Immunology Letters 187 (2017) 61–67

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

A novel mouse model of chronic prostatitis/chronic pelvic pain syndrome induced by immunization of special peptide fragment with aluminum hydroxide adjuvant

Farhan Ullah Khan[a], Awais Ullah Ihsan[a], Waqas Nawaz[a], Muhammad Zahid Khan[b], Mengqi Yang[a], Gang Wang[a], Xiaoqian Liao[a], Lei Han[c], Xiaohui Zhou[a,d,e,*]

[a] Department of Clinical Pharmacy, School of Basic Medicine and Clinical Pharmacy, China Pharmaceutical University, Nanjing, Jiangsu Province, 211198, China
[b] Department of Pharmacology, China Pharmaceutical University, Nanjing, Jiangsu Province, 211198, China
[c] Department of Pharmacy, Jiangsu Jiankang Vocational College, Nanjing, Jiangsu Province, 211198, China
[d] Department of Surgery, Nanjing Shuiximen Hospital, Nanjing, Jiangsu Province, 211198, China
[e] Zhongda Hospital, Affiliated with Southeast University, Nanjing, Jiangsu Province, 210017, China

ARTICLE INFO

Keywords:
Prostatitis
CP/CPPS
Inflammation
Aluminum
Model
Cytokines

ABSTRACT

Objectives: CP/CPPS is a commonly observed distress in male patients. Because of its little-known etiology, no effective therapy has been developed which has promising outcomes. Therefore, there is a need to develop a valid model which can mimic the etiology of CP/CPPS.
Materials and methods: Fifty male C57BL/6 mice were randomly and averagely divided into 5 groups of 10 mice each. The control group was injected with 0.9% NaCl solution. Aluminum hydroxide and T_2 groups were injected with aluminum hydroxide adjuvant and T_2 peptide. T_2 plus complete Freund adjuvant (CFA) with aluminum hydroxide group was injected with a mixture of T_2, CFA and aluminum hydroxide adjuvant. At the same time, CFA group was injected with complete Freund adjuvant. Hematoxylin-eosin stain and immunohistochemistry were used to investigate inflammatory lesion and expression of IL-β1. Furthermore, TNF-α and CRP protein levels were evaluated by using commercially available ELISA kits. The ANOVA test was used to compare the statistical differences among groups.
Results: Prostates from a mixture of T_2 plus CFA with aluminum hydroxide immunized mice showed elevated lesions and high level of inflammatory cells infiltration compared to the other groups. In addition, the levels of TNF-α, IL-β1, and CRP were also higher in the T_2 plus CFA with aluminum hydroxide group as compared to the other groups.
Conclusion: Our results showed that T_2 with CFA plus aluminum hydroxide adjuvant injection could successfully induce CP/CPPS in mice. This autoimmune novel model provides a useful, economic, safer, and easy tool for exploring the etiology and pathophysiology of CP/CPPS which will improve the therapeutic outcomes.

1. Introduction

Nonbacterial prostatitis, or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) or category III prostatitis, is a commonly observed distress in male patients characterized by genital or pelvic discomfort, with voiding symptoms and sexual dysfunction [1]. It is a poorly understood and the most common genitourinary problem in adult males aged < 50 years [2]. Chronic pelvic pain syndrome accounts for more than 90–95% of all cases of prostatitis [3], affecting 10–14% of men of all ethnic origins. CP/CPPS has a significant impact on the quality of life and health status, as CPPS patients demonstrated poor quality of life and impairment in the daily routine functioning similar to the patients suffering from angina, myocardial infarction, or active Crohn's disease [4].

At present, little is known about the etiology and pathogenesis of CP/CPPS, so no effective treatment of CP/CPPS has been established. Currently, antibiotics, alpha-adrenergic blockers, anti-inflammatories, and neuromodulators are the most commonly prescribed drugs for CP/CPPS treatment [5] but these have limited clinical effects [6,7]. Thus there is a need to develop new therapies to treat CP/CPPS. Because of the little-known pathophysiology, there is controversial with groups postulating that CP/CPPS may be due to infections (bacterial, viral,

* Corresponding author at: Clinical Pharmacy, China Pharmaceutical University, Department of Clinical Pharmacy, School of Basic Medicine and Clinical Pharmacy, 639# Longmian Avenue, Nanjing, Jiangsu Province, 211198, China.
E-mail address: zhxhcpu@163.com (X. Zhou).

http://dx.doi.org/10.1016/j.imlet.2017.05.008
Received 7 April 2017; Received in revised form 28 April 2017; Accepted 10 May 2017
Available online 16 May 2017
0165-2478/ © 2017 European Federation of Immunological Societies. Published by Elsevier B.V. All rights reserved.

bacteria, and viruses such as chlamydia,CMV, HSV) [8,9], uric acid level, inflammation, autoimmunity or neuromuscular mechanisms. However, with the progress made in the etiology of CP/CPPS, the autoimmune basis for CP/CPPS was one of the prominent theory and animal models also supported that view [10–17]. One impediment to the study of new therapeutic approaches to CP/CPPS was the lack of appropriate animal models to mimic the clinical condition [18]. To date, various methods have been used to develop animal models with unique features [12,19].

However, there is still a lack of an explicit animal model for elucidating the aetio-pathogenic mechanisms underlying CP/CPPS. Therefore, it would be a great benefit to develop a proper animal model for CP/CPPS investigation. Previously, complete Freund adjuvant (CFA) together with prostate extract was used to induce prostatitis [13]. Our laboratory has previously established CP/CPPS rat model using prostate extract in Freund adjuvant [14]. This model showed almost all the characteristics of human chronic autoimmune prostatitis: increased expression of TNF-α, interleukin (IL)-2, and IL-1β, the infiltration and histological lesions typically observed in prostate tissue. In the present work, we sought to isolate and use a special peptide sequence called T_2 derived from a TRPM8 protein which is encoded by a prostate specific gene [20]. T_2 is an antigenic epitope of TRPM8 which is a member of the transient receptor potential (TRP) superfamily. Previously in our lab, we extracted a unique sequence of peptides from a TRPM8 protein called SIPT (specific isolated peptides). These are six peptide sequence and all are immunogenic but the most pathogenic is T_2 making it a perfect autoantigen for inducing CP/CPPS (unpublished data). Herein, we described a novel model of experimental autoimmune prostatitis induced by a new peptide T_2. We modeled CP/CPPS by giving a subcutaneous injection of T_2 combined with complete Freund adjuvant plus aluminum hydroxide. Thereafter, we determined the extent of inflammatory changes in the prostate by histopathology, ELISA, and immunohistochemistry. Furthermore, we also observed inflammatory cells infiltration into the prostate and measured the level of C-reactive protein.

2. Materials and methods

2.1. Animals

A total of 50 male C57BL/6 mice (18–22 g body weight) were purchased from the Comparative Medicine Centre of Yangzhou University. Animals were housed at constant temperature and humidity in an animal room under a 12/12 h light-dark cycle. All animal experiments were approved by and conducted in accordance with guidelines of the Committee for Animal Care and Use of the China Pharmaceutical University.

2.2. Reagents

Based on the sequence of the TRPM8 protein, we identified a unique peptide called T_2 which was extracted from the TRPM8 present in the prostate gland. The amino acid sequence of this peptide is CSEEM RHRFR QLDTK LNDLKG and it was considerably synthesized in the laboratory of Bai yan, Wuhan technology, Ltd (Wuhan, China). It was prepared to 1 mg/mL of reserves for use with normal saline. Aluminum hydroxide adjuvant was purchased from Pierce company, USA and was used in accordance with standard protocols while complete Freund's adjuvant (CFA) was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). T_2 was mixed with equal volume aluminum hydroxide adjuvant and CFA. The final concentration of T_2 for mice was 45 mg/mL.

2.3. Immunization

Fifty male C57BL/6 mice were randomly and averagely divided into 5 groups of 10 mice each. All mice were immunized by giving a mixture of 0.2 ml subcutaneously with 5 injections in the back. Every time, each group was immunized by giving injection on the 1st, 14th, and 28th days. The control group was immunized with normal saline. Aluminum hydroxide Al(OH)3 group was immunized with Al(OH)3. T_2 group was immunized with T_2. T_2 plus CFA plus Al(OH)3 group was immunized with a mixture of T_2, CFA, and Al(OH)3. While complete Freund adjuvant (CFA) group was immunized with CFA. All mice were sacrificed by anesthesia on the 7th day after the final injection.

2.4. Histopathology

The anterior and ventral lobes of prostates [15] were fixed in buffered 10% formaldehyde for 48 h. Dehydration was done in ethanol, then cleared in xylene and embedded in paraffin. Thereafter, the paraffin-embedded tissues were sliced into 4-μm thicknesses. Four-micron sections were then taken and stained with hematoxylin and eosin. Tissues were considered positive for prostatitis if one area of inflammatory cell infiltration was observed in a microscopic section. The severity of inflammation was assessed according to the method applied by Bernoulli et al. [21]. In short, inflammation was analyzed on a 4-point grading scale because of its relation to the epithelium, which was defined by 4 grades (0–3): grade 0 meant no contact between inflammatory cells and epithelium; grade 1 some contact; grade 2 periglandular infiltrates adjacent to partially destroyed epithelium, and grade 3 the presence of more than 25% acini [22].

2.5. Immunohistochemistry (IL-1β staining)

The lateral lobe sections of prostates collected after final injection on the 7th day were fixed in 4% paraformaldehyde solution for 48 h, dehydrated in ethanol, embedded in paraffin. They were then cut into 4 μm slices and mounted on slides. The slides were deparaffinized in xylene, rinsed in graded alcohols and hydrated in water. They were then incubated with 3% H_2O_2 for 30 min at room temperature and then washed and blocked with five percent bovine serum antigen for 30 min. Next, the sections were incubated with rabbit anti-mouse IL-1β (Wuhan Boster Biological Engineering, Co, BA0131), (diluted 1:100 in PBS) at 4 °C overnight. On the next day, the sections were washed with 0.01 M PBS and incubated with goat anti-rabbit IgG-HRP as a secondary antibody (Wuhan Boster Biological Engineering, SA1022) at 30 °C for 20 min and then rinsed with 0.01 M PBS. After that, Strept Avidin-Biotin Complex (Wuhan Boster Biological Engineering, SA1022) was added. Color development was done with diaminobenzidine (Wuhan Boster Biological Engineering, AR1022). The sections were then slightly counterstained with Mayer hematoxylin, dehydrated, and mounted. At the end, the slides were evaluated by light microscope.

2.6. Enzyme-linked immunosorbent assay

In order to quantify the expression of TNF-α and C-reactive protein (CRP) in the plasma, we used ELISA. The blood obtained from the carotid artery was put into the heparin for 30 mins. It was then centrifuged at 2500 rpm for 20 min. After that, the supernatant was collected and stored at −80 °C. The contents of TNF-α and CRP were quantified by mouse TNF-α ELISA kit (DRKEWE, China) and mouse CRP ELISA kit (MULTI SCIENCES Biotech, Co., Ltd, China) following the manufacturer's instructions.

2.7. Statistical analysis

Statistical analysis was performed using ANOVA to evaluate statistical differences between data obtained in the normal control and treated groups. Quantitative data were expressed as means ± SD. The P values ($P < 0.05$, $P < 0.01$, $P < 0.001$) were considered significant in all analyses.

Download English Version:

https://daneshyari.com/en/article/5666599

Download Persian Version:

https://daneshyari.com/article/5666599

Daneshyari.com