Effect of alcohol on chronic pelvic pain and prostatic inflammation in a mouse model of experimental autoimmune prostatitis

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Abstract

Background: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is a prevalent disease of the urogenital system. Alcohol has been reported to be closely related to CP/CPPS. Thus, we intended to verify the role of alcohol in CP/CPPS and determine the underlying mechanism.

Methods: We induced experimental autoimmune prostatitis (EAP) mouse model by intradermally injecting a mixture of prostate antigens (PAgs) and complete Freund’s adjuvant on days 0 and 28. Mice were treated with alcohol (control-alcohol and EAP-alcohol groups) or vehicle (control-vehicle, and EAP-vehicle groups) from day 32 to 42. Forty-two days after PAg injection, the pathological appearance of the prostate tissues was evaluated, and histological analyses of the prostate were performed. Chronic pelvic pain was assessed by applying von Frey filaments to the lower abdomen. Proinflammatory cytokines were detected by enzyme-linked immunosor- bent assay test. Then, we explored the effects of the NLRP3 inhibitor MCC950 on chronic pelvic pain and prostatic inflammation in this model.

Results: Histological analyses showed diffuse inflammation in the stromal tissues that were characterized by severe infiltration of neutrophils and mononuclear cells in mice in the EAP-alcohol group compared with EAP-vehicle group. Chronic pain tests showed that the response frequency was significantly increased using a von Frey filament at forces of 0.4, 1.0, and 4.0 g in EAP-alcohol group compared with EAP-vehicle (P < .05). The levels of proinflammatory cytokines, including interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-17, and IL-1β were all significantly elevated in EAP-alcohol group compared with the EAP-vehicle group (P < .05). However, between the control-alcohol and control-vehicle groups, chronic pain tests, histological assays, and cytokine determinations showed no differences. Furthermore, our results demonstrated that MCC950 could decrease the expression level of NLRP3 inflammasome-related proteins including NLRP3, ASC, and caspase-1. The chronic pain tests, histological assays, and cytokine determinations showed that MCC950 could attenuate the chronic pain and prostatic inflammation through the inhibition of the NLRP3 inflammasome.
1 | INTRODUCTION

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS), also known as type III prostatitis is the most common urogenital disease in men less than 50 years old, and it accounts for more than 90% of all cases of prostatitis and affects 8.4% to 14% of males.\(^1\)\(^-\)\(^3\) CP/CPPS is a poorly understood syndrome characterized by perineal or pelvic pain, symptoms of urinary irritation, and sexual dysfunction without infection, lacking a definite etiology that would allow a more reasonable therapy.\(^4\)\(^-\)\(^5\) To date, literature has described potential triggers of CP/CPPS that include a history of urogenital diseases, level of sex hormones, diet, stress, allergies, and marital and psychological status.\(^6\)\(^-\)\(^7\) However, the definite etiology of prostatitis is still largely unknown.

Inflammasomes are complexes that belong to a family of large multi-protein intracellular pattern recognition receptors that respond to various exogenous pathogen-associated molecular patterns (PAMPs) and endogenous danger-associated molecular patterns to promote the secretion of proinflammatory cytokines, including interleukin-1β (IL-1β) and IL-18.\(^8\) One of the most important and widely studied inflammasomes is the NLRP3 inflammasome. NLRP3 inflammasome is also a key tissue damage sensor. The past decade has witnessed many studies, which suggested that the malfunction of NLRP3 inflammasome is a potent trigger of a series of autoinflammatory and autoimmune diseases,\(^9\) including gout,\(^10\) rheumatoid arthritis,\(^11\) and lupus.\(^12\) Moreover, NLRP3 inflammasome has been reported to participate in the chronic inflammation status in the prostate.\(^13\)\(^-\)\(^15\)

Alcohol consumption is widespread all over the world. Alcohol use disorders extensively exist globally.\(^16\)\(^-\)\(^17\) Alcohol uptake affects almost every organ system and is associated with a variety of inflammatory conditions, such as hepatitis,\(^18\) pancreatitis,\(^19\) and atherosclerotic lesions.\(^20\) Epidemiological studies have also shown a close association between alcohol uptake and CP/CPPS,\(^3\)\(^-\)\(^21\) as those who had a habit of drinking alcohol had higher morbidity and more severe symptoms of CP/CPPS. However, the underlying causative mechanisms remain to be elucidated.

Thus, we conducted this study to evaluate the relationships between CP/CPPS and alcohol in experimental autoimmune prostatitis (EAP) mouse model. The development of animal models of EAP is achieved by the immunization of animals with prostate antigen plus an adjuvant.\(^22\) Such a model shows almost all of the previously mentioned characteristics of the human disease: increased levels of cytokines, pelvic pain, inflammation, and infiltration and histological lesions in the target organ.\(^22\) In this study, we designed experiments to evaluate the role of alcohol on EAP and characterized it by measuring a wide range of proinflammatory cytokines in the plasma and inflamed prostate and testing for suprapubic allodynia to assess chronic pain development. Then we used the MCC950 to study whether NLRP3 inflammasome played a role in alcohol-treated EAP. As a result, we found for the first time that the alcohol could aggravate the severity of prostatic inflammation in an EAP model rather than directly induce EAP though activating the NLRP3 inflammasome.

2 | MATERIAL AND METHODS

2.1 | Mice and antigens preparation

The 6 to 8 week-old mice used in our study were NOD/LtJ non-obese diabetic (NOD) mice purchased from Nanjing Biomedical Research Institute of Nanjing University, Nanjing, China. All animals were housed and maintained under specific pathogen-free conditions in the Animal Center of Anhui Medical University. All animal experiments were approved and conducted in accordance with guidelines of the Committee for Animal Care and Use of the Animal Center of Anhui Medical University (NO. LLSC201800488).

The prostate extracts were prepared from prostate glands obtained from Wistar rats that were purchased from the Beijing Vital River Laboratory Animal Technology Co, Ltd. Pooled extracts from 30 rats were homogenized in 0.01 M phosphate-buffered saline (PBS; pH 7.2) containing protease inhibitors in a Precellys Evolution Super Homogenizer (Bertin Technologies, France). The homogenate was centrifuged at 10,000g for 30 minutes at 4°C, and the supernatants were collected as prostate antigens (PAGs). Protein concentrations were determined by using the BCA Protein Assay Kit (cat #P0012; Beyotime Biotechnology, China). The as-prepared PAGs were frozen and stored at −80°C.

The preparation of a mixture of PAGs and the purification of prostatein or prostate steroid-binding protein (PSBP) were performed as previously described.\(^23\) The purity of the PSBP preparation was >95% as evaluated by Western blot analysis and was LPS-free as tested by Gel clot 0.03 endotoxin units/mL sensitivity.
2.2 | Antibodies and reagents

Commercially available antibodies and reagents used in the tests performed and their respective manufacturer were included: complete Freund’s adjuvant (CFA; Sigma-Aldrich, St Louis, MO), anti-NLRP3 (cat #AG-20B-0014; AdipoGen), anti-ASC (cat #E-AB-30582; Elabscience Biotechnology), anti-Caspase-1 (cat #AB1871; EMD Millipore Corporation), anti-IL-1β (cat #12242; Cell Signaling Technology), glyceraldehyde 3-phosphate dehydrogenase (GAPDH; cat #E-AB-20059; Elabscience Biotechnology), MCC950 (cat #CP456773; Selleck), and alcohol (analytical grade reagent).

2.3 | EAP induction

Equal volumes of PAgS or saline solution were emulsified thoroughly in CFA. Six to eight-week-old male NOD mice were intradermally immunized in different locations: bilateral hind footpads, the lower back, and the base of the tail with PAgS (300 μg/mouse, EAP group) or phosphate buffer solution (control group) emulsified in CFA in a total volume of 150 μL per mouse, as previously described. According to the experimental schedule, the mice were immunized at day 0 and day 28 and at last were killed at day 42.

2.4 | Alcohol feeding protocols

Thirty-two days after the immunization of PAgS or saline solution, two groups of mice (control-alcohol and EAP-alcohol group) were subjected to chronic alcohol feeding. 10 days Lieber-DeCarli alcohol liquid diet (ad libitum oral feeding) plus a single binge 35% alcohol feeding (gavage, 5 g alcohol per kg calculated by body weight). Another two groups of mice were fed with control diet (control-vehicle, EAP-vehicle group). The details of alcohol feeding protocols were previously described.

2.5 | Treatment with MCC950

MCC950 was dissolved in deionized water solution to obtain a concentration of 1 mg/mL. MCC950 was injected intraperitoneally into mice (10 mg/kg) at the beginning of treatment with the alcohol on days 32, 33, and 34, and every 2 days thereafter (EAP-alcohol-MCC950 group). The EAP-alcohol group mice were intraperitoneally injected with vehicle (PBS) at the same time points.

2.6 | Histopathological tests

We analyzed the histopathological appearance of prostate tissue sections by hematoxylin and eosin (H&E) staining and microscopy. In detail, the prostate tissues were immersed in 10% neutral formalin and fixed for at least 24 hours, dehydrated in a graded series of alcohol and xylene, embedded in paraffin wax, and then, cut into sections (5-μm thick). The sections were stained with H&E for histopathological analysis under a light microscope. The histopathological appearance was assessed by a point-counting method for the severity of inflammation though an ordinal scales according to the methods as previously described. The histopathological changes were graded on a four-point scale from 0 to 3 as follows: 0, no inflammation; 1, mild but definite perivascular cuffing with mononuclear cells; 2, moderate perivascular cuffing with mononuclear cells; 3, marked perivascular cuffing, hemorrhage, and numerous mononuclear cells in the parenchyma.

2.7 | Assessment of chronic pelvic pain

Mice in the study were assessed for cutaneous allodynia on day 42 after immunization. As previously described, tests were performed in isolated transparent plastic chambers with a stainless steel wire grid floor. At first, a 30-minute acclimation period was allowed before testing in all mice. Then, tactile allodynia and hyperalgesia were measured by von Frey filaments with forces of 0.04, 0.16, 0.4, 1.0, and 4.0 g in every mouse, respectively. The filaments were applied 10 times in total for 1 to 2 seconds each time with an interval of 2 minutes. Stimulation was detected in the lower abdominal area nearby the prostate and being attention to test different areas within the region to avoid desensitization or “windup” effects. As a result, three types of responses were affirmed as positive responses to filament stimulation including (1) sharp retraction of the abdomen; (2) immediate licking or scratching of the area of filament stimulation; and (3) jumping. The response frequency was calculated as the percentage of positive responses (eg. 2 responses out of 10 applications = 20%). Data are reported as the mean ± SEM.

2.8 | Cytokine quantification

The concentrations of cytokines were detected in mouse plasma and prostate tissue homogenates samples from immunized mice by an enzyme-linked immunosorbent assay (ELISA) according to the user manual (TNF-α: CSB-E04741m, CUSABIO, China; INF-γ: CSB-E04578m, CUSABIO; IL-1β: CSB-E08054m, CUSABIO; IL-17: CSB-E04608m, CUSABIO). The linear ranges of the assays were 15.6-100 pg/mL for TNF-α and INF-γ, 31.25-2000 pg/mL for IL-1β, 47-3000 pg/ml for IL-17. The inter-assay and intra-assay coefficients of variation were less than 8% and 10%, respectively.

2.9 | Western blotting

Mice prostate tissues were collected and frozen in an −80°C refrigerator until homogenization. The total protein was extracted from the prostates and the protein concentrations were determined using a BCA Assay Kit. Then, samples were denatured at 95°C in boiling water. After denaturation, samples were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes with a semi-dry transfer apparatus (Bio-Rad). Nonspecific binding sites were blocked with 5% (w/v) defatted milk. Then, the membranes were probed with antibodies specific for GAPDH, NLRP3, ASC, caspase-1, and IL-1β (at 1:1000). Horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) were applied, and the membranes were developed using an EZ-ECL Kit (Biological Industries, Israel). The chemiluminescence was visualized using a...
ChemScope 5600 (Clinx Science Instruments, China). Arbitrary density of immunopositive bands were quantified using the ImageJ software (National Institutes of Health).

2.10 | Immunohistochemistry tests

Prostate tissues were fixed in 10% formalin for at least 24 hours, embedded in paraffin, and cut into 5-μm sections. Sections were dewaxed with xylene and rehydrated, and then microwave antigen retrieval was performed at 95°C for 15 minutes using 0.01 M citric acid buffer (pH 6.0). Then, the sections were incubated with a 3% hydrogen peroxide solution (SP 9000; Beijing Zhongshan Jinqiao Biotechnology Co, Ltd.) for 10 minutes at room temperature. After three washes in PBS (pH 7.4), sections were blocked with 10% bovine serum albumin and then incubated overnight with primary antibodies ([1:300] NLRP3, [1:50] caspase-1, and [1:300] ASC) overnight at 4°C. After three washes with PBS, sections were incubated with biotinylated goat anti-rabbit IgG (1:200) at 37°C for 30 minutes. Subsequently, horseradish peroxidase-labeled streptavidin was added after three washes with PBS, followed by 30 minutes of incubation at 37°C. After additional washes with PBS, immunoreactivity was detected with a Diaminobenzidine Staining Kit (Beijing Zhongshan Jinqiao Biotechnology Co, Ltd.), and sections were counterstained with H&E.

2.11 | Statistical analysis

Statistical analysis was performed using one-way analysis of variance with Bonferroni post hoc test analysis. Unless stated otherwise, data are shown as mean ± (standard error of the mean) SEM. We used the GraphPad Prism 6.0 Software for Windows (GraphPad) to perform statistical analyses. Differences were considered statistically significant at \( P<.05 \).

3 | RESULTS

3.1 | Induction of EAP model

The EAP mouse model was successfully induced with a mixture of PAgs and CFA as shown in Figure 1. The EAP model group was observed with pathological changes, including multifocal perivascular and stromal mononuclear cell infiltration accompanied by edema and

![Figure 1](https://wileyonlinelibrary.com)
severe tissue disorders. The histopathological scores for the control and EAP groups were 0.33 ± 0.07 vs. 2.13 ± 0.15 (Figure 1B,C).

To test the chronic pelvic pain, tactile allodynia over the lower abdominal area nearby the prostate was tested on day 42 after immunization. Significant increases of response frequency were detected when using a von Frey filament with a force of 0.4, 1.0, and 4.0 g (P < 0.05) on day 42 in EAP group compared with the control group in immunized NOD mice (Figure 1D).

NOD/LtJ mice have a tendency to develop diabetes. Females generally begin to develop diabetes at 12 to 14 weeks and males begin to develop diabetes several weeks later than females. Thus, we tested the fasting blood glucose of the NOD mice on day 42 to exclude the possible effect of diabetes on the experiment. The results were shown in Figure S1.

### 3.2 Alcohol aggravated EAP severity

H&E staining assays showed that the inflammation in immunized mice prostate tissues was more severe in EAP-alcohol group than EAP-vehicle group. The pathological changes in EAP-alcohol group showed that diffuse inflammation in the stromal tissue characterized by severe infiltration of neutrophils and mononuclear cells was observed and EAP-alcohol mice showed fibrosis and hemorrhage in the stromal tissue when compared with EAP-vehicle mice. However, mice in the control-vehicle group and control-alcohol group showed no inflammatory infiltration (Figure 2A). The EAP histopathological scores for the four groups were 0.13 ± 0.03 for control-vehicle, 0.17 ± 0.03 for control-alcohol, 1.97 ± 0.18 for EAP-vehicle, and 2.80 ± 0.12 for EAP-alcohol (Figure 2B).

Then we tested chronic pain development in each of the groups. Compared with the EAP-vehicle group, the response frequency of mice in the EAP-alcohol group was significantly increased with a force of 0.4, 1.0, 4.0 g (P < 0.05) at 42 day after immunization. However, the results of response frequency showed no difference between the control-vehicle and control-alcohol group (Figure 2C).

Then, we detected the expression levels of several inflammatory cytokines in mouse plasma and prostate tissue homogenates from immunized mice using ELISA tests. Plasma from mice in the EAP-alcohol group showed higher expression levels of IFN-γ (6.94 ± 0.73 vs. 4.20 ± 0.42 ng/mL), TNF-α (2.50 ± 0.28 vs. 1.55 ± 0.18 ng/mL), IL-17 (5.09 ± 0.43 vs. 3.67 ± 0.23 ng/mL), and IL-1β (0.97 ± 0.07 vs. 0.69 ± 0.07 ng/mL) compared with EAP-vehicle group (Figure 2D).

Similarly, inflammatory cytokines of 10 mg prostate tissue homogenates in EAP-alcohol group were higher for IFN-γ (2.55 ± 0.30 vs. 1.76 ± 0.08), TNF-α (9.58 ± 0.40 vs. 7.99 ± 0.30), IL-17 (2.42 ± 0.32 vs. 1.71 ± 0.19), and IL-1β (5.14 ± 0.24 vs. 3.54 ± 0.31). The results showed that alcohol treatment was able to aggravate the severity of EAP (Figure 2D).

### 3.3 The NLRP3 inflammasome activated after the treatment of alcohol

To explore the mechanisms underlying the effects of alcohol on EAP, proteins involved in NLRP3 inflammasome signaling pathways were detected by Western blotting and Immunohistochemistry (IHC) assays. Activation of the NLRP3 inflammasome assembly is required to trigger caspase-1 cleavage and subsequent IL-1β release. Caspase-1 and IL-1β are key inflammatory molecules. We determined whether alcohol can induce NLRP3 inflammasome activation. In the EAP-vehicle group, the upregulation of NLRP3, caspase-1, and ASC were observed. And after treatments with alcohol in EAP, the expression of inflammasome proteins, NLRP3, ASC, and caspase-1 were significantly increased detected by Western blotting (Figure 3A,B) and IHC assays (Figure 3C).

### 3.4 MCC950 inhibited NLRP3 inflammasome activation and attenuated alcohol-treated EAP severity

We determined whether treatment with MCC950 affected the activation of NLRP3 inflammasome and inflammation severity in EAP-alcohol mice. First, we examined the expression of proteins NLRP3, ASC, caspase-1, and IL-1β by Western blotting. The results showed that the NLRP3 inflammasome-related proteins NLRP3, ASC, and caspase-1 were decreased in EAP-alcohol-MCC950 group compared with EAP-alcohol group (Figure 4A,B). Then the IHC assays were conducted to evaluate the level of NLRP3-related proteins. The results showed that the levels of these proteins were reduced in the EAP-alcohol mice, demonstrating that the NLRP3 inflammasome inhibitor MCC950 blocked the NLRP3 inflammasome pathway (Figure 4C).

Next, we investigated the effects of MCC950 on the severity of inflammation in mice. The H&E staining showed that the histological appearance of tissues from EAP-alcohol mice was alleviated after the treatment of MCC950, as: multifocal perivascular and stromal mononuclear cell infiltration alleviated (Figure 5A). The EAP histopathological scores for the four groups were 1.93 ± 0.15 for EAP-vehicle, 2.77 ± 0.12 for EAP-alcohol, 0.53 ± 0.03 for EAP-MCC950, and 0.77 ± 0.07 for EAP-alcohol-MCC950 (Figure 5B).

Moreover, the chronic pain development test reported that the response frequency was attenuated in EAP-alcohol-MCC950 group at forces of 0.4, 1.0, and 4.0 g (P < 0.05) compared with EAP-alcohol group (Figure 5C).

Then, ELISA detection showed that the levels of inflammatory cytokines in mice plasma from EAP-alcohol-MCC950 group for IFN-γ (4.90 ± 0.36 vs. 6.98 ± 0.56 ng/mL), TNF-α (0.98 ± 0.02 vs. 2.51 ± 0.36 ng/mL), IL-17 (3.59 ± 0.20 vs. 5.31 ± 0.54 ng/mL), and IL-1β (0.57 ± 0.07 vs. 1.05 ± 0.09 ng/mL) and 10 mg prostate tissue homogenates IFN-γ (1.17 ± 0.23 vs. 2.51 ± 0.29), TNF-α (4.68 ± 0.44 vs. 9.72 ± 0.59), IL-17 (0.91 ± 0.24 vs. 2.57 ± 0.24), and IL-1β (2.17 ± 0.33 vs. 3.55 ± 0.44) were significantly reduced after MCC950 treatment compared with EAP-alcohol group (Figure 5D). Thus, we demonstrated that treatment with MCC950 could reduce the inflammation severity of EAP-alcohol group mice.

### 4 DISCUSSION

This is the first study to investigate the mechanisms underlying the effects of alcohol on CP/CPPS in an EAP mouse model. In this study,
Alcohol treatment could aggravate the severity of EAP. 1: control-vehicle group; 2: control-alcohol; 3: EAP-vehicle; 4: EAP-alcohol group. A. Representative H&E staining assays performed in prostate tissue sections from immunized NOD in the four groups. Original magnification: ×400. The scale bars indicate 20 μm. B. Inflammation scores for four groups were analyzed. The EAP histopathological score for four groups were 0.13 ± 0.03 for control-vehicle, 0.17 ± 0.03 for control-alcohol, 1.97 ± 0.18 for EAP-vehicle, and 2.80 ± 0.12 for EAP-alcohol. C, Chronic pelvic pain development in NOD mice between the four groups. The response frequency of the EAP-alcohol group increased compared to EAP-vehicle group, while the response frequency showed no difference between control-alcohol and control-vehicle. D, Relative protein expression of cytokines IFN-γ, TNF-α, IL-17, IL-1β in plasma and 10 mg prostate tissue from immunized mice in the four groups. EAP, experimental autoimmune prostatitis; H&E, hematoxylin and eosin; IFN, interferon; IL, interleukin; NOD, non-obese diabetic; TNF, tumor necrosis factor. *P < .05 [Color figure can be viewed at wileyonlinelibrary.com]
we demonstrated that alcohol could aggravate the severity of prostatic inflammation in an EAP model by activating the NLRP3 inflammasome. With the treatment of NLRP3 inhibitor MCC950, the activity of NLRP3 inflammasome-related proteins could be blocked and the prostatic inflammation could be alleviated.

CP/CPPS is a very prevalent urological problem worldwide. During clinical practice, we observed that patients with CP/CPPS have worse symptoms after drinking alcohol. Additionally, epidemiological studies have reported an association between alcohol consumption and CP/CPPS.\(^3\)\(^3\)\(^0\) Our study group conducted a large multi-center epidemiological investigation, figuring out the morbidity of prostatitis-like symptoms in China and finding out that alcohol drinking was associated with an increased risk of prostatitis.\(^3\)\(^0\)\(^1\)\(^7\)\(^0\)\(^3\)\(^0\)\(^3\)\(^6\) Zhang et al\(^3\)\(^0\)\(^1\)\(^7\)\(^0\)\(^3\)\(^0\)\(^3\)\(^6\) also reported a correlation between alcohol consumption and prostatitis. However, the mechanism underlying the effects of alcohol on prostatitis has not yet been revealed.

Many clinical and experimental studies have indicated that alcohol uptake has dose-dependent modulatory effects on the immune system by influencing the two branches of the immune response.\(^3\)\(^1\) The integrated human immune response has been generally divided into two parts: innate immunity and adaptive immunity. The innate immune system is in charge of the initial task of identifying and eliminating dangerous pathogens. An important property of the mediators of inflammation in the innate immune system is its ability to distinguish microorganisms from itself through recognition of conserved microbial structures called PAMPs, such as LPS, flagellin, peptidoglycan, and microbial nucleic acids.\(^3\)\(^2\) Inflammasomes are important parts of the innate immune response that activates a cascade of inflammation. When the inflammasome is activated, it can bind to the former pro-caspase-1 and trigger auto-catalytic cleavage and re-assembling into the active form, cleaved caspase-1. Subsequently, it could induce cleavage of pro-IL-1β into IL-1β and promote inflammation. IL-1β has been proven to be a critical marker of CP/CPPS in many previous clinical studies\(^3\)\(^3\)\(^-\)\(^3\)\(^5\) and is also crucial in our mouse model of EAP.\(^3\)\(^6\) In the present study, the IL-1β level was significantly elevated in EAP

**FIGURE 3** The NLRP3 inflammasome pathway was activated in EAP and after the treatment of alcohol. A: control-vehicle group; 2: control-alcohol group; 3: EAP-vehicle group; 4: EAP-alcohol group. A, Alcohol induced the activation of NLRP3, ASC, caspase-1, and IL-1β showed by Western blotting. B, Relative density analysis of each band compared with the corresponding GAPDH band. C, The activation of NLRP3, ASC, caspase-1, and IL-1β showed by Immunohistochemistry Assays. The scale bars indicate 20 μm. EAP, experimental autoimmune prostatitis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin. *P < .05 [Color figure can be viewed at wileyonlinelibrary.com]
mice after alcohol treatment. The same tendency of caspase-1 expression provided strong evidence that inflammasome plays a significant role in the EAP-alcohol mice. Alcohol has been demonstrated to be involved in the pathogenesis of inflammation. Alcohol uptake increases the production of proinflammatory cytokines including IFN-γ, TNF-α, and IL-17, which contributes to the pathogenesis of inflammation. Lopez reported that alcohol treatment increased the levels of IFN-γ and IL-17 in the small intestine. Qin et al found that the expressions of TNF-α and IL-1β induced by LPS based on the pretreatment with alcohol. In our study, we also found that alcohol treatment promoted the secretion of inflammatory cytokines, including IFN-γ, TNF-α, IL-17, and IL-1β in mouse plasma and prostate tissue homogenates. Previous studies reported that drinking alcohol could aggravate allergic asthma and allergic dermatitis, in which autoimmune factors play an important role. Similar to these two kinds of disease, the EAP model also represents a kind of autoimmune inflammation. In our study, alcohol also aggravated the EAP severity though the immune system related inflammasome pathway.

MCC950 is a powerful, highly specific inhibitor of both canonical and non-canonical activation of NLRP3 inflammasome that has been demonstrated in a series of NLRP3-related inflammatory diseases. Rebecca et al demonstrated that MCC950 could inhibit NLRP3 inflammasome, reduce IL-1β secretion and attenuate the severity of experimental autoimmune encephalomyelitis. In addition, Perera et al reported the ability of MCC950 in the therapy of murine ulcerative colitis. In our study, we also found that MCC950 could alleviate the severity of inflammation in alcohol-treated EAP mice. Thus, MCC950 may be a promising therapeutic for NLRP3-related diseases, including CP/CPPS, and will be an important tool for further study of the NLRP3 inflammasome in various diseases.

**FIGURE 4** The treatment of NLRP3 inhibitor MCC950 inhibited NLRP3 inflammasome activation. 1, EAP-vehicle group; 2, EAP-alcohol group; 3, EAP-MCC950 group; 4, EAP-alcohol-MCC950 group. A, The inhibition of NLRP3, ASC, caspase-1, and IL-1β by MCC950 showed by Western blotting. B, Relative density analysis of each band NLRP3, ASC, caspase-1, and IL-1β compared with corresponding GAPDH band after the treatment of MCC950. C, The inhibition of NLRP3, ASC, caspase-1, and IL-1β by MCC950 showed by Immunohistochemistry Assays. The scale bars indicate 20 μm. EAP, experimental autoimmune prostatitis; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin. *P < .05 [Color figure can be viewed at wileyonlinelibrary.com]
FIGURE 5  The treatment of NLRP3 inhibitor MCC950 attenuates EAP severity. 1, EAP-vehicle group; 2, EAP-alcohol group; 3, EAP-MCC950 group; 4, EAP-alcohol-MCC950 group. A, Representative H&E staining assays performed in prostate tissue sections from the mice in the four groups after the treatment of MCC950. Original magnification: ×400. The scale bars indicate 20 μm. B, Inflammation score for each group was analyzed. C, Relative protein expression of cytokines IFN-γ, TNF-α, IL-17, IL-1β in plasma and 10 mg prostate tissue in the four groups. EAP, experimental autoimmune prostatitis; H&E, hematoxylin and eosin; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor. *P < .05 [Color figure can be viewed at wileyonlinelibrary.com]
5 | CONCLUSION

In conclusion, our study demonstrated that alcohol could aggravate the severity of prostatic inflammation in an EAP model through activating the NLRP3 inflammasome. In addition, the role of MCC950 in inhibiting NLRP3 inflammasome activation and IL-1β secretion and in alleviating the alcohol-treated EAP severity may demonstrate that it is a promising novel therapeutic agent for human CP/CPPS after drinking alcohol. Nevertheless, more studies will be needed to shed light on the mechanism underlying the effects of alcohol on CP/CPPS. And the pharmacological prospect of MCC950 in the therapy for CP/CPPS patients drinking alcohol deserves any attention in future studies.

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CONFLICT OF INTERESTS

All authors declare there is no conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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