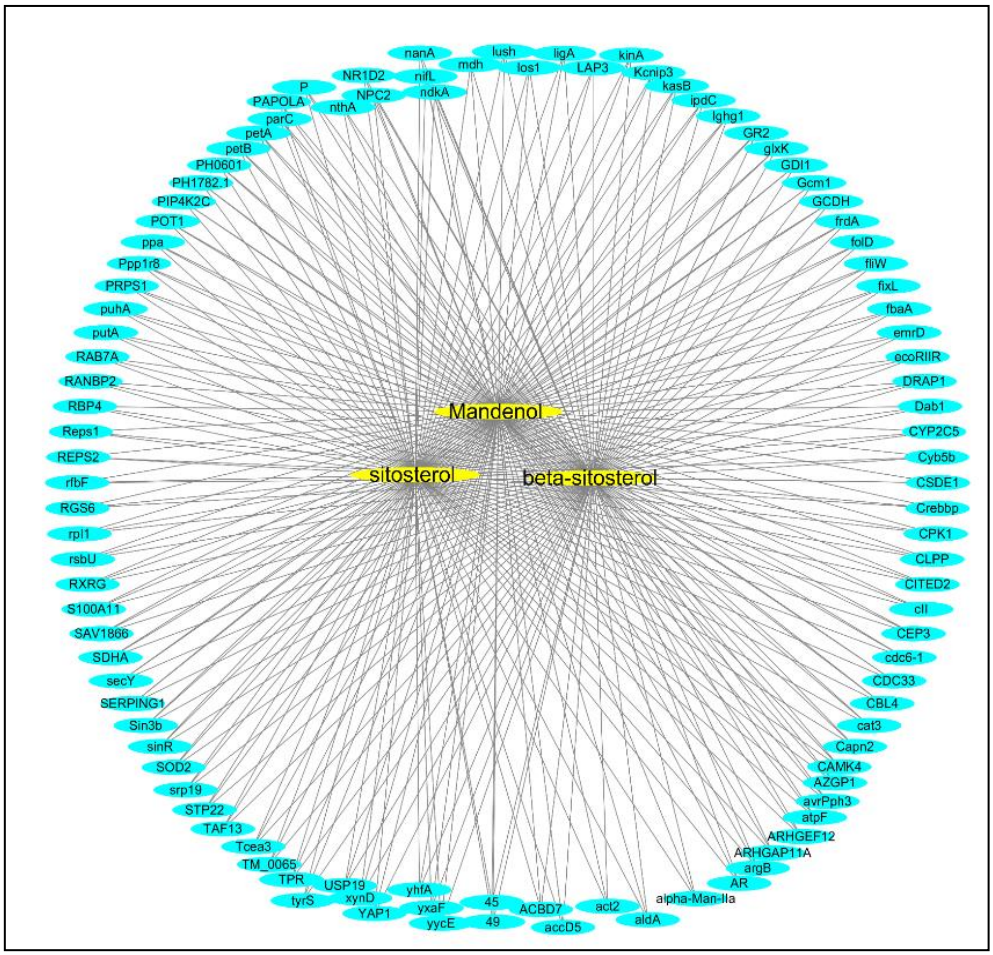

Figure 2. Target network of Rhizoma typhonii extract

Figure 3. Venn diagram of action targets and pain related targets of Rhizoma typhonii extract (LIST1 is the corresponding target of compound, List2 is the pain related target)

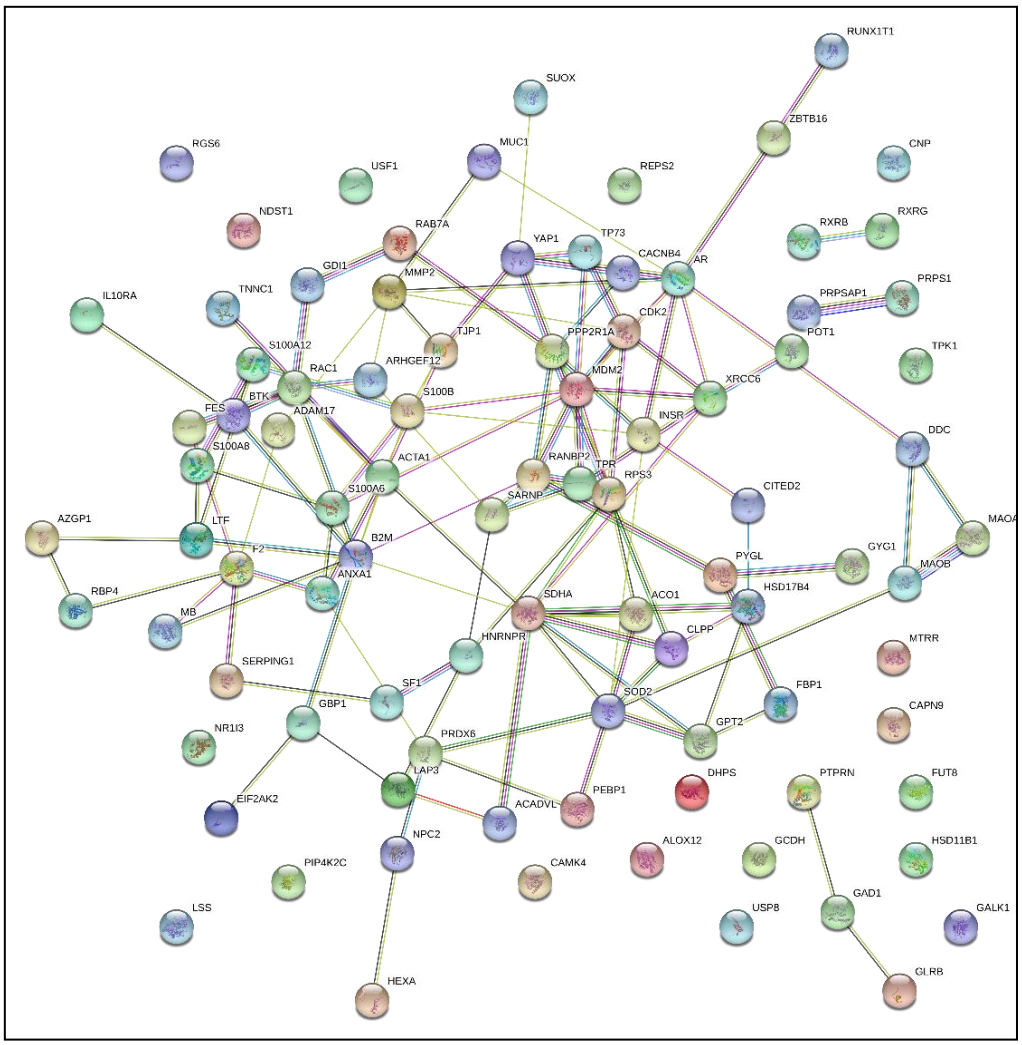

Figure 4. Network diagram of the effects of extracts from Rhizoma typhonii on pain related targets

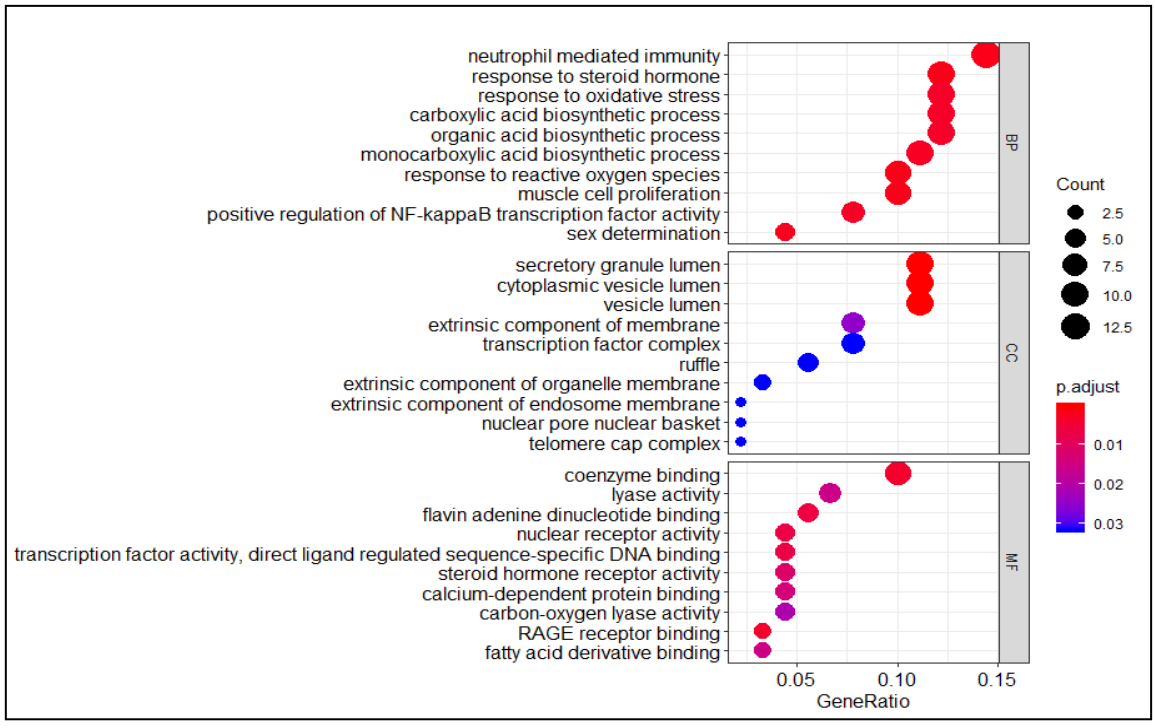

Figure 5. GO function annotation of Rhizoma typhonii extract on pain related targets

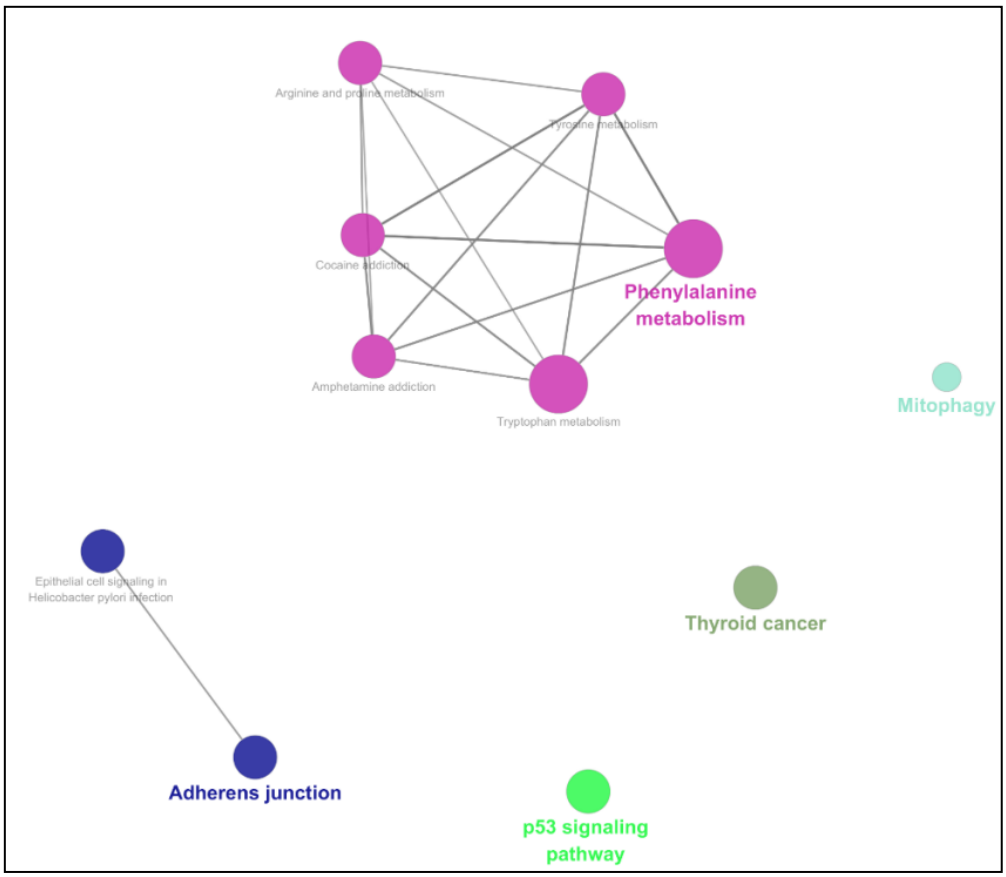

Figure 6. KEGG enrichment analysis of extracts from Rhizoma typhonii on pain related targets

3.5 Enrichment analysis of biological process and pathway

R language programming is used to annotate and analyze the go function of the target, and the results are shown in figure 5; KEGG pathway enrichment analysis is carried out through the ClueGO of Cytoscape software, and the results are shown in figure 6. The biological process network diagram of target enrichment shows that the related targets of Rhizoma typhonii participate in multiple biological processes, and different biological process involve different types of diseases. These targets play an important biological function in the body by regulating multiple biological process. [9-23]

4. Discussion

Pain is a kind of feeling of human disease caused by tissue injury, which stimulates the sensory system. It is of great significance and research value in clinical diagnosis and treatment. Usually the clinical pain mainly comes from the noxious stimulation of internal and external conditions caused by disease or surgery, which not only includes the defense response of the human body to noxious stimulation, but also a clinical manifestation of a variety of diseases and postoperative reactions. Most of the patients have severe pain or chronic long-term pain and need to be treated with analgesic drugs. Cancer pain usually accompanies the whole course of cancer patients, so that patients are in pain, anxiety and even depression and other negative emotions for a long time, causing some patients to lose confidence in life, which seriously affects the quality of life of patients. Therefore, how to effectively control the degree of cancer pain of cancer patients has great significance for the treatment of cancer and the improvement of life quality of cancer patients. We need to understand the meaning of this. Therefore, the research of analgesic drugs has become one of the hot directions of modern drug research, but the clinical use of chemical drugs for acute or chronic pain, such as opioids and non-opioids: morphine, codeine, pethidine, aspirin, indomethacin, ibuprofen and so on. Although the above methods have achieved good clinical efficacy, the side effects are often more serious due to

the continuous and large dose of use. Traditional Chinese medicine believes that pain is due to body deficiency, deficiency of healthy qi and invasion of external pathogens, which eventually leads to heat toxin and internal stagnation, obstruction of meridians and collaterals, qi stagnation and blood stasis. Herbal medicine is one of the most important methods of traditional Chinese medicine in treating cancer pain, and studies have shown that bitter cold herbal medicine has exact anti-tumor effect, with less toxic and side effects and obvious effect. The clinical analgesic effect of chemical drugs has been fully recognized, but for patients with chronic pain, long-term use will produce dependence, peptic ulcer and other adverse reactions. Accordingly, with the development of traditional Chinese medicine industry, the research of natural plant extracts for pain treatment is gradually deepening.

Yubaifu (Aconitum carmichaeli) is the dry tuber of Unicorn lotus, which is mainly produced in Henan, Gansu, Hubei and other places. Yubaifu produced in Yuzhou of Henan Province has the best quality and curative effect, and is considered as a genuine medicinal material. According to the Pharmacopoeia, yubaifu has the effects of relieving pain, dispelling wind and phlegm, calming convulsions, detoxifying and radiating heat. It can be used for the treatment of tanjue headache, pianzheng headache and other diseases. In terms of efficacy, yubai has a long clinical history. In 1963 edition of Chinese Pharmacopoeia, yubai Fu and guanbai Fu were listed separately. In 1985 edition of Chinese Pharmacopoeia, yubai Fu was still marked in brackets under the name of baifuzi, but guanbai Fu was no longer included. Later, baifuzi was listed separately in Chinese Pharmacopoeia, and yubai Fu gradually became the main product source of baifuzi. In recent years, with the development of modern science, yubaifu has been widely used in anti-inflammatory, antibacterial, immune regulation, sedation, analgesic, anticonvulsant, expectorant and other aspects. Because yubaifu contains organic acid, choline, sitosterol and other ingredients, it has sedative and anti-infection effects. It is mainly used for facial paralysis, intractable trigeminal neuralgia, hyperlipidemia, tetanus, tumor and so on. In terms of sedation and pain relief, yubaifu aqueous extract can delay the emergence time and death time of convulsions in mice caused by heat stroke stimulants pentylenetetrazole and strychnine nitrate in varying degrees, and reduce the number of writhing reactions in mice; in addition, yubaifu is also used for the treatment of trigeminal neuralgia, heel pain, eyebrow pain, pain, intractable neuralgia and other diseases, and Moreover, it is used to treat migraine in combination with other medicinal materials.

In the field of clinical analgesia, Shen qinxuan et al. Used yubaifu compound to treat tension type headache patients in 2016, and Luo Shunhong et al. Applied yubaifu to external treatment of rheumatoid arthritis, which showed good analgesic effect of yubaifu. The combination of medicinal materials for migraine authorized by Pan genqi, as well as the related patents of yubaifu for cancer pain, postoperative headache, trigeminal neuralgia, glossopharyngeal neuralgia, etc., have been invented and disclosed. However, the analgesic effect of Rhizoma typhonii alone and the related effective analgesic components of Rhizoma typhonii are not clear. The analgesic experiment results of different extraction methods of effective components of Rhizoma typhonii in mice revealed that Rhizoma typhonii aqueous solution can significantly cause writhing times of mice, and the pain threshold of mice is also improved, but the effect of alcohol solution is not obvious.

In this study, we selected the traditional Chinese medicine in Henan Province, Yuzhou medicine yubaifu, which has been reported on analgesia, as the research object. The network pharmacology analysis method was used to construct and predict the potential action mechanism of effective components of Rhizoma typhonii, such as sitosterol, through the drug target disease pathway, so as to provide a theoretical basis for the analgesic effect and subsequent experimental research of Rhizoma typhonii. Based on the network pharmacology analysis of beta-sitosterol, Mandenol and sitosterol, the corresponding target and gene analysis of the three functional components, combined with the data mining of pain related regulatory targets, the cyposcape 3.6 software was used to construct the signal network of yubaifu - target - pain regulation.

The three functional components of beta-sitosterol, Mandenol and sitosterol were mined in pharmmapper database and 299 important targets were selected. Through combined analysis, there were 109 interaction targets; 4 cooperative targets of beta-sitosterol and Mandenol; 112 cooperative targets of beta-sitosterol and sitosterol; 27 cooperative targets of Mandenol and sitosterol; 3A total of 519 targets were rented for each functional component, and 501 targets were obtained by gene symbol. Combined with 8585 pain related targets from GeneCards database, 92 main pain targets from OMIM database and 405 targets from NCBI-GENE database, a total of 8610 human pain targets were found. there are 91 effective targets in PPI network. By gene annotation of the above targets, we can understand that they are mainly involved in human activities such as neutrophil immunity, response to steroids, oxidative stress, positive regulation of NF KB transcription factor activity, nuclear receptor activity and so on. In the analysis of KEGG enrichment of extracts of Rhizoma typhonii on pain targets, the main signal regulatory pathways involved are mitochondrial autophagy, adhesion junction, p53 signal pathway and so on. According to gene annotation and KEGG enrichment analysis, the relationship between the action targets of beta-sitosterol, Mandenol and sitosterol and pain targets mainly focused on the regulation of pain by NF-kB signaling pathway participated by p53. At present, a large number of studies at home and abroad have reported that NF-kB signaling pathway and related inflammatory factors are involved in the regulation of pain mechanism of various diseases, which also lays a theoretical foundation for the effect of beta-sitosterol, Mandenol and sitosterol, the functional components of Rhizoma typhonii on pain.

Zhang Yong et al. Showed that the regulation mechanism of postherpetic neuralgia may be realized through nuclear factor NF-kB signaling pathway through the study of postherpetic neuralgia. Seon et al. Found in the mouse model experiment of improving neuropathic pain by methane sulfonate that its tricky mechanism is also realized by inhibiting proinflammatory cytokines and NF-kB signaling pathway; and various drugs involving NF-kB participate in the treatment of disease pain, including bone pain, neuropathic pain, cardiac pain, neuropeptide expression lumbar disc herniation, diabetic neuropathy and other inflammatory pain and neuropathy Menstrual pain. Based on the above, through the construction and prediction of drug target disease pathway, the potential mechanism of action of effective components of yubaifu, such as sitosterol, is most likely to participate in the treatment of neuropathic pain through NF-kB signaling pathway and related cytokines, which is also consistent with the pathogenic mechanism of yubaifu mainly Acting on migraine, trigeminal neuralgia, facial paralysis and other pain diseases.

References

[1] Zhao Jishan, Liu Jinfeng, Li Taiyu. Application effect and pain management status of comprehensive treatment in primary cancer patients. Journal of Clinical Rational Drug Use, Vol.10, (2017), No.9, p.155-156.

[2] B. N. Nielsen, S. W. Henneberg, K. Schmiegelow, et al. Peripherally applied opioids for postoperative pain: evidence of an analgesic effect? A systematic review and meta-analysis. Acta Anaesthesiologica Scandinavica, Vol. 59, (2015), No.7, p.830-845.

[3] Zhao Yintao, Jia Lirong. Application of traditional Chinese medicine in the treatment of cancer pain. China Health Nutrition, Vol.22, (2012), No.4, p.232-234.

[4] Liu Shaoqing, Zhou Yangyang, Tu Huiyin. Comparison of traditional Chinese medicine and Western medicine in the treatment of chronic pain. Journal of Henan Medical College, Vol.28, (2016), No.2, p.1058-1060.

[5] Shu Peng Hua, Tang Rong Ping, Li Jun Rong. Analysis of chemical constituents of Rhizoma typhonii. Shi Zhen, Guo Yi Guo Yao, Vol.30, (2019), No.5, p.156-160.

[6] Zhu Tao, Wu Hao, Zhang Lin. overview of research on Rhizoma typhonii. Chinese Journal of Traditional Chinese Medicine, Vol.26, (2008), No.6, p.1176-1178.

[7] Andrew LH,. Network pharmacology: the next paradigm in drug discovery. Nature Chemical Biology, (2008), No.4, p.682-690.

[8] Wang Yanyan, Wang Tuanjie, Ding Linlin. Pharmacological mechanism of Magnolia officinalis based on systematic pharmacology. Chinese Herbal Medicine, Vol.50, (2019), No.25, p.6024-6031.

[9] Louw A, Zimney K, O'Hotto C, et al. The clinical application of teaching people about pain. Physiotherapy Theory and Practice, Vol.32, (2016), No.5, p.385-395.

[10] Bennett M. Mechanism-based cancer-pain therapy. Pain , Vol.158, (2017), No.1, p.S74-S78.

[11] Pak DJ, Yong RJ, Kaye AD, et al,. Chronification of Pain: Mechanisms, Current Understanding, and Clinical Implications. Current Pain and Headache Reports , Vol.22, (2018), No.2.

[12] Diao Xianmin, Kang Zhen, Wang Jiazhu, et al. Clinical study on three-step analgesic therapy for cancer pain. Chinese Medical Guide, Vol.10, (2012), No.11, p.230-231.

[13] Lai Guihua, Yuan Jing, Yan Yuting, et al. Progress of traditional Chinese and Western medicine in the treatment of bone metastasis cancer pain. Journal of Hunan University of Traditional Chinese Medicine, Vol.39, (2019), No.9, p.1166-1170.

[14] Yu Changying, Xu Xue, Zhang Feilong, et al. Analgesics symptoms and meridian tropism. Chinese Journal of Traditional Chinese Medicine, Vol.34, (2019), No.6, p.2661-2663.

[15] Huang Jinyu, Dai Zhong, Ma Shuangcheng. Research progress of Aconitum carmichaeli. Chinese Herbal Medicine, Vol.46, (2015), No.18, p.2816-2822.

[16] Zhang Cuilan. Differentiation between yubaifu and guanbaifu. Clinical Journal of Traditional Chinese Medicine, Vol.28, (2016), No.11, p.1567-1569.

[17] Shi Yanbang, Zhang Zhenling. Research progress on chemical constituents and pharmacological effects of Aconitum carmichaeli. Chinese Practical Medicine, Vol.3, (2008), No.9, p.130-131.

[18] Shen qinxuan. Constitution classification of patients with tension type headache and efficacy observation of modified Qianzheng powder on phlegm syndrome of tension type head gout. 2016

[19] Luo Shunhong. External treatment of rheumatoid arthritis. Journal of External Treatment of Traditional Chinese Medicine, Vol.37, (1995),No.5.

[20] Pan genqi. A medicinal composition for treating migraine and its preparation method: China, cn201210406324.6. 2015-02-25.

[21] Zhang Zhenying, Yang Zhenxiang. Experimental study on sedative and analgesic parts of Rhizoma typhonii . Research of Traditional Chinese Medicine, Vol.20, (2007), No.10, p.14-16.

[22] Zhang Yong, Xue Zhixin, Li Peipei. Effect of plum blossom needle tapping on the content of serum NF KB and IL-6 in patients with postherpetic neuralgia. Journal of Shaanxi University of Traditional Chinese Medicine, Vol.41, (2018), No.5, p.69-71.

[23] Seon HO, Hyun YL, Young JK,. et al,. Gabexate mesilate ameliorates the neuropathic pain in a rat model by inhibition of proinflammatory cytokines and nitric oxidepathway via suppression of nuclear factor-kB. Korean J Pain, Vol.33, (2020), No.1, p.30-39.