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Original article

Establishment of a rat model of chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS) induced by immunization with a novel peptide T2

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ABSTRACT

Background: The exact etiological mechanism of Chronic Prostatitis/chronic pelvic pain syndrome (CP/CPPS) is still unclear however autoimmunity is the most valid theory. We developed a rat model of Chronic Prostatitis/chronic pelvic pain syndrome by using a novel peptide (T2) isolated from TRPM8. This model might be beneficial in elucidating mechanisms involved in the pathogenesis of Chronic Prostatitis/Chronic Pelvic Pain Syndrome (CP/CPPS).
Methods: 40 male Sprague-Dawley rats with an average weight of 180–220 g were equally distributed into five groups. The normal control group was injected with normal saline (.9% NACL), the CFA group with CFA, AL(OH)3 group was given AL(OH)3 injection, T2 group using a novel peptide T2 and T2 + AL(OH)3 + CFA group was injected with T2 + AL(OH)3 + CFA. Dosing to all rat groups were injected subcutaneously. Hematoxylin and eosin staining and Immunohistochemistry were used to investigate inflammatory cell infiltration and IL-1β in the prostate tissue respectively. ELISA technique was used to measure the serum level of CRP and TNF-α. *T*-test was used to analyze the results.
Results: Maximum infiltration of inflammatory cells and the highest level of IL-1β in the prostate tissue was observed in T2 + AL(OH)3 + CFA group as revealed by histopathology and Immunohistochemistry, respectively. Furthermore, T2 + AL(OH)3 + CFA group attained the peak value of serum TNF-α and CRP as determined by ELISA technique.
Conclusion: Our results demonstrated that T2 in combination with AL(OH)3 and CFA induced severe Prostatitis in rats. We believe that our present model will be highly beneficial for investigation of the pathophysiology of Chronic Prostatitis/Chronic Pelvic Pain Syndrome.

1. Introduction

The most recent National Institutes of Health (NIH) classification of Prostatitis adopted in 1995 includes several clinical entities, ranging from acute or chronic bacterial infections, and chronic pelvic pain syndrome (CPPS) and Asymptomatic inflammation of the prostate [1]. Chronic Prostatitis/Chronic pelvic pain syndrome (CP/CPPS) is most common, bothersome and enigmatic disease with an estimated prevalence rate of up to 15% of the male population and most diagnosis occurs between the age of 35–45 years [2,3]. Its current definition by the National Institutes of Health (NIH) is a genitourinary pain with or without voiding symptoms in the absence of uropathogenic bacteria or other identifiable causes such as malignancy [1]. Pain is the most prevalent symptom of CP/CPPS which lasts for at least 3 months. Furthermore, Patients of CP/CPPS experience other symptoms such as ejaculatory pain, sexual dysfunction, depression and psychosocial maladjustment [4]. Currently, the etiology and pathogenesis of CP/CPPS is not clear, therefore no effective therapeutic strategies had been established. Novel therapies including alpha-blocker, anti-inflammatory Phytotherapy, physiotherapy, and neuroleptics offer limited therapeutic benefits [5]. However currently effective

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therapy for CP/CPPS is not available [6] and treatment of this disorder presents numerous challenges for urologists. It has been hypothesized that infection, as well as genetic, anatomical, physiological, neurological and immunological factors, may be involved (alone or combined) in the pathogenesis of CP/CPPS [4]. However, an autoimmune basis for pathogenesis of CP/CPPS is one prominent theory [7]. One impediment to the study of new therapeutic approaches to CP/CPPS that has hampered research in elucidating CP/CPPS was the lack of appropriate animal models to mimic the clinical condition. Reliable experimental animal models of human diseases are critically important for the discovery of molecular pathways, genetic influences and successful management strategies for humans. Animals experimentally affected by such diseases provide a unique opportunity to uncover disease associated pathways, which are complicated or even impossible to define in man. Several animal models of experimental autoimmune Prostatitis have been developed and characterized each with its own unique characteristics and potentials [8]. For example, Chuang et al. [9] reported that intraprostatic capsaicin injection could cause CP/CPPS, but it only induced short-lasting inflammation of less than 7 days. Kim et al. [10] developed chronic prostatitis model using 17 beta-estradiol and dihydrotestosterone in Wistar male rats. Rivero et al. [11] demonstrated that experimental autoimmune models of Prostatitis could be made by using a Male accessory gland (MAG) homogenate, Prostatic acid Phosphatase, Prostatein or steroid binding protein (PSBP) together with complete Freund's adjuvant (CFA). Although a standard model to reflect CP/CPPS has not been established however prostate extract accompanied with adjuvant injection was considered as a valid model to mimic the disease. Previously, our laboratory has established CP/CPPS rat model by using Aluminum hydroxide adjuvant combined with a prostate extract that induced a marked inflammatory cells infiltrating the prostate with increased expression of TNF-alpha and serum IgG [12]. In the present study, we developed another valid CP/CPPS rat model by injection of the novel peptide isolated from TRPM8 called T2 peptide in conjunction with immunoadjuvants.

2. Methods and materials

2.1. Novel peptide and other reagents

The TRPM8 protein comprised of 1104 amino acids in humans and rodents (Tsavaler et al. [23]; McKemy et al., 2002; Peier et al., 2002). An immunogenic peptide of TRPM8 was identified which was named as T2 (1074–1094 amino acid sequence of TRPM8) and used in combination with .9% NACL. It was further synthesized and purified by Wuhan Buyers biotechnology Co Ltd in China. The final concentration of T_2 for the rat was 125 mg/ml. Aluminum hydroxide adjuvant was purchased from Pierce Company while Complete Freund's adjuvant (CFA) was purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and was used according to manufacturer's instructions and standard protocols.

2.2. Rats and immunization

40 SD rats with an average weight of 180–220 g were purchased from Shanghai Jie Sijie experimental animal Co. Ltd. The rats were free to access food and water under controlled temperature (22 °) and humidity (55%). The protocols for this study was approved by the animal ethics committee of China Pharmaceutical University and confirmed with the guidelines for the care and use of laboratory animals published by US national institute of health (NIH, 1996). Rats were randomized into 5 groups (n = 8) including Control group, CFA group, AL(OH)3 group, T2 group and T_2 + CFA + AL(OH)3. Control group was injected with normal saline. CFA Group with CFA, AL(OH)3 group with AL (OH) 3, T2 group with T2 peptide and T2 + CFA + AL(OH) group with T2 + CFA + AL(OH)3. Dosing (1 ml) to all groups were administered subcutaneously on 1st, 14th and 28th day. All rats were sacrificed on day seven after immunization.

2.3. Histopathology

The prostate tissues were fixed in neutral buffered 10% formaldehyde for 48 h. They were dehydrated in ethanol, and then infiltrated with embedding agent, paraffin. Tissues were then sliced into extremely thin tissue sections (5 μm) and stained with hematoxylin and eosin. Grading scales were used to critically access the extent of inflammation in prostate tissues. The grading scales for histopathological assessment included: grade 0 represented no contact of inflammatory cells with prostate epithelium; grade 1 indicated slight contact with epithelium; grade 2 indicated significant peri glandular infiltrates adjacent to destroy epithelium while in grade 3, more than 25% inflammatory acini were present.

2.4. Immunohistochemistry

Immunohistochemistry was performed to investigate interleukin IL-1β level in the prostate tissues. Shortly, 5-μm formalin-fixed and paraffin-embedded prostate tissue slides were rehydrated in ethanol and deparaffinized with xylene. Slides were treated with Citric acid buffer for antigen retrieval at 95 °C for 30 min, and then cooled to room temperature. They were then incubated with 3% H_2O_2 for blocking peroxidase activity and blocked with 5% bovine serum antigen for an hour. Later on, the slides were treated with 1:100 dilutions of rabbit anti-mouse IL-1β (Wuhan Boster Biological Engineering, Co, BA0131) and goat anti-rabbit IgG-HRP which is a secondary antibody (Wuhan Boster Biological Engineering, SA1022). Freshly prepared DAB substrate was added to reveal the color of antibody staining (Wuhan Boster Biological Engineering, AR1022). It was then counterstained by immersing slides in Mayer hematoxylin, dehydrated, and cover slip using mounting solution. Finally, results were analyzed under a microscope.

2.5. Enzyme-linked immunosorbent assay

The quantitative measurement of Serum TNF-α and C-reactive protein (CRP) was performed using ELISA in accordance with manufacturer's recommendations. Shortly, the blood obtained from the carotid artery was drawn into the heparin for 30 min and centrifuged at 2500 RPM for 20 min. Afterward, the supernatant was collected and stored at −80 °C. The contents of TNF-α and CRP were quantified by TNF-α ELISA kit (DRKEWE, China) and CRP ELISA kit (MULTI SCIENCES Biotech, Co., Ltd, China).

2.6. Statistical analysis

The statistical differences between control and experimental groups were analyzed by employing *t* test. Data was expressed as the mean ± standard deviation. Statistically significant p values in all groups were $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ as compared to control group.

3. Results

3.1. Histopathology

Histopathological signs of inflammation, severe inflammatory cell infiltration along with congestion in the prostate tissues were observed in the T2 + AL(OH)3 + CFA group as compared to other