ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

Effect of a gout formula on the circumference of the ankle joint and the level of tumor necrosis factor TNF-α in joint fluid of rats with acute gouty arthritis

Zhang Yehui[1,a], Yang Chun[1,b], Jiang Mei[1,c], Lu Shuo[1,d], Song Zifan[1,e], Guo Xianghua[2,*]

[1]Department of Human Anatomy, Guangdong Medical University, Dongguan, China
[2]Department of Pathophysiology, Guangdong Medical University, Dongguan, China
[a]13416708271@139.com, [b]yangchunangel@163.com, [c]jiangm526@outlook.com, [d]neuropge@163.com, [e]454125801@qq.com
**Corresponding author: 370291797@qq.com*

Abstract: *Objective: To reveal part of the mechanism of gout remedy for acute gouty arthritis. Methods: Sixty rats were randomly divided into six groups: blank, model, colchicine, high-dose, medium-dose and low-dose group. After modeling, the colchicine group was given a concentration of 0.1 mg/mL colchicine per 200 g rat per gavage, and the low, medium and high dose group were given 0.5, 1 and 2 g/mL of raw herbs containing the gout formula per gavage for 5 d, respectively. The swelling of joints and the change of TNF-α in joint fluid were observed in each group. Results: Except for the blank group, the swelling of joint circumference was obvious in all the groups 1 day after modeling, and the difference was statistically significant before and after modeling (P<0. 05). On the 6th day after modeling, the circumference of the joint in the model group was significantly larger than that in the colchicine, high-dose, medium-dose and low-dose groups (P<0. 05), respectively, and there was no statistically significant difference in the circumference of the joint between the blank group and each drug treatment group (P>0. 05). The concentration of the cellular inflammatory factor TNF-α in the joint fluid of the model group was significantly higher than that of the blank group and the drug treatment group (P<0.01), and there was no significant difference in the concentration of TNF-α between the high-dose group, the colchicine group and the blank group (P>0.05). Conclusion: Gout test formula can significantly reduce the concentration of inflammatory factor TNF-α in the joints of rats with acute gouty arthritis, block the further development of inflammation and relieve the joint swelling of rats, which is one of the mechanisms of its ability to treat acute gouty arthritis.*

Keywords: *Traditional Chinese medicine; Gout; Acute gout arthritis; Inflammatory factor*

1. Introduction

In recent years, the global incidence of gout has increased significantly and the age of onset is trending towards a lower age. Colchicine, NSAIDs, and glucocorticoids can effectively relieve the symptoms of acute gouty arthritis, but do not reduce blood uric acid levels and have many side effects [12].Western drugs are effective in the treatment of gout, but have significant toxic side effects, while Chinese herbal medicine has better efficacy and lower incidence of adverse effects in the treatment of gout [5][7]. Therefore, it is important to discover highly effective and less toxic drugs for the treatment of gouty arthritis in traditional Chinese medicine. Folk Chinese medicine gout prescription has cured a large number of gout patients [1]: 100 g of Root of Vetchleaf Sophora, 2 g of Chinese Alangium Root, 35 g of Chinese Clematis Root, 15 g of Roughleaved Spikemoss , 15 g of Radix Angelicae Sinensis wrapped in gauze, put in a clay pot and add 1 kg of cold water to soak for 2 hours, then put 500 g of chicken, boil over high heat and then change to low heat and stew until the chicken is cooked, wait for the temperature to eat the chicken and drink the soup, take 3 times a day, 2 days to finish 1 dose to finish The pain will be eliminated in some patients after the first dose of medicine, while some patients will have increased pain, which will generally be relieved after a few hours, and the symptoms will be eliminated. After 5 days of pain relief, the second dose is taken. Eat gout-inducing foods such as animal offal, seafood, and beer before taking the medication to prompt a gout attack. At this time, the attack symptoms are not as severe as before. After the second dose of medicine, take the third dose of medicine after 10 days, and eat gout-inducing food before taking the medicine. After taking 4 doses of this medicine, the gout will

ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

no longer attack and will be cured. I have collected a total of 34 cases of gout patients who took this formula since 2013, and 27 cases could effectively relieve the symptoms without obvious adverse reactions, and its efficacy on gouty arthritis is definite. Therefore, animal experiments were conducted on this formula to reveal the mechanism of its treatment of acute gouty arthritis and to lay the foundation for the later development of Chinese medicine.

2. Materials and methods

2.1. Experimental materials

2.1.1. Laboratory animals

Sixty healthy male SD rats, free of specific pathogens (SPF), with an initial body weight of 200±10 g, were selected and provided by the Animal Experiment Center of Guangdong Medical University.

2.1.2. Experimental drugs Gout remedy

100 g of Root of Vetchleaf Sophora, 2 g of Chinese Alangium Root, 35 g of Clematis chinensis Osbeck, 15 g of Roughleaved Spikemoss, 15 g of Radix Angelicae Sinensis, the above drugs can be purchased from regular Chinese medicine stores.

2.1.3. Experimental reagents

Sodium urate, produced by Shanghai Sayue Biotechnology Co. Colchicine tablets, produced by Xishuangbanna Banna Pharmaceutical Co. TNF-α ELISA Kit, Zhengzhou Botai Biological Co.

2.1.4. Experimental instruments

GANGTUO TOOL digital vernier calipers, Tissue homogenizer, magnetic stirrer, PH meter, distilled water machine, low-temperature high-speed centrifuge, ultra-low temperature refrigerator, ice machine, electronic balance, dehydrator, ultra-clean bench, etc. provided by Guangdong Medical University Experimental Center.

2.2. Experimental method

2.2.1. Animal grouping

Sixty healthy male SD rats were selected and randomly divided into six groups by random grouping method. 60 rats were randomly divided into blank group, model group, colchicine group, high dose group, medium dose group and low dose group, 10 rats in each group.

2.2.2. Experimental drug preparation

Preparation of gout prescription: put the gout prescription 334 g gauze wrapped in a clay pot, add cold water 6000 ml and soak for 1 hour, add chicken meat 1000 g and boil on high heat, then cook on low heat until the chicken meat is cooked for 10 minutes, remove the dregs of the medicine, mix the chicken meat into minced meat with the medicine in a blender, put the medicine in a 60 ℃ water bath and concentrate it into three kinds of liquid containing raw medicine 0.5 g/ml, 1 g/ml and 2 g/ml. The liquid was sealed and stored in a refrigerator at 4 ℃.

Preparation of colchicine solution: put colchicine tablets 0.5 mg/tablet, 20 tablets into a mortar and grind them into fine powder, dissolve them in 100 mL distilled water, mix thoroughly and prepare colchicine suspension with a concentration of 0.1 mg/mL, store them in a refrigerator at 4℃.

Preparation of sodium urate solution: 500 mg of sodium urate crystals was added to 20 mL of 0.9% sodium chloride injection and stirred to make a sodium urate solution with a concentration of 25 mg/mL. Ultrasonically shake and mix well.

2.2.3. Animal modeling

Referring to the modified rat animal model modeling method [2]. After ether anesthesia, the joint cavity between the ankle joint and the tibiofibula in the dorsal side of the right hind limb was used as the entry point, and the ankle joint was positioned at a right angle to fully expose the gap between the ankle joint and the tibiofibula, and the injection needle was inserted into the joint cavity at an angle of 45 ° to the tibia. 25 mg/mL sodium urate crystal suspension was injected into the joint cavity with 0.2 mL for each group. The rats were less active, restless, and less frequently fed and watered 2 h after the modeling,

ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

and the right ankle joint was obviously congested and swollen with redness and heat, and some of them even lifted the right foot off the ground, which confirmed the success of the modeling.

2.2.4. Animal drug delivery

After modeling, the colchicine group was given l mL of colchicine per 200 g rat per gavage, and the low, medium and high dose groups were given 1 mL of the medicine containing 0.5, 1 and 2 g/mL of the raw gout formula per gavage, respectively, while the blank and model groups were given l mL of saline per gavage, all once a day for 5 d.

2.2.5. Observation of joint swelling

The swelling of the right lower limb of the rats was observed. The circumference of the ankle joint of the right hind limb was measured in each rat before modeling, 1 day after modeling, and before execution: the circumference value at 0.5 mm below the ankle joint of the right hind foot of each group was measured with a 2- to 3-mm-wide paper strip and a digital vernier caliper.

2.2.6. Measurement of cytokines

The rats were executed 5 days after drug administration, and the rats were stripped below the ankle joint of the right lower limb. The ankle joint tissues of the test foot of each group of rats were taken on ice and transferred to a 1.5 mL EP centrifuge tube, the joint cavity was cut open, diluted with 0.5 mL of pre-chilled 0.9% sodium chloride solution, and centrifuged at 3 000 r-min-1 for 10 min after 15 min of low-temperature sonication, and the supernatant obtained after centrifugation was assayed by ELISA kit to determine tumor necrosis factor-α (TNF-α).

2.2.7. Statistical Methods

The data were expressed as mean ± standard deviation ($x \pm s$) using SPSS 17.0 statistical software, and the joint circumference of rats was analyzed by repeated data ANOVA, and the comparison of TNF-α concentration in joint fluid was analyzed by one-way ANOVA, with $P<0.05$ as a statistically significant difference.

3. Results

3.1. Comparison of ankle circumference in each group of rats

Table 1: Ankle circumference and swelling coefficient of each group of rats ($x \pm s$, mm, n= 10).

Group	Pre-molded joint circumference	1 day after moulding	6 days after moulding
blank group	25.42±0.389*	26.81±0.394○	25.56±0.404√
model group	25.85±0.326*△	36.06±0.353△○	30.13±0.310▲
colchicine group	25.44±0.580*△	35.87±0.438△○	26.56±0.564▲√&
low dose group	25.43±0.613*△	35.86±0.422△○	26.85±0.546▲√&
medium dose group	25.45±0.534*△	35.83±0.357△○	26.75±0.483▲√&
high dose group	25.51±0.561*△	36.23±0.224△○	26.58±0.526▲√&

Note: *P>0.05 for comparison between groups before modeling, △P<0.05 for comparison between groups 1 day after modeling and before modeling, ○P<0.05 for comparison between blank group 1 day after modeling and other groups, √P>0.05 for comparison between blank group 6 days after modeling and each drug treatment group, ▲P<0.05 for comparison between model group 6 days after modeling and each drug treatment group, &P<0.05 for comparison between colchicine group 6 days after modeling and high, medium and low drug treatment groups P>0.05

There was no statistically significant difference in joint circumference between rats in each group before modeling (P>0. 05). Except for the blank group, the swelling of joint circumference was obvious in all the groups 1 day after modeling, and the difference was statistically significant before and after modeling (P<0. 05). On the 6th day after modeling, the circumference of the joint in the model group was significantly larger than that in the colchicine, high-dose, medium-dose and low-dose groups (P<0. 05), respectively, and there was no statistically significant difference in the circumference of the joint between the blank group and each drug treatment group (P>0. 05). This indicates that all the drugs in each group could effectively reduce joint edema. There was no significant difference in joint circumference between the colchicine group and the high-dose, medium-dose and low-dose groups (P > 0. 05), indicating that the gout test formula could effectively treat joint swelling and the efficacy was

ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

close to that of colchicine. The data are shown in Table 1.

3.2. ELISA for the determination of TNF-α

From the data in Table 2, it can be seen that the concentration of the cellular inflammatory factor TNF-α in the joint fluid of the model group was significantly higher than that of the blank group and the drug treatment group (P<0.01), and there was no significant difference in the concentration of TNF-α between the colchicine group, the high-dose group and the blank group (P>0.05), but the low-dose group and the medium-dose group were still higher than that of the blank group (P<0.05), indicating that the high-dose group of the gout test formula can effectively reduce the intra-articular concentration of inflammatory factor TNF-α.

Table 2: Concentration of inflammatory factors in ankle joint fluid of rats in each group ($x \pm s$, pg/ml).

Group	Number of samples	TNF-α
blank group	10	152.539±3.865△
model group	10	170.795±7.659*
colchicine group	10	153.866±2.862▲
low dose group	10	157.204±1.407◆
medium dose group	10	156.036±1.538◆
high dose group	10	153.526±1.966▲

Note: *P<0.01 between model group and other groups, △P>0.05 between blank group and colchicine group and high dose group, ◆P<0.05 between low dose group and medium dose group and colchicine group, respectively.

4. Discussion

To date, the mechanism of acute gouty arthritis (AGA) is the increase of uric acid in the blood from various causes and the deposition of urates in muscles, joints and internal organs, especially the urinary system. This deposition depends on multiple factors such as blood uric acid concentration, local temperature of the body, pH of the tissues, local inflammatory state of the tissues and concentration levels of other ions. The sodium urate formed by its deposition is strongly inflammatory and can further lead to macrophage activation and synovial cell inflammatory response, which in turn leads to the production of large amounts of cytokines. The large amount of cytokines further leads to an inflammatory response of synovial cells, resulting in gouty arthritis. Such inflammatory factors lead to the activation of a large number of phagocytes in the synovial tissue, the detection of activated intrinsic immune cells in the synovium, and the accumulation of a large number of inflammatory response factors in the synovial fluid, including IL-1β, IL-6, IL-18, IL-8 (CXCL8), MM and TNF-α [3] [2] [5].TNF-α, as a primary cytokine in the proinflammatory reticulum, not only accelerates inflammatory progression and release inflammatory substances such as prostaglandins, leukotrienes and oxygen radicals, but also promote the production of collagenase and other neutral proteases, causing serious consequences such as cartilage matrix disintegration, cartilage resorption and bone destruction, which play an important role in the development and progression process of acute gouty arthritis[6] .

In this experiment, by using randomized grouping and setting up a blank group control, a rat model of acute gouty arthritis was successfully manufactured by referring to a modified modeling method [2]. By comparing the statistical analysis of the herbal treatment groups (high, medium and low doses) with the model group and the blank group, all the herbal groups could significantly reduce the content of TNF-α in the joint fluid, and the circumference of the swollen joints of rats was significantly improved, which was close to the efficacy of colchicine. The clinical efficacy of the folk Chinese herbal gout formula is exact. By inhibiting the production of TNF-α, an inflammatory mediator in the joint cavity, it avoids further local inflammatory lesions in the joints that lead to swollen and painful joints.

In Western medicine, acute gouty arthritis belongs to the category of bi syndrome in Chinese medicine, while modern Chinese medicine scholars believe that the causes of gout are internal and external. The internal causes are either congenital endowment deficiency, or eating a lot of fat, sweet and strong food, or damp and hot constitution. Insufficient congenital endowment can easily lead to deficiency of liver and kidney, and the desire to eat fatty, sweet and greasy food, which can lead to loss of spleen health and internalization of dampness and heat; the external cause is the feeling of wind, cold, dampness and heat, which invade the meridians. The combination of internal and external evil, resulting in dysfunction of qi

ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

and blood, phlegm and dampness in the muscles and bones, then clinical evidence of localized redness, swelling and heat pain in the joints, serious cases may lead to joint deformation and functional disorders. Patients in the acute stage are seen to have red, swollen and hot joints, mostly due to cold and dampness, stagnant heat and toxicity, damp heat, phlegm and turbid toxicity, and treatment is based on clearing away heat and toxic materials, relieving dampness and phlegm, and activating blood circulation to dissipate blood stasis [9][10][11]. Root of Vetchleaf Sophora has the effect of clearing heat and detoxification, relieving dampness and swelling, cooling blood to stop bleeding; Chinese Clematis Root has the effect of curing rheumatism and dredging meridian; Roughleaved Spikemoss can activate blood to promote menstruation; Radix Angelicae Sinensis have tonifying and harmonzing blood, Regulating menstruation to relieve pain. The combined effect of these drugs is to clear away heat and toxic materials, promote diuresis and dissolve phlegm, promote blood circulation to remove blood stasis and relieve pain. Chinese Alangium Root dispels wind-evil and wetnese-evil, relaxes tendons and activates the meridians, disperses blood stasis and relieves pain. According to Chinese medicine, chicken meat, slightly warm in property and sweet in taste, can warm the middle warmer and tonify spleen, supplement the qi and nourish the blood, reinforce kidney essence, and improve the body's healthy energy. This experiment provides theoretical support for the clinical treatment of Acute gouty arthritis with this formula, and also provides the basis for further search of its active ingredients.

The present experiment also has some shortcomings: previous studies [8][13] have shown that sodium urate can stimulate TNF-α secretion through several pathways, which in turn are involved in the pathogenesis of gout. However, this theory of sodium urate as a pathogenic signal cannot explain the phenomenon that some hyperuricemia does not develop gout for life, suggesting that other signaling pathways are involved in gout pathogenesis. With advances in molecular biology and immunology, it has been found that gout, as an autoimmune inflammatory disease, synovial inflammatory factors and their constituent synovial immune microenvironment play an important role in its pathogenesis, and they can mediate the pathogenesis of gout through multiple signaling pathways. Clarifying how sodium urate interacts with immune cells in the pathogenesis of gout will open new avenues for the study of acute gouty arthritis pathogenesis and provide new ideas and entry points for inflammatory intervention in gout. The unique method of taking this formula: patients are required to drink beer and eat seafood to trigger gout before taking the drug, which further indicates its effect on human immune function, a direction that can be studied in the future.

Acknowledgements

Guangdong Provincial Bureau of Traditional Chinese Medicine Project (No.20182071). Guangdong Medical Research Fund Project (A2017559).

References

[1] Pen NG. 2013, Folk remedies to cure gout. New Rural Technology, 2013, 3, 47.
[2] CaiTY, Wang X, He ZH, et al., 2017, Establishment of acute gouty arthritis rat model and observation of model maintenance time. Chinese Journal of Laboratory Animal Science, 2017, 24(2): 494–499.
[3] Alexander SO, Alexandre DUMUSC, Sonia NASI. 2018, The role of IL-1 in gout: from bench to bedside. Rheumatology, 2018, 42(57):112–119.
[4] Cavalcanti NG, Marques CD, Lins E LTU, et al. 2016, Cytokine profile in gout: inflammation driven by IL-6 and IL-18. Immunol Invest, 2016, 45(5): 383–395.
[5] Zhao MS. 2018, Advances in the treatment of gout from the new classification criteria guidelines. Armed Forces Medicine, 2018, 29(3): 217-220.
[6] Huang JQ, Sun WJ, Wang SW, et al. 2012,Anti-inflammatory and antioxidant activity of quercetin against gouty arthritis in rats. Chinese Journal of Experimental Formulary, 2012, 18(2):169-173.
[7] Zhou Z, Liao T, Fei HX, et al. 2016,Research progress of Chinese medicine in the treatment of gouty arthritis. Heilongjiang Science, 2016, 7(3):22-23.
[8] Guo YX, Zhu SHY, Kang PZ, et al. 2021, Effects of Gout Clearing Formula on TNF-α, IL-1β and IL-18 in the synovial tissue of rats with acute gouty arthritis. Journal of Hunan University of Traditional Chinese Medicine, 2021, 41(5): 673-677.
[9] Long W, Jin JS.2016, Advances in Chinese medicine treatment of gout. Hubei Journal of Traditional Chinese Medicine, 2016, 38(9):80-82.
[10] State Administration of Traditional Chinese Medicine. Diagnostic efficacy criteria for Chinese medical evidence. Nanjing University Press, 1994:50-51.

ISSN 2618-1584 Vol. 4, Issue 7: 1-6, DOI: 10.25236/FMSR.2022.040701

[11] Yuan X, Chen XL. 2019, Understanding of acute gouty arthritis in Chinese medicine. Hubei Journal of Traditional Chinese Medicine, 2019, 41(3):64-66.
[12] An Y, Liu ZH. 2016, Evolution of gout diagnostic criteria and interpretation of new criteria. Journal of Kidney Disease and Dialysis Kidney Transplantation, 2016, 2:165-169.
[13] Yang F. Effect of dispelling paralysis soup on serum levels of IL-6 and TNF-α in patients with acute gouty arthritis (damp-heat paralysis evidence). Shandong University of Traditional Chinese Medicine, 2021.