ISSN 2618-1584 Vol. 5, Issue 10: 137-142, DOI: 10.25236/FMSR.2023.051020

Mechanism of Xuanhuang Ointments for the Acute Soft Tissue Injury Based on Network Pharmacology

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Abstract: *Screen the therapeutic target of XuanHuang Ointment on acute soft tissue injury by network pharmacology method, and to clarify its mechanism. The main chemical constituents and their targets of XuanHuang Ointment were obtained by using TCMSP database. The disease targets of acute soft tissue injury were searched by GeneCards .A compound-target-disease network was constructed using Cytoscape3.8.2 software. The targets were analyzed by GO analysis and KEGG enrichment analysis based on Metascape database. The core active components of XuanHuang Ointment were Quercetin, beta-sitosterol, Stigmasterol. The core target spots are: NCOA2, PTGS2, PTGS1, PGR and NR3C2 .Pathways in cancer, Lipid and atherosclerosis, AGE-RAGE signaling pathway in diabetic complications may play a potential role in XuanHuang Ointment in the treatment of acute soft tissue injury Signaling pathway. Conclusion XuanHuang Ointment has the characteristics of multiple components, multiple targets and overall regulation in the treatment of acute soft tissue injury .*

Keywords: *XuanHuang Ointment; Acute soft tissue injury; Network pharmacology*

1. Introduction

Acute soft tissue injury refers to a kind of trauma syndrome caused by a variety of acute direct or indirect violent injury factors, which is a common orthopedic disease[1], most present with local edema of soft tissue, muscle fiber rupture, pain and dysfunction[2]. Acute soft tissue injury Traditional Chinese medicine belongs to the category of "acute tendon injury", which is due to external external and internal damage to qi and blood, qi stagnation and blood stasis, meridians obstruction, resulting in pain, swelling, subcutaneous bleeding, movement obstruction[3]. With the continuous development of medicine, the Chinese and western medicine treatment methods for acute soft tissue injury are numerous. Conservative treatment of western medicine and the treatment of acute soft tissue injury is based on the basic principles of internal and external treatment, both internal and external treatment, and the treatment methods of internal and external treatment, acupuncture and manipulation[4].

Xuanhuang ointment is the effect of the long-term experience of treating acute tendon injury, namely acute soft tissue injury, in Shaanxi Provincial Hospital of Traditional Chinese Medicine. Xuanhuang ointment consists of rhubarb, gardenia, frankincense, myrrh, dandelion, and turtle insects.Rhubarb bitter, cold, working in clearing away heat and detoxification, cooling blood and removing blood stasis, external use can detoxify, reduce swelling and relieve pain[5]. Gardenia is bitter and cold; it is "the main medicine to relieve heat and cool blood", with the effect of clearing heat and detoxification, promoting blood circulation and dispersing knot, external use of swelling and pain, treating contusion and pain[6]. Frankincense and myrrh belong to the drugs of promoting blood circulation and removing blood stasis, frankincense, bitter, warm, activating blood circulation, swelling, no muscle, pain, flat, swelling, pain, Zhang Sichun believes that frankincense is good at measure the body and qi; Myrrh is good at regulating blood stasis, the effect of promoting viscera and meridians[7]. Dandelion bitter, sweet, cold, heat detoxification, swelling and knot. Ground turtle insect into the blood, good walk channeling, can promote the blood swelling and pain, continuous bone healing, especially for fracture injury, hematoma and pain[8]. Prescription drug bitter cold, co-treatment of early qi stagnation and blood stasis type of acute soft tissue injury[9].

Network pharmacology is a discipline that integrates the relationship between drugs and diseases from the perspective of system and whole, and shows the systemic pharmacological mechanism of drugs[10]. Network pharmacology studies TCM from the perspective of "multi-component, multi-target and multi-channel", which is consistent with the overall concept of TCM and the treatment concept of

ISSN 2618-1584 Vol. 5, Issue 10: 137-142, DOI: 10.25236/FMSR.2023.051020

syndrome differentiation and treatment[11]. Many studies have found that xuanhuang ointment has a significant effect in the treatment of acute soft tissue injury. The author uses network pharmacology to identify the main active components and core targets of xuanhuang ointment and acute soft tissue injury, and further analyzes the molecular mechanism of the treatment of acute soft tissue injury.

2. Method

2.1 Search and screening of the active ingredients of Xuanhuang ointment

Using the TCMSP database (http: // tcmspw.com/tcmsp. Search php) for the prescription ingredient: rhubarb, gardenia, frankincense, no medicine, dandelion, all the active ingredients of the turtle worm. All the active ingredients of the searched drug were selected as oral availability (OB) 30%, drug-like (DL) 0.18 for the active ingredients and their protein targets. Then in the Uniprot Protein Data Bank (https://www.uniprot. The org/) to normalize the protein targets.

2.2 GO pathway enrichment analysis and KEGG pathway enrichment analysis

In order to explore the role of the target protein of Xuanhuang ointment in gene function and to find the core pathway of its action on acute soft tissue injury, this study used the Metascape database (https://metascape.Og/) conducted GO pathway enrichment analysis and KEGG pathway enrichment analysis on core targets,and used microxin cloud platform (http://www.bioinformatics.com.Cn/) to alizee the results.

2.3 Construction of the component-target-disease network map

The drug active ingredients and the screened core targets were uploaded to the Cytoscape 3.8.2 software to generate the "component-target-pathway network map". The built-in tools of CytoScape3.8.2 is used to analyze the active components and the network topological parameters of the targets, and to judge the core targets and the main active components according to the relevant parameters.

2.4 Molecular docking

Selecting the PDB database (https://www.rcsb.Org/packages/search_features) download the PDB structure of key targets, and the MOL 2 structure of the active components of Xuanhuang ointment in the TCMSP database to docking the molecules and targets with AutoDock Vina 1.1.2. If the binding energy is less than 0, then there is a binding activity between the ligand and the receptor, which is less than-5 kcal / mol^{-1}Good docking. Finally, the results were visualized using Pymol 2.2.0.

Table 1: XuanHuang Ointment information on active ingredients

number	MOLID	active principle	OB/%	DL	source
DH1	MOL002235	Obustine	50.8	0.41	rheum officinale
DH2	MOL002268	parietic acid	47.07	0.28	rheum officinale
DH3	MOL002281	Tularenoester	46.46	0.24	rheum officinale
DH4	MOL002297	Turnip sterol _ qt	35.89	0.7	rheum officinale
A	MOL000358	β-sitosterin	36.91	0.75	rheum officinale
DH5	MOL000471	aloe-emodin	83.38	0.24	rheum officinale
DH6	MOL000096	(-)-catechin	49.68	0.24	rheum officinale
ZZ1	MOL001406	crocin	35.3	0.26	Cape jasmine
ZZ2	MOL001941	Aminamide	34.55	0.22	Cape jasmine
ZZ3	MOL004561	Sudan III	84.07	0.59	Cape jasmine
C	MOL000098	meletin	46.43	0.28	Cape jasmine
A	MOL000358	β-sitosterin	36.91	0.75	Cape jasmine
ZZ4	MOL000422	kaempferol	41.88	0.24	Cape jasmine
B	MOL000449	stigmasterol	43.83	0.76	Cape jasmine
ZZ5	MOL001494	mannitol	42	0.19	Cape jasmine
ZZ6	MOL001942	auraptin	45.46	0.23	Cape jasmine
ZZ7	MOL002883	Ethyl oleate (NF)	32.4	0.19	Cape jasmine
ZZ8	MOL003095	5-hydroxy-7-methoxy-2- (3,4,5-trimethoxyphenyl) chromoone	51.96	0.41	Cape jasmine
ZZ9	MOL007245	3-methyl Kemp o	60.16	0.26	Cape jasmine
RX1	MOL001215	Trucarol	42.12	0.75	Boswellia arterii
RX2	MOL001241	And O-acetyl- α -lactocentic acid	42.73	0.7	Boswellia arterii

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RX3	MOL001243	3alpha-Hydroxy-olean-12-en-24-oic-acid	39.32	0.75	Boswellia arterii
RX4	MOL001255	mastic acids	39.55	0.75	Boswellia arterii
RX5	MOL001295	chlorophyll	33.4	0.27	Boswellia arterii
MY1	MOL001001	Quercetin-3-O- β -D-glucuronic acid	30.66	0.74	myrrh
MY2	MOL001002	ellagic acid	43.06	0.43	myrrh
MY3	MOL001004	pelargonin	37.99	0.21	myrrh
MY4	MOL001006	poriferasta-7,22E-dien-3beta-ol	42.98	0.76	myrrh
MY5	MOL001009	guggulsterol-VI	54.72	0.43	myrrh
MY6	MOL001013	glycyrrhizic acid	48.1	0.32	myrrh
MY7	MOL001026	bisabolol C	39.96	0.58	myrrh
MY8	MOL001028	(8R)-3-oxo-8-hydroxy-polypoda -13E,17E,21-triene	44.83	0.59	myrrh
MY9	MOL001029	Andosterone B	34.39	0.67	myrrh
MY10	MOL001031	Table mannitol	61.81	0.4	myrrh
MY11	MOL001033	Diayangabin	63.84	0.81	myrrh
MY12	MOL001040	(2R)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one	42.36	0.21	myrrh
MY13	MOL001045	(13E,17E,21E)-8-hydroxypolypodo-13,17,21-trien-3-one	44.34	0.58	myrrh
MY14	MOL001046	(13E,17E,21E)-polypodo-13,17,21-triene-3,18-diol	39.96	0.58	myrrh
MY15	MOL001049	16-hydroperoxymansumbin-13(17)-en-3β-ol	41.05	0.49	myrrh
MY16	MOL001052	mansumbin-13(17)-en3,16-dione	41.78	0.45	myrrh
MY17	MOL001061	(16S, 20R)-dihydroxydammar-24-en-3-one	37.34	0.78	myrrh
MY18	MOL001062	15 α -hydroxylygenenone	37.51	0.44	myrrh
MY19	MOL001063	28-acetyl-15 α -hydroxyglygenone	41.85	0.67	myrrh
MY20	MOL001095	Isofuranone	40.95	0.78	myrrh
MY21	MOL001126	[(5 AS, 8aR, 9R) -8-oxo-9- (3,4,5-trimethoxyphenyl) -5,5a, 6,9-tetrahydroisobenzofuran [6,5-f] [1,3]benzodioxol-8a-based] acetate	44.08	0.9	myrrh
MY22	MOL001131	morin _qt	56.6	0.39	myrrh
MY23	MOL001138	(3R, 20S) -3,20-dihydroxydama-24-ene	37.49	0.75	myrrh
MY24	MOL001156	3-methoxuran guaiac-9-en-8-one	35.15	0.18	myrrh
MY25	MOL001175	Gugu steroidone	42.45	0.44	myrrh
A	MOL000358	β-sitosterin	36.91	0.75	myrrh
B	MOL000449	stigmasterol	43.83	0.76	myrrh
MY26	MOL000490	Short morning ox flower element	30.05	0.31	myrrh
C	MOL000098	meletin	46.43	0.28	myrrh
MY27	MOL000988	4,17(20)-(cis)-pregnadiene-3,16-dione	51.42	0.48	myrrh
MY28	MOL000996	Archaeosterol IV	33.59	0.74	myrrh
PGY1	MOL000006	Syracuse	36.16	0.25	dandelion
C	MOL000098	meletin	46.43	0.28	dandelion
PGY2	MOL000359	sitosterin	36.91	0.75	dandelion
A	MOL000358	β-sitosterin	36.91	0.75	dandelion
B	MOL000449	stigmasterol	43.83	0.76	dandelion
TBC1	MOL005030	arachidonic acid	30.7	0.2	cockroach
TBC2	MOL005573	genkwanin	37.13	0.24	cockroach

3. Results

3.1 Acquisition of active ingredients and targets of xuanhuang ointment

The active ingredients of rhubarb, gardenia, frankincense and myrrh can be directly collected through TCMSP, including 92 kinds of rhubarb, 98 kinds of gardenia, 127 kinds of frankincense and 276 kinds of medicine. The active components of dandelion and turtle worm cannot be found directly by the TCMSP database; retrieve the database and found the relevant components of dandelion and turtle worm[12-13].

Combined with the TCMSP database, after ADME, set the threshold and select the effective components of dandelion and turtle worm. The effective ingredients of all the prescriptions of Xuanhuang ointment were summarized, and the effective ingredients of xuanhuang ointment were obtained, including 62 kinds, 7 kinds of rhubarb, 14 kinds of gardenia, 5 kinds of frankincense, 31 kinds of no medicine, 5 kinds of dandelion and 2 kinds of turtle worm, among which there were 3 kinds of repeated effective ingredients between drugs, see Table 1. After the merger, 201 active components of xuanhuang ointment were obtained.

3.2 Acquisition of targets associated to acute soft tissue injury

The search results for disease-related targets of acute soft tissue injury was: 4448 GeneCards

ISSN 2618-1584 Vol. 5, Issue 10: 137-142, DOI: 10.25236/FMSR.2023.051020

database, 207 OMIM database, removing duplicate targets, and totaling 4600 targets related to acute soft tissue injury.

3.3 Results of GO pathway enrichment and KEGG pathway enrichment analysis

GO pathway enrichment analysis and KEGG pathway enrichment analysis were performed on 148 targets using the Metascape gene annotation and analysis resource platform, and the enrichment analysis results were visualized using the microcloud platform.The results show that multiple target functions are closely related to the treatment of acute soft tissue injury. Among them, Xuanhuang ointment mainly involved in biological processes includes: hormone response, response to inorganic substances, cell response to lipids, response to external stimuli; cell component analysis results mainly include: membrane raft, transcriptional regulatory complex, dendrites, etc.; molecular function analysis results mainly include: DNA transcription binding factor, nuclear receptor activity, protein domain specific binding, kinase binding bond, protein kinase activity, etc. The results of KEGG pathway enrichment analysis were 203 pathways, which showed that the main pathways of xuanhuang ointment for acute soft tissue injury were: cancer pathway, lipid and atherosclerosis pathway, AGE-RAGE signaling pathway in diabetic complications, and PI3K-Akt signaling pathway.

3.4 Construction of the component-target-pathway network map

The 62 active ingredients of Xuanhuang ointment and 167 common targets of acute soft tissue injury were uploaded to Cytoscape3.8.2 software to construct a "component-target-pathway network map". Through CytoScape3.8.2 built-in NetworkAnalyzer tool, analyze the network topological parameters for acute soft tissue injury, and obtain the core components and core targets. CytoScape3.8.2 Analysis results showed that the main component of acute soft tissue injury was quercetin with 372, resolution is 0.2176, tightness is 0.5995; β -sitosterol has 108, resolution is 0.0160, 0.3982; stigsterol was 66,0.0121, tightness is 0.3912; predicted that β -sitosterol and prosterol also play an important role in the treatment of acute soft tissue injury. In the target analysis results, NCOA2 was the first target with connectivity 38, resolution of 0.0504, and tightness of 0.5068. NCOA2 was predicted to be the core target of acute soft tissue injury. In addition, PTGS 2, PTGS 1, PG, GR, and NR 3 C 2 were predicted to play an important role in the treatment of acute soft tissue injury.

3.5 Molecular docking results

Docking of the active ingredients: quercetin, β -sitosterol and core targets: PGR, NCOA2 and PTGS 2. The results showed that the binding energy between the active components and the core target sites was less than-5kcal / mol^{-1}, all have strong binding activity, as shown in Table 2,3,4. Finally, the molecular docking results.

Table 2: Characteristic parameters of main active ingredient network nodes of XuanHuang Ointment

MOLID	**name**	**connectivity**	**Intermittivity**	**compactedness**
MOL000098	meletin	372	0.217589532	0.599462366
MOL000358	β-sitosterin	108	0.01601209	0.398214286
MOL000449	stigmasterol	66	0.012102938	0.39122807
MOL000006	Syracuse	50	0.041353099	0.43385214
MOL000422	kaempferol	46	0.035887406	0.425572519
MOL000471	aloe-emodin	19	0.009520825	0.387152778
MOL003095	5-hydroxy-7-methoxy-2- (3,4,5-trimethoxyphenyl) chromoone	19	0.007542084	0.387152778
MOL001002	ellagic acid	16	0.006337286	0.383161512
MOL001004	pelargonin	14	0.003999655	0.380546075
MOL001126	[(5 AS, 8aR, 9R) -8-oxo-9- (3,4,5-trimethoxyphenyl) -5,5a, 6,9-tetrahydroisobenzofuran [6,5-f] [1,3]benzodioxol-8a-based] acetate	12	0.00342738	0.377966102

Table 3: Characteristic parameters of the core target network nodes of Xuanhuang ointment

Core targets	connectivity	Intermittivity	compactedness	Core targets	connectivity	Intermittivity	compactedness
NCOA2	38	0.050366534	0.506818182	GABRA1	17	0.006149495	0.462655602
PTGS2	37	0.040694183	0.504524887	CHRM1	16	0.005734414	0.464583333
PTGS1	28	0.021539745	0.484782609	PIK3CG	15	0.005378949	0.462655602
PGR	27	0.028106676	0.486899563	RXRA	15	0.00852935	0.464583333
NR3C2	19	0.019333259	0.476495726	SCN5A	14	0.003207155	0.456967213

ISSN 2618-1584 Vol. 5, Issue 10: 137-142, DOI: 10.25236/FMSR.2023.051020

Table 4: Molecular docking of core components and targets of XuanHuang Ointment

ingredient	NCOA2	PTGS2	PTGS1
	Binding energy / kcal · mol-1	Binding energy / kcal · mol-1	Binding energy / kcal · mol-1
meletin	-7.3	-7.9	-9.7
β-sitosterin	-7.4	-8.0	-8.9

4. Conclusions

At present, with the development of society, acute soft tissue injury has become more and more frequent in the fast pace of life and work, and the influence on people's work and life is increasing year by year. With the continuous progress of medical technology and more attention to their own health, the treatment of acute soft tissue injury is increasing in traditional Chinese and western medicine. Internal and external treatment of traditional Chinese medicine, acupuncture and massage, modern Chinese medicine, oral medicine, western medicine, physical therapy, regenerative medicine, all achieve acute soft tissue injury local detumescence, analgesic, anti-inflammatory and even the whole body under the action of various principles.

Xuanhuang ointment is used in the treatment of acute soft tissue injury such as flash tendons, falling and injury for a long time. It is an important compatibility of seven li powder, nine dispersion, and activating blood circulation and removing blood stasis. This paper analyzed the molecular mechanism of xuanhuang ointment, and analyzed the molecular mechanism of acute soft tissue injury. After analysis, the main effective component of acute soft tissue injury is quercetin. Various studies have shown that quercetin has the effects of antioxidant, tumor suppressor, hypoglycemic, anti-inflammatory, anti-inflammatory, antiviral, antioxidant, lipid regulation, cardiovascular protection and bone protection. NCOA2 is the core target of Xuanhuang ointment for the treatment of acute soft tissue injury. The pathway prediction of xuanhuang ointment for acute soft tissue injury may be related to cancer pathway, lipid and atherosclerosis pathway, AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, etc.

The results show that the compound regulatory targets are complex, and the target intervention in biological processes and signaling pathways are diverse, which fully reflects the characteristics of multi-target and multi-pathway interaction of Xuanhuang ointment. It suggests that the possibility and feasibility of important targets in the regulatory network can regulate the whole network, which provides a scientific basis for the clinical use of xuanhuang ointment to treat acute soft tissue injury, and also provides a new direction for exploring the potential mechanism of xuanhuang ointment. However, this paper only predicts the effective components, targets and pathway information of xuanhuang ointment in the treatment of acute soft tissue injury from the perspective of network pharmacology, without the support of relevant clinical trials. Follow-up studies should complete the relevant research content of the treatment of acute soft tissue injury from basic and clinical aspects.

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