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Effect of *Tripterygium Wilfordii* Polyglycoside on Experimental Prostatitis Caused by *Ureaplasma Urealyticum* in Rats

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Background: Prostatitis is a common and refractory urological disease with complicated etiology. *Ureaplasma urealyticum* (UU) has a close relationship with human urinary tract infection that can induce nonbacterial prostatitis. *Tripterygium wilfordii* polyglycoside (TWP) is a non-steroidal immune inhibitor that causes significant immune suppression and anti-inflammatory effects. Its role in prostatitis caused by UU has not yet been established. The aim of this study was to investigate the effect of TWP on UU-infected prostatitis in a rat model.

Material/Methods: UU-infected prostatitis SD model rats were randomly divided into 2 groups: the prostatitis group (model group) and the TWP treatment group (treatment group). At 7 days after treatment, prostate weight, leucocyte count, lecithin corpuscles, UU infection rate, and UU microbe count were compared between the 2 groups. Serum inflammatory cytokines TNF-α was determined by ELISA, and ICAM-1 and NF-κB expression were detected.

Results: UU infection rate was 80% after modeling. The rat prostate weight and leucocyte count in the model group increased significantly, while lecithin corpuscles decreased. Compared with controls, inflammatory factor TNF-α, ICAM-1, and NF-κB expression were obviously higher ($P<0.05$). TWP markedly reduced prostate weight and leucocyte count, increased lecithin corpuscles, and decreased UU microbe count and TNF-α, ICAM-1, and NF-κB expression ($P<0.05$).

Conclusions: TWP can inhibit expression of inflammatory factors and may be useful in treating UU-infected prostatitis through reducing UU infection rate.

MeSH Keywords: Prostatitis • Tripterygium • Ureaplasma Infections

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Background

Prostatitis is a common urological disease causing urination abnormalities, including urinary urgency, frequent urination, odynuria, dysuria, and micturition. It also can cause suprapubic, lumbosacral, and perineum pain, together with sexual dysfunction, which is also known as prostatitis syndrome [1,2]. There are millions of prostatitis outpatients every year, mostly younger than 50 years of age. Prostatitis patients accounted for one-third of all urology outpatients, with a steadily increasing incidence rate [3,4]. According to the etiology and onset time, it can be divided into acute bacterial prostatitis, chronic bacterial prostatitis, acute nonbacterial prostatitis, and chronic nonbacterial prostatitis, of which chronic nonbacterial prostatitis is the most common type [5]. The cause of prostatitis is complicated and the etiology of nonbacterial prostatitis is still unclear. Chronic nonbacterial prostatitis is easy to diagnose but difficult to cure, leading it to be a clinically refractory disease [6].

Different types of mycoplasma exist in the human urogenital system, respiratory system, and joints. *Mycoplasma hominis*, *Mycoplasma genitalium*, and *Ureaplasma urealyticum* (UU) have been found in various tissues. Thus, mycoplasma infection has a close relationship with prostatitis [7]. UU, which belongs to *Mycoplasmaceae ureaplasma*, can be divided into 14 serotypes belonging to 2 different biotas [8]. Under normal circumstances, UU parasitizes on the mucous membrane of the genitourinary tract. Specific circumstances such as immunity reduction, hormone level changes, mucous membrane damage, surgery, and trauma can lead to different serotypes of UU in opportunistic infection, causing non-gonococcal urethritis, reactive arthritis, and even infertility or abortion [9,10]. UU has a close relationship with human urinary tract infection and nonbacterial prostatitis [11].

Tripterygium wilfordii, also known as *Daemonorops margaritae* root, xanthate, *Gelsemium elegans*, or *Daemonorops margaritae* grass, is a bitter herb. As one of the extracts of *Tripterygium wilfordii* plants, *Tripterygium wilfordii* glycoside (TWP) is a non-steroidal immune inhibitor with many pharmacological activities. Its intraperitoneal injection can inhibit graft versus host reaction (GVHR). It also can inhibit delayed-type hypersensitivity, and suppress peripheral blood mononuclear cells (PBMC) proliferation response induced by PHA [12,13]. TWP also has significant anti-inflammatory effects [14]. The present study analyzed the effect of TWP through establishing a UU-infected prostatitis rat model.

Material and Methods

Experimental animal

Sixty healthy male SD rats at SPF grade, 2–3 months old, and weighing 250±50 g were purchased from the Laboratory Animal Center of ZheJiang University. The rats were housed in a specific pathogen free (SPF) animal experiment center with constant temperature (21±1°C) and humidity (50–70%). The day/night cycle was maintained at 12 h.

Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Shaoxing County Central Hospital.

Main materials and instruments

Experimental surgical instruments were purchased from Suzhou Medical Instrument Co. Serum type IV UU standard strains and medium were bought from a strain collection center in the United States. TNF-α, ICAM-1, and NF-κB ELISA kits were from R&D. TWP was from Fujian Huitian Biological Pharmaceutical Co., LTD. Other common reagents were purchased from Sangon.

Methods

UU culture and titration

UU standard strain kept in freeze-dried powder form was recovered under aseptic condition, and cultured in UU special medium maintained in 37°C for 18–24 h. Medium that is orange-red and clear without turbid indicates good growth. UU in logarithmic phase were used for the following investigation. Titer of the UU was determined according to the medium color (CCU/ml). UU was diluted and seeded in 40-well plates for 18 h to observe the results. Red color indicated that the UU titer was at the highest dilution. The UU titer used in this study was 9.2×10^7 CCU/ml.

Establishment of the UU-infected prostatitis model

All rats tested negative for mycoplasma before the experiment. Fifty rats were randomly selected for UU-infected prostatitis model establishment. Rats were anesthetized by intraperitoneal injection of 10% pentobarbital sodium at 0.6 mg/kg [15]. After opening the abdominal cavity, 0.6 ml of UU strain liquid was injected into the ventral prostate.

UU infection rate detection

The rat prostate fluid was collected at 1 week after vaccination, and seeded in UU liquid medium and solid medium plate. The UU in liquid medium was counted based on eluent color

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Table 1. Rat body and prostate weight change ($\bar{\chi}$±S).

Group	n	Weight (g)	Prostate wet weight (g)	Wet prostate/body weight ratio
Control	10	402±21.6	0.57±0.049	1.47×10^{-3}
Model group	20	328±19.2*	0.79±0.038*	2.41×10^{-3}*
Treatment group	20	376±22.1#	0.66±0.051#	1.75×10^{-3}#

* $P<0.05$, compared with control; # $P<0.05$, compared with model group.

changes (CCU/ml). Solid medium was incubated in 37°C for 48–72 h to observe the granular colonies formation to judge positive UU infection.

Animal grouping and general state observation

Successful modeling rats were randomly divided into 2 groups: the UU-infected prostatitis group (model group) and the TWP treatment group (treatment group). Ten mg/kg TWP was given to treatment group rats by gastric gavage 3 times/d, while another 10 rats served as normal controls. Rats in the control and model group received equal amounts of normal saline for 7 continuous days. General status, including weight, hair condition, and food intake were observed, and the rats were killed on the 7th day.

Specimen collection

Abdominal aortic blood was collected in a vacuum biochemical tube and kept at room temperature for 30 min. Then the sample was centrifuged at 3600 rpm and 4°C for 10 min. The supernatant was cryopreserved at –20°C. Prostate tissue was collected for weighing and analysis.

White blood cell count and lecithin corpuscle density analysis

We mixed 1 mg prostate tissue with 4 µl saline. After adding the leucocyte diluent, we counted the total number of white blood cells and density of lecithin corpuscles under low-power magnification. Lecithin corpuscle density can be divided into 4 levels based on clinical inspection standard: full vision field, level 4; 3/4 vision field, level 3; 1/2 vision field, level 2; and 1/4 vision field, level 1.

ELISA

Serum TNF-α, ICAM-1, and NF-κB expression levels were detected by ELISA according to the manufacturer's instructions. Major steps include: put 50 µl diluted standard product into the corresponding reaction holes to prepare the standard curve; add 50 µl samples to each hole in triplicate; after washing the plate 5 times, add 50 µl enzyme standard reagent; wash the plate 5 times after incubation at 37°C for 30 min; insert 50 µl agent A and 50 µl agent B to each hole and incubate the plate at 37°C for 30 min; terminate the reaction after adding 50 µl color agent A and 50 µl color agent B; and measure the plate at 450 nm wavelength to get the absorbance value (OD value). The sample concentration was calculated according to the OD value and standard curve.

Statistical treatment

All statistical analyses were performed using SPSS16.0 software. Measurement data are presented as mean ± standard deviation (mean ±SD). One-way ANOVA and chi-square test were used for mean comparisons. $P < 0.05$ was considered as a significant difference.

Results

UU infection rate and general morphological observation

No UU was detected in the control group. After modeling, 40 rats showed UU infection. UU-infected prostatitis caused rats to lose weight, reduce activity, and eat less, and their hair became dull. TWP treatment caused weight gain, increased activity, normal eating, and lustrous hair. At 7 days after treatment, wet prostate/body weight ratio increased obviously in the model group compared with controls ($P<0.05$), and it decreased significantly in the treatment group significantly compared with the model group ($P<0.05$) (Table 1).

Prostate white blood cell number and lecithin corpuscle density changes

We found that WBC numbers in the prostate increased significantly in the model group ($P<0.05$), while in the TWP treatment group there were obviously fewer WBCs ($P<0.05$) (Table 2). Lecithin corpuscle density analysis showed that its grade declined markedly compared with the control group ($P<0.05$), whereas TWP treatment significantly improved lecithin corpuscle density classification ($P<0.05$) (Table 2).

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Table 2. Prostate white blood cell number and lecithin corpuscle density changes ($\bar{\chi}$±S).

Group	n	WBC number (×10⁹)	Lecithin corpuscle density			
			Level 1	Level 2	Level 3	Level 4
Control	10	1.92±0.67	0	1	2	7
Model group	20	7.21±0.97*	9	7	3	1
Treatment group	20	3.61±0.81*#	3	3	10	4

* $P<0.05$, compared with control; # $P<0.05$, compared with model group.

Table 3. UU microbe amount changes ($\bar{\chi}$±S).

Group	n	Before treatment (CCU/ml)	After treatment (CCU/ml)
Model group	20	(6.2±0.8)×10⁶	(5.4±1.6)×10⁶
Treatment group	20	(6.4±0.6)×10⁶	(3.4±1.1)×10³*#

* $P<0.05$, compared with control; # $P<0.05$, compared with model group.

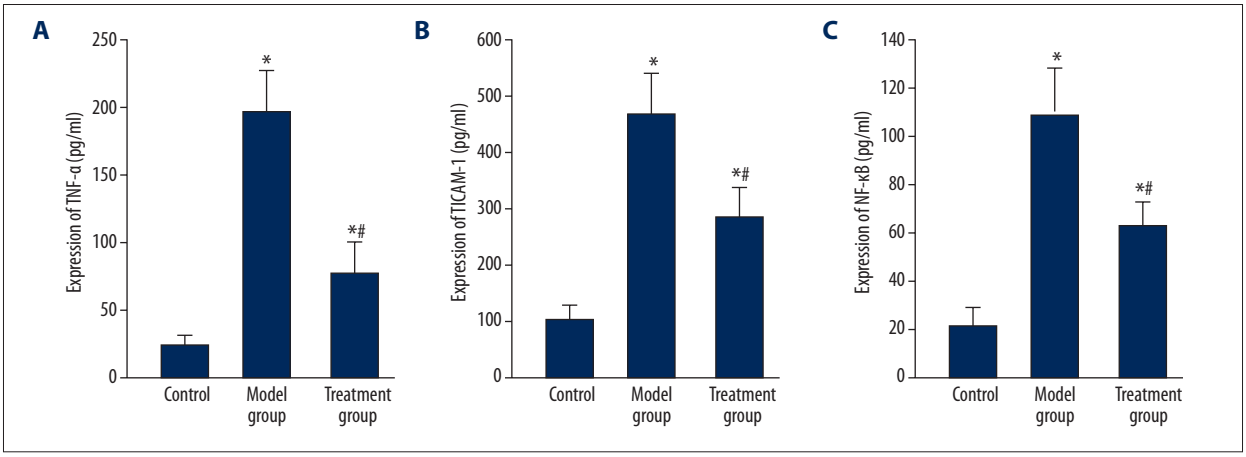

Figure 1. Inflammatory factor expression (**A**) TNF-α; (**B**) ICAM-1; (**C**) NF-κB. * $P<0.05$, compared with control; # $P<0.05$, compared with model group.

UU microbe number changes

Comparison of UU microbe numbers showed they were obviously decreased after TWP treatment compared with the model group and before treatment (P<0.05) (Table 3).

Inflammatory factors TNF-α, ICAM-1, and NF-κB expression changes

ELISA detection revealed that UU infection caused significantly increased expression of TNF-α, ICAM-1, and NF-κB (P<0.05), whereas TWP treatment obviously reduced TNF-α, ICAM-1, and NF-κB levels compared with the model group (P<0.05) (Figure 1).

Discussion

In recent years the incidence of UU-induced chronic nonbacterial prostatitis has been increasing [16]. However, the treatment effect of chronic nonbacterial prostatitis is still poor at present, and the quality of life has not been improved [17]. Establishing a UU-infected prostatitis animal model is the precondition of effective prevention and treatment of UU-infected prostatitis.

This study used UU strains cultured bacteria liquid injected into the prostate, and obtained a UU-positive rate of 80%. WBC and lecithin are important qualification standards in prostatitis detection and diagnosis. Prostate inflammation can cause considerable phagocytosis of lipids by macrophages, leading to lecithin corpuscle decline and density reduction. However, because inflammation results in higher WBC numbers, they have

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an important reference value in prostatitis diagnosis [18,19]. In this study, lecithin corpuscles obviously declined and WBC numbers increased in our established UU-infected prostatitis model, suggested that our method can successfully establish a UU-infected prostatitis animal model. Chronic nonbacterial prostatitis can increase the secretion of inflammatory mediators and cytokines, thus promoting inflammation.

TNF-α is mainly derived from mononuclear macrophages, which is the earliest-secreted cytokine in the inflammatory response. It can induce chemokine production, promote adhesion molecules expression in epithelial cells and lymphocytes, and gather inflammatory cells to the inflammation region [20]. As a member of the adhesion molecules immunoglobulin family, intercellular adhesion molecule 1 (ICAM-1) mediates adhesive attraction between the cells and extracellular matrix (UU), and participates in inflammatory response. Nuclear transcription factor B (NF-κB) is also a transcription factor involved in the early stage of inflammatory reaction; it can play a role in the processes of immune response, inflammatory reaction, and oxidation reaction [21]. TNF-α can promote ICAM-1 expression in lymphatic cells and vascular endothelial cells, and activates NF-κB expression through phosphorylation reaction to promote ICAM-1 transcription [22]. In this study, 3 types of inflammatory factors secretion were increased markedly in UU-infected prostatitis, further indicating that inflammatory factors promote UU-infected prostatitis occurrence and development. TWP plays a critical role in inflammation through increasing inflammatory vascular permeability and suppressing platelet aggregation [14]. However, its role in treating UU-infected prostatitis is still unclear. Our results confirmed that TWP treatment can significantly relieve prostate weight, reduce WBC numbers, increase lecithin corpuscles, decrease the amount of UU, and downregulate TNF-α, ICAM-1, and NF-κB expression in a rat model.

Conclusions

TWP can treat UU-infected prostatitis and improve inflammation through reducing UU infection rate and inhibiting expression of inflammatory factors. Our methods and findings may contribute to creation of new drugs for clinical treatment of chronic nonbacterial prostatitis.

Disclosure of conflict of interest

None.

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