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The Effect of Resveratrol on Rats Acute Gouty Arthritis*

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ABSTRACT Objective: To investigate the effects of resveratrol on rats acute gouty arthritis. **Methods:** According to random number table, 36 wista rats were divided into six groups. Control group, model group, Colchicine group, high dose Resveratrol group(HR), medium dose Resveratrol group (MR), low dose Resveratrol group (LR), were administrated of normal saline, Colchicine suspension, high dose Resveratrol, medium dose Resveratrol, low dose Resveratrol, respectively, once a day for 7days. Rats acute gouty arthritis modles were estalished on the fourth day by monosodium urate solution (concentration of 25 mg/mL, 0.05 mL) injected into the ankle cavity, while 0.05 mL normal saline was injected into joint cavity of rats in control group. 72 hours later, model establishment synovial fluid was collected from ankel to detect level of IL-1β, CXCL10. Synovium were fixed to do Histopathologic with 10% formalin. **Results:** The level of IL-1β, CXCL10 in resveratrol group were lower than that in model group ($P<0.05$). The Histopathologic results showed that resveratrol might reduce acute gouty arthritis joint edema, improve inflammatory cells infiltrating. **Conclusion:** The levels of IL- 1β and CXCL10 were raised in acute gouty arthritis. The resveratrol can effectively control acute gouty arthritis attack ,and the curative effect is dose dependent .

Key words: Gouty arthritis; Resveratrol; IL-1β

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Introduction

Gout is an inflammatory joint disorder characterized by hyperuricaemia and precipitation of monosodium urate crystals in the joints[1]. During hyperuricaemia, oversaturation of serum urate and formation of monosodium urate crystals activates the inflammasome, which results in the maturation of pro-inflammatory cytokine IL-1β followed by neutrophil influx into the joint fluid[2]. Neutrophils accumulate in both the joint fluid and the synovial membrane, where a small fraction of these cells actively phagocytes monosodium urate crystals. Gout affects more than 1% of adults in the world, and this is the most common form of inflammatory arthritis in men. Accumulating data support an increase in the prevalence of gout that is potentially attributable to recent shifts in diet and lifestyle, improved medical care, and increased longevity[3]. Resveratrol has received tremendous attention over the past couple of decades because of its benefits in several human disease models, including cardioprotection, antioxidant effect, anti-inflammatory effect, immune regulation, and cancer chemoprevention [4-7]. Recent studies have shown that Resveratrol also has good anti-inflammatory effects in rats with osteoarthritis, but its role in acute gouty arthritis is not yet clear[7,8]. Therefore, the objective of the study was to investigate the effects of Resveratrol on on mice with MSU-induced acute gouty arthritis.

1 Materials and methods

1.1 Experimental animals and groups

SPF Wistar male mice were from Qingdao Drug Control Institute, Qingdao, China (License number: SCXKLU20080002). All animals used for the experiments were ages 6-10 weeks with weight 180± 30 g. Thirty-six of SPF Wistar mice were randomized into six groups (6 of each), including control group, model group, colchicine group, high dose Resveratrol group (HR), medium dose Resveratrol group(MR), low dose Resveratrol group(LR). All mice were bred conventionally one week and then weighed to determine the dose of gavage. The mice in Control group and model group were given saline according to the weigh. Mice in the Colchicine group was given colchicines ($1mg \cdot kg^{-1} \cdot d^{-1}$), while mice in the low dose Resveratrol group, medium dose Resveratrol group, and high dose Resveratrol group were given low dose Resveratrol ($250 mg \cdot kg^{-1} \cdot d^{-1}$), middle dose Resveratrol ($500 mg \cdot kg^{-1} \cdot d^{-1}$), and high dose Resveratrol ($1000 mg \cdot kg^{-1} \cdot d^{-1}$), respectively. The experiments were approved by the Qingdao University Animal Ethics Committee and carried out in accordance with the Committee's guidelines for the care of animals.

1.2 Reagents

Resveratrol was obtained from Shanxi Zhongxin Biotechnology Co., Ltd. (HPLC determination of purity ≥ 98%, Batch NO: 121102). MSU was obtained from Alfa Aesar Company (Batch NO: A13348). Sodium hydroxide was provided by Sinopharm Chemical Reagent Co., Ltd. (Batch NO: F20070307). Polysorbate -80 was from Sigma-packing (Batch NO: C283). Colchicine was obtained from Xishuangbanna Pharmaceutical Co., Ltd. (Batch

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NO: 120709). Enzyme-linked immunosorbent assay (ELISA) kits of mouse IL-1β and CXCL10 were obtained from R&D Company.

1.3 Preparation of MSU solution

1 g uric acid was boiled in 200 ml of NaOH (6 ml, 1N)/double-distilled water (194 ml) until completely dissolved. The PH of uric acid solution was adjusted to 7.2 with HCL (1N). The uric acid solution was then filtered sterile (0.2-μm filter) and stored at 4 ℃ for 24 hours to allow crystal formation. The MSU crystals were then washed and dried under sterile conditions. 250 mg MSU crystals were added to 10 mL of saline (9 mL)/ Polysorbate -80 (1 mL), and the solution was further heated and stirred, leading to 10 mL solution of MSU (25 mg/ml).

1.4 Model of MSU-induced acute gouty arthritis

4-day after gavage, all mice were weighed again. 1h after the last gavage and prior to MSU injection, the right ankle circumference was measured with tie-line method. In the model control group, Colchicine group and three Resveratrol groups, acute gouty arthritis was induced by injection of 0.05 mL MSU solution (25 g/mL) into the right ankle joint cavity. In the Control group, the mice were injected with 0.05ml saline instead.

1.5 Joint swelling index

After MSU injection, the right ankle circumference was measured at the same position with tie-line method at 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 24 h, 48 h, and 72 h. The degree of joint swelling was measured by the joint swelling index, which was calculated as the following: (Circumference at test point - Initial Circumference) /Initial Circumference. The bigger joint swelling index was the more serious joint swelling.

1.6 Measurement of IL-1β and CXCL10

The levels of IL-1β and CXCL10 in the synovial fluid were measured at 72h after MSU injection.

1.7 Synovial biopsy

Synovial biopsy was carried out to assess the histopathological changes in the ankle joint,. The mice was anesthetized at 72h after MSU injection, and synovium from right ankle joint was collected. The collected synovium was fixed by 10% neutral formalin solution, decalcificated by 5% nitric acid, dehydrated conventionally, hyalinized, buried, and then sliced. The tissue sections were finally stained by using Hematoxylin-Eosin (HE) staining, the local histopathological changes were analyzed under the light microscope.

1.8 Statistical analysis

The statistical analyses were performed by using SPSS 17.0. All data were expressed as mean ± SEM. Statistical analysis was performed using a one-way analysis of variance followed by Dunnett's multiple comparison tests to determine the level of significance. A value of $P< 0.05$ was considered statistically significant.

2 Results

2.1 Variation of joint swelling index

From 1 h to 24 h after MSU injection, compared with the mice from Control group, there were obvious increased joint swelling indexes in the right ankle joints of mice from model group, Colchicine group, and three Resveratrol groups ($P<0.05$, Table 1). However, compared with model group, Resveratrol significantly suppressed the degree of MSU-induced joint swelling, especially in the high dose Resveratrol group ($P<0.05$, Table 1).

Table 1 Comparison of joint swelling index in rats of each group (x± s,n=6)

Group	Time(h)								
	1	2	3	4	5	6	24	48	72
Control	0.043± 0.01	0.061± 0.19	0.057± 0.04	0.063± 0.04	0.057± 0.04	0.044± 0.03	0.031± 0.03	0.029± 0.02	0.028± 0.02
Model	0.088± 0.06	0.220± 0.06[a]	0.391± 0.10[a]	0.351± 0.09[a]	0.391± 0.10[a]	0.425± 0.09[a]	0.414± 0.10[ab]	0.228± 0.06[a]	0.081± 0.05[a]
Colchicines	0.143± 0.06[a]	0.209± 0.09[a]	0.279± 0.11[ab]	0.293± 0.11[a]	0.279± 0.11[ab]	0.332± 0.12[a]	0.143± 0.07[ab]	0.056± 0.07[b]	0.048± 0.05
LR	0.146± 0.10[a]	0.196± 0.10[a]	0.296± 0.07[a]	0.303± 0.10[a]	0.296± 0.07[a]	0.354± 0.07[a]	0.228± 0.11[ab]	0.151± 0.12[a]	0.086± 0.10[a]
MR	0.137± 0.05[a]	0.254± 0.06[a]	0.346± 0.11[a]	0.378± 0.10[a]	0.346± 0.11[a]	0.397± 0.09[a]	0.164± 0.07[ab]	0.146± 0.08[a]	0.059± 0.03
HR	0.115± 0.07[a]	0.227± 0.08[a]	0.277± 0.10[ab]	0.308± 0.09[a]	0.277± 0.10[ab]	0.283± 0.10[ab]	0.128± 0.07[ab]	0.096± 0.07[ab]	0.051± 0.02

Note: compared with the control group, a$P< 0.05$; compared with the model group, b$P< 0.05$.

2.2 Variation of IL-1β and CXCL10 level in the synovial fluid Tab 2

Compared with that in the model group, resveratrol significantly reduced the IL-1β level in synovial fluid at 72h after MSU injection in all three does groups ($P<0.05$). In addition, resveratrol could also significantly reduce the CXCL10 level in synovial fluid at 72h after MSU injection in all three does groups ($P<0.05$).

2.3 Histopathological changes in the synovium (HE staining, ×200 Figure 1)

Synovial biopsy from the Model group revealed there were obvious edema and inflammatory cell infiltration in the synovium of MSU-induced acute gouty arthritis. The edema andinflammatory cell infiltration reduced in the mice from Colchicine group and Resveratrol groups.

Table 2 Comparison of IL-1β and CXCL10 level in the synovial fluid in rats of each group (x± s, pg/mL,n=6)

Group	IL-1β	CXCL10
Control	5.78± 0.25	57.52± 2.83
Model	11.85± 0.45[a]	110.13± 4.02[a]
Colchicines	8.11± 0.61[ab]	86.10± 3.20[ab]
LR	9.63± 0.71[ab]	107.93± 4.57[a]
MR	7.67± 0.48[ab]	86.79± 2.90[ab]
HR	6.66± 0.29[ab]	89.61± 5.47[ab]

Note: IL-1β :Inteleukin-1β ; vs Control group, aP＜0.05;vs Model group,bP＜0.05.

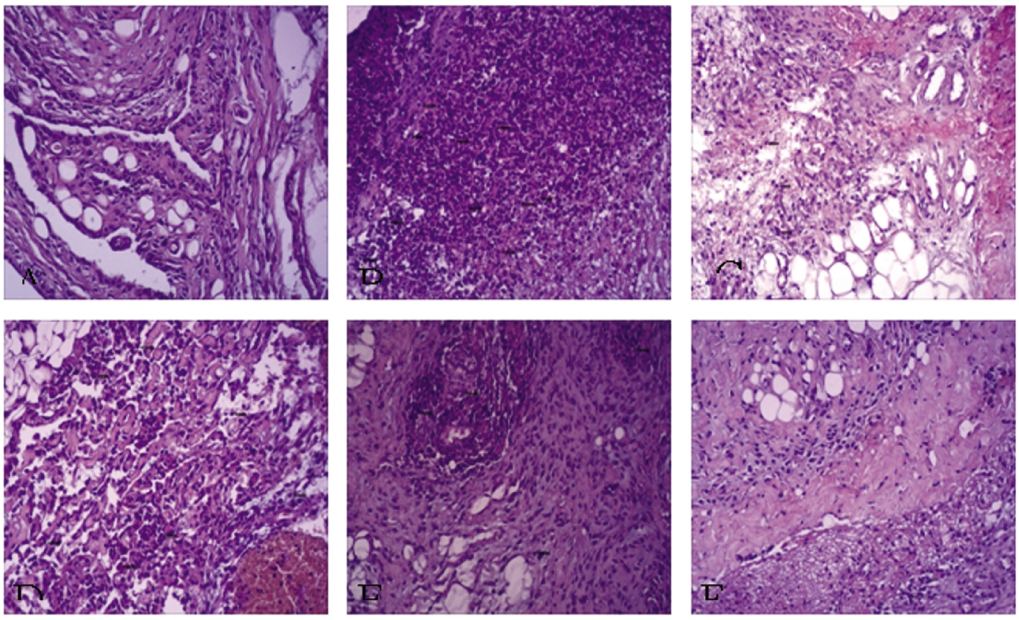

Fig.1 Histopathological changes in the synovium of mice from those six groups (HE staining, × 200):(A: Control group; B: Model group; C: Colchicine group; D: Low dose Resveratrol group; E: Middle dose Resveratrol group; F: High dose Resveratrol group)

3 Discussion

Acute gouty arthritis is an acute inflammatory arthritis caused by the deposition of MSU crystals within the joints [9,10]. MSU crystals can act as a proinflammatory cytokine and trigger a series of serious inflammations during the development process of gouty arthritis [11]. In acute gouty arthritis, MSU crystals activate the NLRP3 inflammasome which further leads to IL-1β secretion [10]. It has been well known that IL-1β is a key mediator of the inflammatory response and it plays a central role in the MSU-induced inflammation in the development of acute gouty arthritis[9].

IL-1β can induce the expression of endothelial cells, increase the activity of macrophages and granulocytes, and stimulate monocyte-macrophages to express IL-6, IL-8 and TNF-α and other secondary inflammatory cytokines [9]. Therefore, inhibition of IL-lβ is a promising treatment of acute gouty arthritis, and some effective biologic agents targeting IL-1β have been developed against acute gouty arthritis such as IL-1β antagonist Canakinumab [12,13]. In present study, Resveratrol could significantly reduce the levels of IL-1β in the synovial fluid in mice with MSU-induced acute gouty arthritis (P<0.05) . The inhibitory effect of Resveratrol on IL-1β expression in synovial fluid suggests that Resveratrol is a promising and new therapeutic method for acute gouty arthritis by the anti-inflammatory effect.

Chemokines coordinate leukocyte migration in immunity and inflammation and have been implicated in the pathogenesis of many human diseases. So far, there are over 50 chemokines and 20 chemokine receptors identified. Chemokines can be divided into four subfamilies, namely the CXC subfamily (α subfamily), CC subfamily (β subfamily), CX3C subfamily (δ subfamily), and C subfamily (γ subfamily)[14]. CXC subfamily was major in the activation and recruitment of neutrophilic granulocytes. Previous studies have shown that CXCL10 expression increased in various autoimmune diseases including rheumatoid arthritis [15]. In this study, Resveratrol significantly reduced the levels of CXCL10 in the synovial fluid (P<0.05) in mice with MSU-induced acute gouty arthritis.

In summary, Resveratrol can reduce the levels of IL-1β and

CXCL10 in the synovial fluid in mice with MSU-induced acute gouty arthritis. This study provides a new and promising therapeutic method for acute gouty arthritis, and further clinical researches are needed to assess the therapeutic potential of Resveratrol on acute gouty arthritis.

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白藜芦醇对急性痛风性关节炎大鼠的影响*

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摘要 目的：观察白藜芦醇对急性痛风性关节炎大鼠的影响。**方法：**选取 Wista 大鼠 36 只，随机分为正常对照组、模型组、秋水仙碱组、白藜芦醇低剂量组、白藜芦醇中剂量组、白藜芦醇高剂量组，各组相应采用生理盐水、秋水仙碱、白藜芦醇低、中、高剂量灌胃 7 天（1 次 / 日），模型组及各实验组于灌胃第四天把 25g / mL(0.05mL)浓度的尿酸盐溶液注射到大鼠踝关节腔内，制备急性痛风性关节炎模型，正常对照组大鼠关节腔内注射生理盐水 0.05 ml，72 h 后留取踝关节关节液及关节滑膜，应用 ELISA 法观察关节液中 IL-1β、CXCL10 的变化。关节滑膜用 10%福尔马林固定待做病理。**结果：**与模型组比较，白藜芦醇能显著降低关节液中 IL-1β、CXCL10 水平（P<0.05），病理结果显示，白藜芦醇可减轻急性痛风性关节炎大鼠踝关节组织的水肿和炎性细胞浸润。**结论：**急性痛风性关节炎发病过程中 IL-1β，CXCL10 明显增高，白藜芦醇可有效抑制急性痛风性关节炎发作，且该作用呈一定的剂量依赖性。

关键词：痛风性关节炎；白藜芦醇；白介素 1-β

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