# Effects of febuxostat on serum cytokines IL-1, IL-4, IL-6, IL-8, TNF-α and COX-2

GUOHUA HAO, WEI DUAN, JIANPING SUN, JINGYAO LIU and BO PENG

Department of Endocrinology II, Affiliated Zhongshan Hospital of Dalian University, Dalian, Liaoning 116001, P.R. China

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**Abstract.** Effects of febuxostat on serum cytokines interleukin (IL)-1, IL-4, IL-6, IL-8, tumor necrosis factor-α (TNF-α) and cyclooxygenase-2 (COX-2) in patients with gout were investigated. A total of 80 patients with gout admitted and treated in the Affiliated Zhongshan Hospital of Dalian University from January 2015 to September 2017 were selected and divided into two groups by virtue of a random number table, with 40 patients in each group. All the enrolled patients received strict gout diet adjustment and took colchicine at the same time. Patients in the control group were additionally treated with allopurinol, while those in the the observation group were administered with febuxostat. The serum uric acid levels were compared between the two groups. The number of gout attacks and adverse reactions were recorded, and the variations in COX-2 positive value integral were clarified. At different time-points of observation, the serum uric acid levels in the observation group were significantly lower than those in the control group ($p<0.05$). Moreover, at 3 months after treatment, the levels of inflammatory cytokines in the serum in the observation group were decreased compared with those in the control group ($p<0.05$). The IL-1 and TNF-α levels were lower in the observation group at 1 week, 1 and 3 months after treatment compared with those in the control group ($p<0.05$). Furthermore, it was discovered that at 3 months after treatment, the COX-2 positive value integral in the observation group was superior to that in the control group ($p<0.05$). During follow-up, the number of gout attacks that needed medical intervention in the observation group was smaller than that in the control group ($p<0.05$). Compared with allopurinol therapy, febuxostat therapy can remarkably inhibit inflammatory responses in the body, relieve clinical symptoms and reduce relapse of the patients with gout.

*Correspondence to:* Dr Wei Duan, Department of Endocrinology II, Affiliated Zhongshan Hospital of Dalian University, 6 Jiefang Road, Dalian, Liaoning 116001, P.R. China
E-mail: menpin229@163.com

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## Introduction

The pathogenesis of gout is an inflammatory joint disease caused by sodium urate crystal deposited in the joint *in vitro*. The disease is mainly triggered by hyperuricemia (1), which occurs more frequently in men aged >40 years and post-menopausal women (2). Previous studies have suggested that ~10% patients with raised uric acid have inflammatory responses resulting from deposition of serum uric acid in the form of sodium salt at the joints, finally having the onset of gout (3).

Currently, the treatment of gout mainly focuses on lowering the uric acid level, and it is generally advised to control the uric acid level at 6.0 mg/ml or below, so as to alleviate the patients' clinical symptoms (4). Allopurinol is the most commonly applied medicine in clinic, which can effectively suppress the production of uric acid in the patients. However, the clinical application of the drug is restricted among the yellow race, especially the Chinese Han population, because of the existence of positive rate of human leukocyte antigen-B (HLA-B)*5801 allele that may result in hypersensitivity reactions and even death of the patients after the use of allopurinol (5). Studies have confirmed that febuxostat, as a new type of xanthine oxidase inhibitor, can effectively decrease the uric acid level in the body of patients, and it becomes increasingly recognized in clinical practice (6). In order to better investigate the clinical effects of febuxostat on treating gout, the major purpose of this study is to analyze the influence of febuxostat on the primary inflammation-associated cytokines and cyclooxygenase-2 (COX-2) in the serum of gout patients.

## Patients and methods

*General data*. A total of 80 patients with gout admitted and treated in the Affiliated Zhongshan Hospital of Dalian University (Dalian, China) from January 2015 to September 2017 were selected. The diagnosis of all patients was confirmed by virtue of clinical manifestations and laboratory examinations. The patients themselves or their authorized persons signed the consent before enrollment, and this study was approved by the Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University. Patients with the following conditions were enrolled: full capacity for civil conduct, normal mental status, normal audition, language and other expression abilities as well as educational level at or higher than primary education. Patients complicated with other endocrine system

Table I. Comparison of serum uric acid during follow-up between the two groups (means ± SD).

| Groups | Before treatment | 1 week after treatment | 1 month after treatment | 3 months after treatment | F | P-value |
|---|---|---|---|---|---|---|
| Observation | 635.6±15.9 | 415.6±12.1 | 321.1±10.0 | 256.3±5.6 | 13.589 | <0.001 |
| Control | 636.9±16.0 | 568.9±13.3 | 451.5±12.3 | 329.8±7.9 | 8.693 | 0.013 |
| t | 0.364 | 53.923 | 52.026 | 48.005 | - | - |
| P-value | 0.716 | <0.001 | <0.001 | <0.001 | - | - |

diseases, systemic immune system diseases, diabetes mellitus, limb fracture or allergy to the drugs applied, or pregnant and breast-feeding women were excluded. All the patients were divided into two groups by means of a random number table, with 40 patients in each group. In the observation group, there were 30 males and 10 females aged 18-60 years, with an average age of 42.3±2.1 years. The course of disease was 3 months to 15 years, with an average course of 4.1±0.3 years. A total of 25 patients had apparent crystals of gout, and 15 patients only had pain of limb joints. The control group included 31 males and 9 females aged 18-60 years, with an average age of 42.4±2.0 years. The course of disease ranged from 3 months to 15 years, with an average course of 4.0±0.3 years. There were 24 cases of apparent crystals of gout and 16 cases of mere pain of limb joints. The differences in sex, age, course of disease and major clinical manifestations between the two groups were not statistically significant (p>0.05).

*Methods*. All the enrolled patients received strict gout diet adjustment and took colchicine [national medicine permission number (NMPN) H53021798; Yunnan Haopy Pharmaceutical Co., Ltd., Yunnan, China] (0.5 mg/time, 3 times a day) at the same time. Symptomatic and supporting therapy with non-steroidal anti-inflammatory drugs was conducted when patients had obvious pain. Patients in the control group were additionally treated with allopurinol (NMPN H31020334; Shanghai Sine Pharmaceutical Co., Ltd., Shanghai, China) (100 mg/time, 3 times a day). Furthermore, the consent of medication was signed by the patients before laparotomy and drug administration, and they were informed of the possibility of hypersensitivity reactions in detail. In the observation group, patients were administered with febuxostat (NMPN H20130081; Jiangsu Hengrui Medicine Co., Ltd., Jiangsu, China) (40 mg/time, once a day). Treatment conducted for 8 consecutive weeks was regarded as a course of treatment.

*Observation indexes*. All the enrolled patients were followed up and observed through out- and in-patient follow-up for 3 consecutive months. The serum uric acid levels before treatment and at 1 week, 1 and 3 months after treatment were compared between the two groups. Moreover, the levels of interleukin (IL)-1, IL-4, IL-6 and IL-8 at 3 months after treatment as well as changing trends of IL-1 and tumor necrosis factor-α (TNF-α) during the observation period in both groups were compared. The gout attacks that needed medical intervention during the follow-up period were recorded, and the variations in COX-2 positive value integral before and after treatment were clarified.

*Evaluation criteria*. Serum uric acid (phosphotungstic acid deoxidizing method: 149-416 μmol/l). Testing methods and normal values of related inflammatory factors: IL-1 [enzyme-linked immunosorbent assay (ELISA): 0.13-0.25 μg/l], IL-4 (ELISA: ≤31.2 μg/l), IL-6 (ELISA: 67.37-142.33 ηg/l), IL-8 (ELISA: 0.317-0.329 μg/l) and TNF-α (ELISA: 1.10-1.18 g/l). The COX-2 level was detected using reverse transcription-polymerase chain reaction (RT-PCR), whose expression was evaluated through positive scores, that is, the staining distribution under every high-power field was scored: 0 point (no staining), 1 point (light yellow staining), 2 points (yellowish brown staining), 3 points (tawny staining) and 4 points (brown staining). Higher scores indicated stronger COX-2 expression.

*Statistical analysis*. Statistical Product and Service Solutions (SPSS) 21.0 software (IBM Corp., Armonk, NY, USA) was applied. Measurement data in the enrolled information, such as inflammatory cytokine (IL-1, IL-4, IL-6, IL-8 and TNF-α) levels and COX-2, were presented as mean ± standard deviation (means ± SD). t-test was adopted for intergroup comparisons, repeated measures analysis of variance was conducted for intragroup comparison of means and the post hoc test was Dunnett's test. $\chi^2$ test was performed for comparison of adverse reaction rate. P<0.05 suggested that the difference was statistically significant.

## Results

*Comparison of serum uric acid during follow-up between the two groups*. There was no statistically significant difference in the serum uric acid among all the enrolled patients before treatment (p>0.05). At 1 week, 1 and 3 months after treatment, the serum uric acid level was decreased markedly in all the enrolled patients (p<0.05), and the level in the observation group was obviously lower than that in the control group (p<0.05) (Table I).

*Comparison of IL-1, IL-4, IL-6 and IL-8 levels at 3 months after treatment between the two groups*. Compared with those in the control group, the levels of IL-1, IL-4, IL-6 and IL-8 were notably lower at 3 months after treatment in the observation group (p<0.05) (Table II).

*Change in trends of IL-1 at different time-points of observation in both groups*. In the observation group, the IL-1 level was 1.16±0.12 μg/l before treatment, 0.31±0.06 μg/l at 1 week after treatment, 0.21±0.03 μg/l at 1 month after treatment and 0.16±0.01 μg/l at 3 months after treatment.

Table II. Comparison of IL-1, IL-4, IL-6 and IL-8 levels at 3 months after treatment between the two groups (means ± SD).

| Groups | IL-1 (μg/l) | IL-4 (μg/l) | IL-6 (ng/l) | IL-8 (μg/l) |
|---|---|---|---|---|
| Observation | 0.16±0.01 | 28.5±1.5 | 124.1±2.7 | 0.312±0.001 |
| Control | 0.86±0.11 | 59.8±2.6 | 205.3±5.9 | 0.419±0.012 |
| t | 40.082 | 65.950 | 79.149 | 56.199 |
| P-value | <0.001 | <0.001 | <0.001 | <0.001 |

IL, interleukin.

Table III. Comparison of COX-2 positive value integrals before and after treatment between the two groups (point, means ± SD).

| Groups | Before treatment | After treatment | F | P-value |
|---|---|---|---|---|
| Observation | 3.1±0.2 | 0.6±0.1 | 70.711 | <0.001 |
| Control | 3.1±0.2 | 2.1±0.3 | 17.541 | <0.001 |
| t | 0.000 | 30.000 | - | - |
| P-value | >0.001 | <0.001 | - | - |

COX-2, cyclooxygenase-2.

In the control group, the IL-1 levels before treatment and at 1 week, 1 and 3 months after treatment were 1.16±0.12, 1.05±0.10, 0.96±0.08 and 0.86±0.11 μg/l, respectively. At 1 week, 1 and 3 months after treatment, the observation group had significantly lower IL-1 levels than the control group in the same time period (p<0.05) (Fig. 1).

*Change in trends of TNF-α at different time-points of observation in the groups*. In the observation group, the TNF-α level was 1.26±0.03 g/l before treatment, 1.15±0.03 g/l at 1 week after treatment, 1.13±0.02 g/l at 1 month after treatment and 1.11±0.01 g/l at 3 months after treatment. In the control group, the TNF-α levels before treatment and at 1 week, 1 and 3 months after treatment were 1.26±0.03, 1.20±0.03, 1.18±0.01 and 1.18±0.01 g/l, respectively. The TNF-α levels in the observation group at 1 week, 1 and 3 months after treatment were decreased remarkably compared with those in the control group in the same time period (p<0.05) (Fig. 2).

*Comparison of COX-2 positive value integrals before and after treatment between the two groups*. The difference in the COX-2 positive value integral was not statistically significant between the two groups before treatment (p>0.05). After treatment, however, the COX-2 positive value integral in the observation group was better than that before treatment and that in the control group after treatment (p<0.05) (Table III).

*Comparison of gout attacks that needed medical intervention during follow-up*. There was no statistically significant

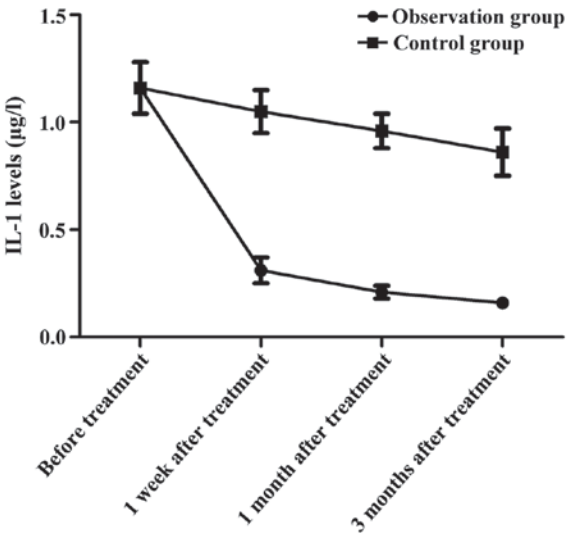


Figure 1. Change in trends of IL-1 at different time-points of observation in both groups. At 1 week, 1 and 3 months after treatment, the observation group has significantly lower TNF-α levels than the control group in the same time period (p<0.05). IL, interleukin; TNF-α, tumor necrosis factor-α.

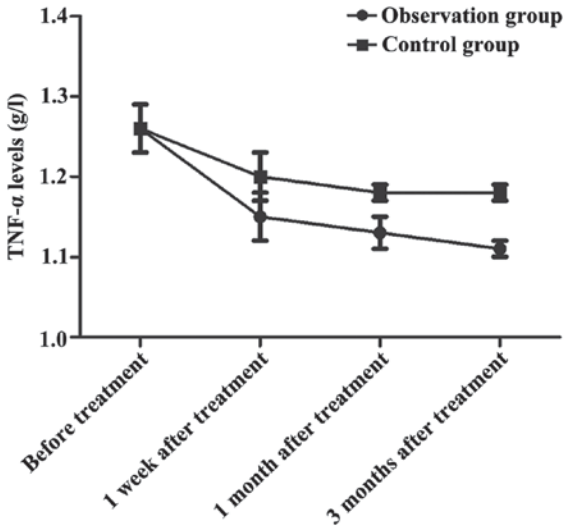


Figure 2. Change in trends of TNF-α at different time-points of observation in both groups. The TNF-α levels in the observation group at 1 week, 1 and 3 months after treatment are decreased remarkably compared with those in the control group in the same time period (p<0.05). TNF-α, tumor necrosis factor-α.

difference in the frequency of gout attacks that needed medical intervention among the enrolled patients before treatment (p>0.05). At 1 week, 1 and 3 months after treatment, the number of gout attacks that needed medical intervention was decreased markedly in all the enrolled patients compared with that before treatment (p<0.05), and the number in the observation group was apparently smaller than that in the control group (p<0.05) (Table IV).

*Comparison of adverse reactions that occurred during treatment between the two groups*. Comparisons of adverse reactions or complications detected during treatment between the two groups showed no statistically significant differences, with no comparability (p>0.05) (Table V).

Table IV. Comparison of gout attacks that needed medical intervention during follow-up (time/month, (means ± SD).

| Groups | Before treatment | 1 week after treatment | 1 month after treatment | 3 months after treatment | F | P-value |
|---|---|---|---|---|---|---|
| Observation | 3.1±0.2 | 1.3±0.2 | 1.1±0.1 | 0.9±0.1 | 21.362 | <0.001 |
| Control | 3.1±0.3 | 2.3±0.1 | 1.8±0.1 | 1.5±0.2 | 12.305 | <0.001 |
| t | 0.000 | 28.284 | 31.305 | 16.971 | - | - |
| P-value | >0.001 | <0.001 | <0.001 | <0.001 | - | - |

Table V. Comparison of adverse reactions that occurred during treatment between the two groups [n (%)].

| Groups | Hyperlipidemia | Gastrointestinal discomfort | Liver and kidney injury | Hypersensitivity reaction | Total incidence |
|---|---|---|---|---|---|
| Observation group | 1 | 2 | 1 | 2 | 6 (15.0%) |
| Control group | 1 | 1 | 1 | 1 | 4 (10.0%) |
| t | | | - | | 0.114 |
| $\chi^2$ | | | - | | 0.735 |

## Discussion

In recent years, with the increase in China's national economy and changes in people's living standard and diet style, the incidence rate of hyperuricemia is obviously increasing (7). Studies have demonstrated that (8) hyperuricemia is associated with inheritance, medicine, past history of renal diseases, and intake of high purine and protein diet. When purine metabolism disorder occurs in the body, it causes overproduction and excretion reduction of uric acid *in vitro* at the same time (9). At this time, ~1/10 patients with hyperuricemia have uric acid deposited in the joints, soft tissues and kidneys in the form of sodium salt, which triggers inflammatory responses at the above-mentioned positions with uric acid deposition, further manifesting as gout attacks (10). As a kind of metabolism-related joint disease induced by sodium urate deposition, gout is mainly a local inflammatory response that results from disorders of the purine metabolism (11). In severe cases, it can lead to the occurrence of renal lesions and damage to joint functions, thus affecting the quality of life and even threatening the life safety of the patients (12). As a result, inflammatory response factors have close correlations with the occurrence and development of gout. In previous treatments, the two most classic and important drugs (allopurinol and colchicine) are utilized, of which allopurinol is especially widely applied in clinic. However, it is used with vigilance in clinical practice due to its inevitable hypersensitivity reactions (13). Therefore, it is urgent in clinic to find an alternative drug for patients unsuitable for allopurinol.

In this study, all the enrolled patients were definitely diagnosed with gout, and colchicine as well as symptomatic and supporting therapy with non-steroidal anti-inflammatory drug was utilized on the basis of diet for gout. Patients in the control group were treated with allopurinol, while those in the observation group were given febuxostat. The study on the serum uric acid level after intervention discovered that in spite of the urate-lowering effects of the drugs, the serum uric acid levels in the observation group were decreased significantly compared with those in the control group at 1 week, 1 and 3 months after treatment. It demonstrated that patients treated with febuxostat have better effectiveness in lowering the uric acid. The changes in the inflammation-associated cytokines at 3 months after treatment in both groups were investigated, and it was revealed that the levels of IL-1, IL-4, IL-6 and IL-8 at 3 months after treatment in the observation group were remarkably lower than those in the control group, implying that febuxostat plays a positive role in reducing inflammatory responses in the body. Moreover, the study findings of the change in trends of IL-1 and TNF-α at different time-points of observation in the two groups indicated that at 1 week, 1 and 3 months after treatment, the observation group had significantly decreased IL-1 and TNF-α levels than the control group in the same time period. Furthermore, it indicated that as for gout patients, the febuxostat therapy is more effective in suppressing inflammatory responses in the body, and the effects start at 1 week after medication. In addition, the COX-2 positive value integrals before and after treatment were compared between the two groups, and the results revealed that the COX-2 positive value integral in the observation group after treatment was superior to that before treatment and that in the control group after treatment. It implied that on treating patients with gout, febuxostat can selectively inhibit the activity of prostaglandin-endoperoxide synthase 2 in the body, thereby reducing local inflammatory responses and relieving the clinical symptoms of the patients. Finally, the comparison of gout attack frequency and adverse reactions that occurred during the treatment indicated that during follow-up, the number of gout attacks in the observation group was obviously smaller than that in the control group, and the incidence of adverse reactions of medication were <15% in both groups. Besides, the differences were not statistically significant.

For gout patients, febuxostat was applied in the observation group based on the conventional treatment in this research (14), and it had more prominent clinical effects in patients than allopurinol. As a novel xanthine oxidase inhibitor (15), febuxostat can repress the activity of xanthine oxidase in an efficient manner and further avoid the allopurinol-induced adverse reactions in a selective way (16). The possible mechanism of action is that it inhibits the transformation of hypoxanthine into xanthine by means of oxidation (17) and reduces the formation of uric acid as much as possible. Compared with allopurinol (18), febuxostat averts the inhibitory effects on nucleotidase and deaminase in the processes of purine or pyrimidine metabolism through the selective inhibitory effect (19), enhances the efficacy of medical treatment and reduces the occurrence of hypersensitivity reactions of allopurinol (20).

In conclusion, compared with allopurinol therapy, febuxostat therapy can remarkably inhibit inflammatory responses in the body, relieve clinical symptoms and reduce relapse of the patients with gout.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

GH and WD were responsible for treating patients and collecting data of patients. JS detected the serum uric acid levels. JL contributed to PCR. GH, WD and BP contributed to ELISA. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

The study was approved by the Ethics Committee of the Affiliated Zhongshan Hospital of Dalian University (Dalian, China) and informed consents were signed by the patients or their guardians.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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