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Review

Experimental rodent models of chronic prostatitis and evaluation criteria

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ABSTRACT

Chronic prostatitis (CP) is a common disease in urology and can be develop in all age groups. It is more commonly seen in men over the age of 50. It's cure rate is low, the recurrence rate is high, the symptoms are complicated, the duration of disease is prolonged, the lingering is difficult to heal, the pain site is extensive and the associated symptoms are more, which bring great physical pain and mental burden to the patient. At present, the etiology, pathology and pathophysiology of prostatitis are not clear yet, and it is still a difficult problem in medical research. The establishment of an effective animal model for experimental research has become an important way to explore its pathogenesis. There are currently several popular modeling methods that vary in degree of operation, success rate, and time length. It would become a trend to study chronic prostatitis through different modeling methods in the future. The successful preparation of animal models can provide the treatment of CP with the corresponding theoretical basis. This article reviews the recent advances in research on rodent models and analyzes the advantages, limitations, and evaluation criteria of various models for reference.

1. Introduction

Chronic abacterial prostatitis (CAP) or chronic pelvic pain syndrome (CPPS) is the most common type of clinical prostatitis, accounting for more than 90% of cases, and is a frequently-occurring disease in urology. It refers to the inflammatory state of the prostate. The incidence rate is about 3–16% [1–4]. According to statistics, there are more than 8 million CP outpatients in the world every year, and there are 2 million new prostatitis patients every year in the United States. The average age of prostatitis patients is decreasing year by year [5,6]. In North America, Europe and Asia half of whom will have repeat episodes emphasizing that prostatitis is an important worldwide health problem. Moreover, prostatitis accounts for more clinic visits than either benign prostatic hyperplasia or prostate cancer.

Prostatitis is classified into 4 categories by the National Institutes of Health (NIH), including acute and chronic bacterial prostatitis (types I and II), chronic prostatitis (type III) and asymptomatic prostatitis (Type IV). Among them, type III chronic non-bacterial prostatitis (CNP) or chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is the most common, accounting for about 90% [7–9]. According to the presence or absence of leukocyte infiltration in prostate samples, CP/CPPS is further divided into IIIA and IIIB [10].

The etiology, pathogenesis, and pathophysiology of CNP are currently diverse. Some studies have suggested that the incidence might be related to unidentified microbial infections, autoimmune abnormalities, oxidative stress, endocrine disorders, neurological disorders, and social psychology [11,12]. Histologically, prostatitis is characterized by poly- and mononuclear cell infiltrates (neutrophils, lymphocytes, macrophages and plasma cells) in the stromal connective tissue around the acini or ducts. Factors related to the clinical diagnostic criteria have not been clarified yet and remains a fortress that has not been clinically overcome so far. The typical clinical features are frequent urination,

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urgency, dysuria, urinary urination, voiding of urine or after stool, and other urination abnormalities and the pain sitewide (lumbar sacrum, lower abdominal pain, as well as perineum, testis, medial thigh, *etc*.) can affect normal sexual function in severe cases [13]. Chronic abacterial prostatitis has a greater impact on the QOL of patients. Human and rodent prostates are anatomically quite different. In order to better understand CAP, a good animal model is essential. Currently, many human noncancerous diseases have appropriate animal models that serve as sources for obtaining research data and testing therapeutic strategies. The evaluation criteria of animal models of CP will have different detection methods and evaluation criteria because of different modeling methods. Therefore, this article has been combined with some classical modeling methods to make a systematic review of the modeling and evaluation criteria of CP for reference.

2. Animal models

The first step in a good animal model is the successful study of the pathogenesis of prostatitis. At present, there is no universally accepted standard method for the immunity of prostatitis for production of animal models. Many researchers at home and abroad use rodents as experimental animals, including Wistar rats, SD rats, Lewis rats, C57BL/6 mice, and NOD mice. There are different modeling methods for different animal species.

2.1. Experimental autoimmune prostatitis (EAP) models

2.1.1. EAP rat models

At present, there is no universally accepted standard method for the production of animal models of EAP. Many researchers at home and abroad have used rats as experimental animals. This model was proposed by Pacheco-Rupil et al. [14] purified protein from the gonads of Wistar rats is used and injected complete Freund's adjuvant (CFA) into the subcutaneous tissue of the same type of rats to induce autoimmunity. As a result, prostatitis was found in which 3 out of 8 rats (38%) exhibited a proinflammatory reaction on the 21st day after receiving a single MAG inoculation, and the same kind of rat was repeatedly tested, and 9 out of 20 (45%) showed prostate symptoms after 30 days. This classic modeling method has been used until now. However, there are many limitations to the success of this method. At present, there are many reports in the literature that the severity of inflammation is not only related to the concentration of protein purification solution but also related to the time, place and amount of injection. The general injection time is 0, 15 and 30 days. A total of 5 subcutaneous injections were made in the sole, inguinal and cervix of rats. The concentration of the protein purification solution was too high, which may cause the death of rats; if the concentration is too low, it is insufficient for autoimmunity. Some people used different concentrations of proprotein purification solution to evaluate the dose-effect relationship and found that the optimal injection volume was 40–60 mg/mL. Donadio et al. [15] found that prostatic acid phosphatase (PAP) may also be a major antigen that induces autoimmune prostatitis. They immunized rats with recombinant rat or human PAP for 4 weeks and found humoral immunity in the rat model, and there were no cytotoxic T cells response (CTL) and tissue changes were observed. However, CTL responses and tissue-specific prostatitis were able to be produced after intravenous injection of recombinant vaccinia expressing human PAP in Copenhagen rats, but no specific antibodies to PAP observed. It suggested that whether PAP induces cellular or humoral immune responses may be related to the type of rat.

In addition, in Wistar rats, spleen mononuclear cells from a rat model of syngeneic EAP can also be used to induce an inflammatory reaction in the prostate [11]. Chinese scholar X. Zhou et al. [16], also successfully established the CP/CPPS model of Wistar rats by using prostate homogenate protein and CFA for the first time in 2005. Compared with other modeling methods, X. Zhou et al. [16,17] used prostate homogenate protein to establish the CP/CPPS model of animal prostate pathological changes and changes in related indicators are more common in CP/CPPS patients. In the EAP model, the main antigenic substance that causes autoimmunity in rat prostatitis is the prostate steroid binding protein (PSBP) [18], which can cause both cellular and humoral immunity. The study found that mononuclear cells began to infiltrate 7 days after the first injection. After that, the number increased rapidly and reached its maximum value on the 28th day. Inflammation occurred when the infiltrating mononuclear cells were mainly concentrated in the interstitial space of the prostate, accompanied by hemorrhage and some tissue fibrosis, and mast cells appeared at the same time. The infiltrating cells are mainly CD4 + T cells and CD8 + T cells and a few macrophages [14].

2.1.2. EAP mouse models

The first mouse model for establishing EAP was proposed by Keetch et al. [19]. They inoculated the homologous mouse's ventral prostatic lobe extract with CFA into C57BL/6 mice with a maximum dose of 0.75 mg protein extract supplemented with pertussis toxin, it was found out that 100% of C57BL/6 mice developed prostatitis with a wide range of inflammation, concentrated in the dorsal part of the prostate, and the degree of inflammation was related to the dose size. The results of SJL and A/J mice of different species were not satisfactory. It was found that SJL and A/J mice can develop some degree of inflammation. After 30 days of immunization, mononuclear cell infiltration and inflammation were found. The site was concentrated in the interstitial and near blood vessels. Later, Jackson et al. [20] also found that the C57BL/6 mouse species had a good analog to the characteristics of CP by injecting proproteinogens with adjuvants, and that the proinflammatory reaction of the prostate was significant at the 4th and 8th week observations, and there was an increasing trend that the inflammation was persistent. This study shows that compared with other species of mice, using C57BL/6 mice to establish EAP models have a higher success rate.

X. Zhou et al. [21–24] developed a model of CP/CPPS by using T2 peptide, which was extracted from the TRPM8 present in the prostate gland. They injected T2 + CFA + $AL(OH)_3$ group with T2 + CFA + $AL(OH)_3$. Dosing (1 mL) to an experimental group was administered subcutaneously on 1st, 14th and 28th day. The previous successful models of CP/CPPS were narrated by enhanced accumulation of T cells in prostate and recognition of prostate antigens by antibodies [25]. In this model, enhanced T cells, macrophage infiltration into the prostatic stroma and glandular epithelium in experimental rats was observed, indicating that CP/ CPPS model was established successfully. Moreover, higher levels of pro-inflammatory cytokines (IL-1β and TNF-a) and nonspecific inflammatory marker (CRP) was detected in the seminal plasma of semen in CP/CPPS patients. In this model, significantly increased levels of TNF-α and IL-β along with the elevated amount of CRP in the T2 + CFA + $AL(OH)_3$ group making it a valid model of CP/CPPS was also observed. That is to say, groups immunized with T2 + CFA together with $AL(OH)_3$ adjuvant showed more severe inflammation than the other groups, which leads to the conclusion that T2 mixed with CFA + $AL(OH)_3$ adjuvant could induce experimental autoimmune prostatitis (EAP) with a 100% incidence rate.

Rivero et al. [26] established an EAP model using NOD mice. They also mixed MAG homogenate with CFA and injected subcutaneously at multiple sites. It was found that NOD mice developed obvious prostate inflammatory reaction on the 10th and 20th days after inoculation. The extent of inflammatory infiltration was very large, moderate and severe inflammatory lesions can be observed in the lateral pages of the prostate, the dorsal page, the seminal vesicles, and the coagulation glands. Not only the humoral immune response but also the cellular immune response in the inflammatory reaction was found. Further experiments in the same group were also conducted, which showed that twice injection of1.0 mg Wistar rat MAG or 30 mg Wistar rat PSBP could induce 80–100% of young male NOD mice to have prostatitis, but only 30% C57BL/6 mice developed inflammation. Mice vaccinated with MAG

were more edematous than those vaccinated with PSBP.

In summary, the autoimmune modeling method may not be well adapted to each rodent. Wistar rats were selected to establish an EAP model, mainly because of the spontaneous, age-dependent and histopathological changes of prostatitis, which are very similar to human chronic prostatitis in many aspects, and when the concentration of the injected prostate protein purified solution is 40–60 mg/mL, the success rate of modeling can reach 38%.NOD mice were selected to establish an EAP model, mainly because they were more sensitive than C57BL/6 mice and Wistar rats, and the success rate of modeling was 100%. It was confirmed that NOD mice are suitable for EAP modeling. In general, compared to the EAP rat model, the advantage of the NOD mouse model is that mouse species are readily available and easier to raise, suitable for experiments requiring large sample size data, and there are several immunological techniques that can be used to study prostatitis. Due to its immunological characteristics, many researchers have tended to select the mouse EAP model in studies of the mechanisms and treatments of prostatitis. The main drawback is that the tissue autoantigens that induce autoimmune prostatitis are not yet clear, and NOD mice can develop diabetes spontaneously, which interfere with prostatitis [27].

2.2. Age-related prostatitis models

2.2.1. Rat prostatitis models

Age-related spontaneous prostatitis is a commonly used model for the study of autoimmune prostatitis. Previous studies had shown that non-bacterial prostatitis occurred spontaneously when certain ages were reached in rats of different races. There was moderate mononuclear cell infiltration in spontaneous prostatitis, mainly CD4 + T cells, and inflammation mainly occurred in the prostate interstitium, around the acinar and in the lateral lobes [28–30]. The study found that these rats had a defect in their own mechanisms of tolerance to their own prostate antigens, and as age increased and the body's environment changed, tolerance mechanisms can be further weakened, leading to age-related spontaneous autoimmune prostatitis. The occurrence of different types of rats had a different probability of spontaneous autoimmune prostatitis. Vykhovanets et al. [30,31] found that 60–70% of male Lewis rats developed spontaneous inflammation in the lateral lobe of the prostate at approximately 12 weeks; SD rats at 22–24 weeks of age had abdominal and lateral prostate glands. The incidence of inflammation was low (approximately 16%); the incidence of spontaneous CNP in elderly Wistar rats was 27%; 88% of Copenhagen rats developed spontaneous lateral lobes prostatitis at 20 weeks of age.

2.2.2. Mouse prostatitis models

The probability of spontaneous prostatitis among mice is small, but in the late 1990s, the researchers found that aged NOD mice not only spontaneously develop autoimmunity in the pancreas, thyroid, parathyroid, and adrenal glands but also the prostate. It can produce spontaneous autoimmunity. Jackson et al. [32] found that NOD male mice may develop spontaneous autoimmune prostatitis at 20 weeks and remain stable. This NOD mouse has type I insulin-dependent diabetes mellitus. Prostate leukocyte antigen is 2–4 times more common in these male mice than in 8-week-old mice at 20–30 weeks. By the age of 40 weeks, 70% of NOD mice will have prostatitis reaction, and both cellular and humoral immunity exist [32,33]. However, 8-week-old NOD mice did not show any cellular and humoral immune responses to the prostate. Studies have shown that high serum titer of IgG antibodies against prostate antigen can occur in the serum of elderly male NOD mice, with IgG2b as the major component. The prostate antigen that has been identified is mainly PSBP. The occurrence of autoimmune prostatitis in aged male NOD mice may be related to the gradual decline of the body's resistance to prostate antigen as the age increases [33], eventually leading to the occurrence of spontaneous autoimmune prostatitis. Another study found that Kunming mice can also develop spontaneous autoimmune prostatitis by giving passive abstinence for a long time [34].

Previous studies have shown that non-bacterial prostatitis occurs spontaneously in certain species of rats when they reach a certain age. The establishment of this model is related to the type and age of rats and mice. Lewis, Wistar, and Copenhagn rats and NOD male mice that developed autoimmune prostatitis with age provided a good model for the study of the disease. This model has good stability and long-term maintenance. Its pathological appearance and clinical manifestation are similar. It is suitable for the study of therapeutic drugs for human CP, the pathological specificity is good. However, the modeling time is more than 3 months, relatively long and costly, and the repeatability is not ideal so it is less used. If it is for the study of human chronic prostatitis, it is best to use Copenhagen rats as model animals, the success rate can reach 88% at 20 weeks of modeling.

2.3. Hormone and castration induced prostatitis models

2.3.1. Rat prostatitis models

It is well known that the prostate is an organ that depends on male hormones. Androgens play a key role in regulating the growth, function, and disease of the prostate. This model uses estrogen and castration methods to cause abnormal hormone levels and destruction of the equine androgen balance in animals, resulting in a non-bacterial inflammatory response to the prostate. The estrogens can induce male accessory sex organ inflammation under other conditions has been documented in immature rabbits treated with estradiol benzoate [35] and in testosterone-stimulated adult rats that were treated neonatally with 17β-estradiol [36]. The sub acute administration of estradiol-17β was shown to be a potent inducer of an inflammatory response specific to the lateral prostate of the castrated Wistar's rat. The subsequent administration of dihydrotestosterone restored the wet weight of the gland while maintaining the inflammation established with estrogen treatment. These changes are histologically similar to a spontaneously arising nonbacterial prostatitis previously reported by others in the aging rat lateral prostate [37].

Some researchers used 17β-estradiol combined with ovariectomy to model Westar rat's results showed that three different concentration of the group can successfully induce prostatitis inflammatory changes.

In addition, it was found that Wistar rats were treated with 100 μg of 17β-estradiol for 2–5 days can also develop prostatitis successfully. After testosterone undecanoate capsules (2.0 mg/d) were treated for 10 weeks, 10–12 weeks old neonatal rats developed severe prostatitis [37]. By observation, severe edema and infiltration of lymphocytes, mononuclear cells, and extensive fibrosis were observed in both prostate glands in the affected prostate lobules. This showed that this method is 100% successful.

2.3.2. Mouse prostatitis models

Pakarainen et al. [38] found that 67% of testosterone-treated LH receptor-depleted LuR-KO mice exhibited significant prostatitis, characterized by the presence of large numbers of lymphocytes in the prostate gland between interstitial and stromal tissues. Eight weeks of testosterone replacement therapy began to restore the function of gonadal function and accessory organ of LuR-KO male mice on the 21 st day. Aromatase overexpression (AROM +) can lead to an increase in endogenous estrogen levels in mice. AROM + mice are normal at the beginning of the prostate. As the level of estrogen increases, mast cells, macrophages gradually appear in the prostate, and inflammatory manifestations are mainly granulocytes and T lymphocytes infiltration [39].

The estrogen-induced CNP model was simple, the rat species was easily available, the modeling reagents were easy to obtain, and the modeling cost was low. Pathological changes were similar to spontaneous CNP in aged rats. The age-dependent spontaneous rat CNP is pathologically similar to human CP, so this kind of rat model is very

suitable for the study of human CP treating drugs; pathological specificity is better [40]. From the point of perspective of shortcomings, estrogen combined with castration mode has high requirements for aseptic technique and complicated operations. The acquired prostate specimens have been reduced to varying degrees, which makes it more difficult to obtain materials for future studies. It is still unclear whether this model can represent experimentally induced prostatitis (prostatitis is spontaneous or genetically predetermined, or simply stimulated by androgen deprivation and estrogen therapy).

2.4. Chemical prostatitis models

In this model, chemicals are directly injected into the animal's prostate to cause aseptic CP. Chemicals currently used include carrageenan, amidraphane, glycerin, 2% agar, formaldehyde-croton oil, FCA, and alike [21,22]. Among them, carrageenan preparations are the most commonly used and have the advantage of less damage to the prostate tissue and modeling is more similar to chronic inflammation [8,9]. Radhakrishnan and Nallu [9] used SD rat intraprostatic injection of 3% carrageenan to construct the CNP model and tested its degree of reduction of the perineal pain threshold during thermal and mechanical stimulation to reflect the rat model (Fig. 1A). They found that the model rats showed infiltration of inflammatory cells which were mainly monocytes and lymphocytes, fibrous connective tissue hyperplasia, interstitial hyperemia, edema, and other chronic inflammation. The comparison between the experimental group and the control group showed that the pain threshold of the thermal stimulation was significantly reduced in the experimental group at 48–72 h, and 1 week after the injection of 3% carrageenan, and the pain threshold of the mechanical stimulation was in the injection group. After 72 h and 1 week, there was a significant decrease in the two-time points and kept a long time; the results confirmed that this method is effective in simulating pelvic floor pain. However, this modeling method also has its drawbacks. The chemical modeling method is to directly inject chemicals into the prostate to induced inflammation, which will inevitably cause great damage to the prostate tissue; and this model mimics the mechanism of prostatitis. There is a certain gap between acute prostatitis and CP. Chen's et al. a study [41] have found that the effective duration of chemical modeling methods is mostly within one month. Longer and long-lasting effects have not been studied in depth. The CP/CPPS rats were given 100 μL carrageenan suspension (3% w/v) injection on each side of the prostatic lobe. And the rats were tested for tactile allodynia before prostate injection (baseline), and 3 days after the surgery, they found that static tactile allodynia was confirmed in the rat model. The bearing threshold of withdrawal response in carrageenan treated rats was 80.0 ± 11.0% of the initial pre-treatment threshold. Hypersensitivity to pain at the scrotal base was ameliorated by CHA treatment. After injection with carrageenan and CHA (CP-T group), the rats became less responsive to stimulation and the threshold

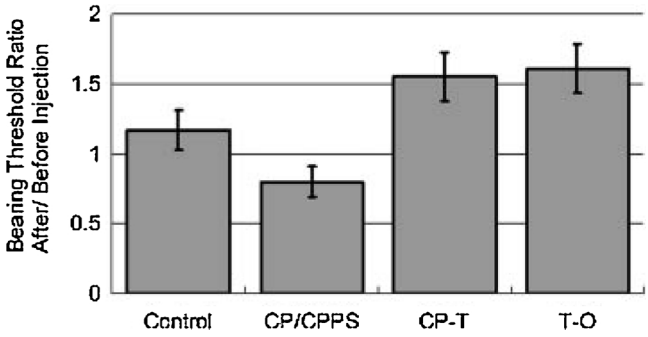

Fig. 2. Mechanical allodynia confirmed by von Frey filament examination. The chronic pelvic pain was shown in the rat after prostate injection with carrageenan. Bearable pressure ratio was reduced by 32.8% in rats with CP/CPPS. With a synchronous injection of CHA solution, the rats had similar bearable pressure ratio as the controls. The ratio did not change in groups with only CHA injection [41].

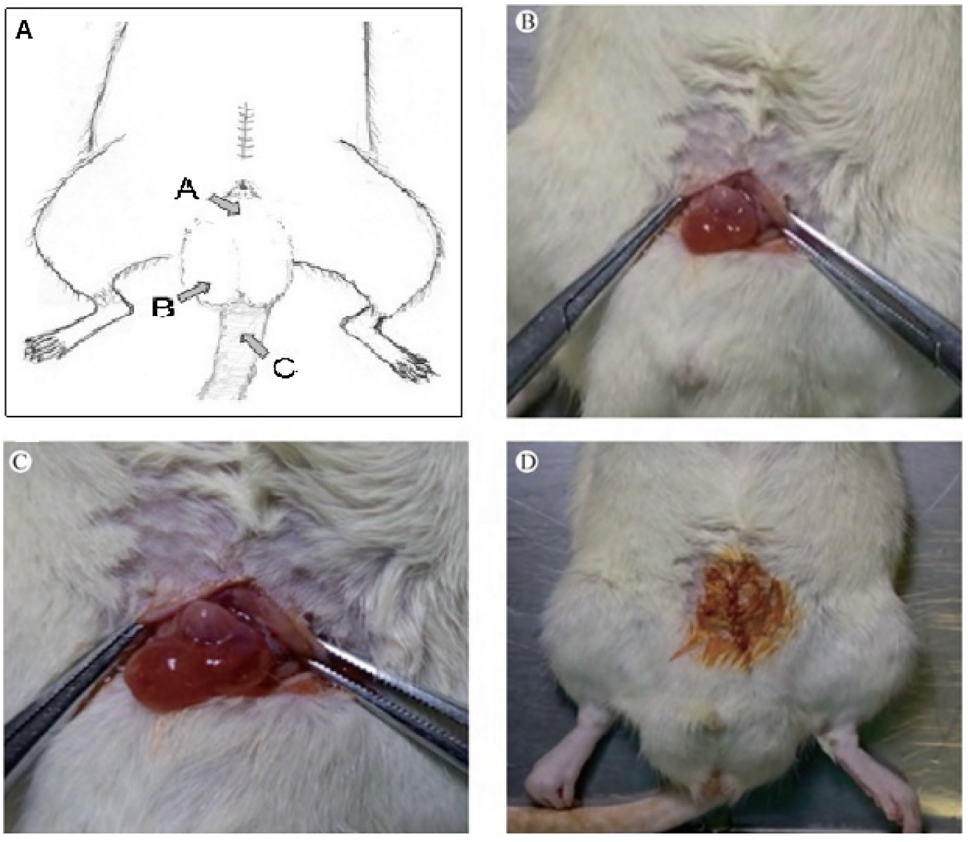

Fig. 1. (A), Illustration of the pelvic areas where the heat and mechanical stimuli were applied in the rat. Reductions in heat and mechanical thresholds were observed in the skin overlying the scrotum area B, but not in areas A and C [9]. (B), The process of surgical operation to inject 1% carrageenan solution into the rat prostate lobes. Routine skin preparation and cut through the abdominal wall, and exposure of the anterior surface of the prostate. (C), Bilateral injection of carrageenan solution or saline into the prostate lobes. (D), suture of the surgical incision and local use of antibiotics.

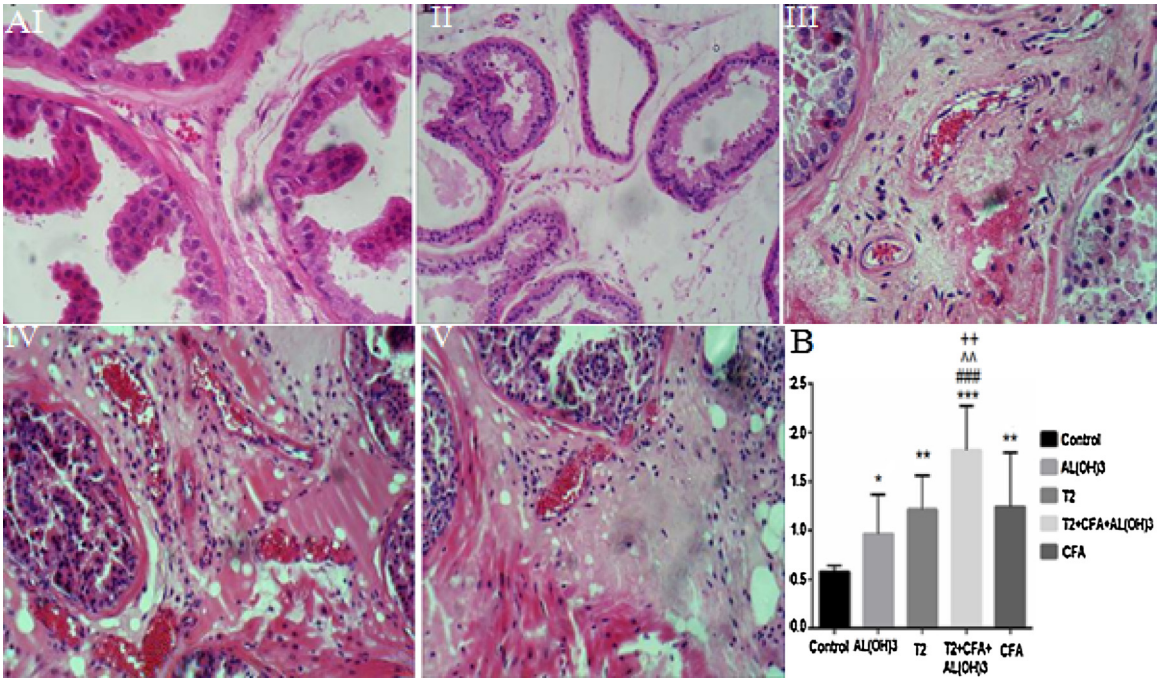

Fig. 3. (A) H&E staining of prostate tissue from C57BL/6 mice in the different groups (magnification: ×300). (I) Control group, (II) $Al(OH)_3$group, (III) T2 group, (IV) T2 plus CFA plus $Al(OH)_3$ group was observed, and (V) CFA group. Extensive inflammatory cells infiltration and inflammation lesion in the group (IV) and the group (V). The group (IV) showed more severe inflammatory changes than any other groups. (B). Mean inflammation scores of each group. ANOVA test was used to compare statistical differences among the groups.*Significant difference from the normal control group at $P < 0.05$. **Greater significant difference from the normal control group at $P < 0.01$. ***The highest significant difference from the normal control group at $P < 0.001$. #Significant difference from the Al(OH)3 group at $P < 0.05$. ##The greater significant difference from the Al(OH)3 group at $P < 0.01$. ###Highest significant difference from the Al(OH)3 group at $P < 0.001$. ^The significant a difference from the T2 group at $P < 0.05$. ^^ Greater significant difference from the T2 control group at $P < 0.01$. +The Significant difference from the CFA group at $P < 0.05$. ++Greater significant difference from the CFA group at $P < 0.01$ [22].

increased by 55.1 ± 17.3% compared to the original. The pain sensation was similar as when the rat prostate was injected with CHA only (T−O group), which was 160.9 ± 17.6% of the original pressure (Fig. 2).

3. Model evaluation index

The evaluation criteria of animal models of CP would have different detection methods and evaluation criteria because of different modeling methods. However, the vast majority of evaluation criteria is based on these aspects: histology, morphology, prostate index, urodynamics, inflammatory factors, and pain.

3.1. Pathology indicators -type I indicators (core indicators)

The most direct indicator of CP is obvious inflammation of the prostate tissue. Pathological observation can accurately confirm the severity of prostatitis, and a light microscope is used to observe whether there is infiltration of inflammatory cells and what kind of the inflammatory cells are, as well as whether there is tissue edema, glandular epithelial necrosis and exfoliation, and glandular epithelium disappeared. In the animal model of CP, the graded reference standard for the pathological changes of prostate tissue [21,22,42]. "0" indicates that most of the prostate gland is at rest, the gland is shrinking, and the glandular epithelium is wrinkled. There is no secretion in the glandular cavity, and the glandular cavity is obviously smaller. "I" indicates that most glands in the prostate are in an expanded state. There are secretions in the glandular cavities, but there are few secretions and a small number of inflammatory cells are visible around them. "II" indicates that the prostate gland is in a markedly expanded state, the glandular epithelium becomes thin and flat, and the glandular cavity is filled with red secretions."III" indicates that the prostate gland is in a state of significant expansion and the gland enlarges by 1–3 times. The glandular epithelium is flat, and the glandular cavity is filled with a large amount of red secretions. The glandular cavity is significantly dilated and scattered around the inflammatory cells. Each prostate pathological section was randomly scored by two people without ignorance according to the above scoring criteria. Pathological indicators can directly reflect the characteristics of CP and are the core indicators for judging the success of the CP model. The weight coefficient is chosen to be 0.4.

X. Zhou et al. [22] used C57BL/6 mice to establish the model of chronic prostatitis, he set up five groups. And the pathological results of each group were as Fig. 3A, inflammation scores of each group as Fig. 3B.

3.2. Biochemical indicators -type II indicators (directly related indicators)

The direct indicator of CP is the marked elevation of white blood cells in the prostatic fluid or its homogenate, and the decrease in the distribution of lecithin bodies [21,24,43]. Based on prostatitis immune factors, IgG, IgA, and IgM in the prostate homogenate are often tested and IgA levels are significantly increased when a model is successfully established. Based on neuroendocrine theory, prostatitis produces pain and excessive secretion of prostatic fluid. The level of NE, PG, histamine, 5-HT and NGF [44] in the homogenate of the prostate increased the levels of IL-6, IL-8, IL-10, TNF-α, and IL-1β. The inflammatory factor arachidonic acid content increased [45]. Based on the immunological theory, when the model is replicated successfully, serum T or prostate tissue homogenates have reduced T levels, E2 levels, serum prostate-specific antigen (PSA) levels, and C-reactive protein (CRP) levels. Biochemical indicators can reflect the development, progression, and rehabilitation of CP, and are directly related indicators with a weight coefficient of 0.3.

Table 1
Rodent models of prostate inflammation.

Model	Species	Inflamed site (s)	Modeling cycle and method	Success rate	Better animal model
EAP animal models T2 peptide	Rat: Wistar, Lewis, and Copenhagen Mouse: C57BL/6 and NOD Rat: SD Mouse: C57BL/6	Male sex accessoy glands, DL, LL, and VL NA Male sex accessoy glands, DL, LL and VL	30 days Multiple injections on days 0, 7, 14, 18 with immunization of homogenate of rat male sex accessory glands, rat prostate tissue, rat or human prostate acid phosphatase, or rat PSBP with CFA. Injected T2 + CFA + $Al(OH)_3$ group with T2 + CFA + $Al(OH)_3$. Dosing (1 mL) to the experimental group was administered subcutaneously on 1 st, 14th and 28th day.	Wistar: 38% [14] NOD: 100% [26] SD: 100% [21,22]. C57BL/6 100% [22]	NOD: mouse species are readily available; easier to raise, suitable for experiments requiring large sample size data, The success rate is 100% SD: Mouse species are readily available; easier to raise, more severe inflammation. The success rate is 100%
Spontaneous	Rat: SD Wistar, Lewis, Copenhagen Mouse: NOD	LL and VL NA	SD: 22-24weeks Wistar: 30weeks Lewis: 12weeks Copenhagen: 20weeks Mouse: NOD 40weeks After reaching a certain age, spontaneous prostatitis is caused by changes in bodily functions	SD:16% [30,31] Wistar:27% [30,31] Lewis: 60-70% [30,31] Copenhagen: 88% [30,31] NOD: 70% [32,33]	Copenhagen: suitable for the study of therapeutic drugs for human CP The success rate is 88%
Hormone and castration	Rat: Wistar, Mouse: LuRKO	LL, LL/DL and VL NA	12weeks After 2-5 days of neonatal, Wistar rats were treated with 100 μg of 17β-estradiol, and 10 to 12 weeks later, testosterone undecanoate (2.0 mg/d) was given for 2 weeks. Testosterone replacement from 21 day for 60 days of age induces severe PMNC and MNC prostate infiltration in 67% of Hypogonodal LuRKO mice.	Wistar: 100% [37] LuRKO: 67% [38]	Wistar: simple, the rat species was easily available, the modeling reagents were easy to obtain, and the cost was low. High requirements for aseptic technique and complicated operations. The success rate is 100%
Chemical	Rat: SD	Male sex accessoy glands, DL, LL and VL	1weeks injection of 3% carrageenan	SD: 100% [41]	SD: inflammation directly in the prostate, Cause damage to prostate tissue The success rate is 100%

Abbreviations: AL, DL, LL, VL, anterior, dorsal, lateral, ventral (coagulating glandlobes of the prostate, respectively;

3.3. Apparent index, urinary fluidity index, pain level-III index (direct correlation index)

Apparent indicators include the animal's prostate index, urine output, water intake, and opening activities. After the model was successfully prepared, the wet weight of prostate and prostate index increased, the amount of urine decreased, the amount of drinking water decreased, the opening activity decreased, and the gloss of hair decreased. Prostatitis can cause changes in urodynamics. Prostatitis animal bladder pressure, urination interval, maximum urinary pressure and other urodynamic parameters can directly reflect the situation of prostatitis, the model After successful preparation of the model, maximum urinary pressure, urination interval, bladder static pressure is significantly improved. Because patients with prostatitis often have pelvic pain syndrome (PPS), the measurement of the degree of pain in the model has become the evaluation standard for the success of the test model. With the development of thermal imaging technology, some authors can further understand the clinical manifestations of autoimmune prostatitis by examining model thresholds for pain [8,46], site of pain, time, mechanical hyperalgesia, and thermal hyperalgesia. Apparent indicators are directly reflected in CP as directly related indicators with a weight coefficient of 0.3.

4. Conclusion and future prospects

The etiology of CP is complex, and the pathophysiological process has not been elucidated yet. Clinical treatment is very difficult. In order to better understand the pathogenesis of autoimmune prostatitis and find the correct diagnosis and treatment methods, it is particularly important to establish an effective animal model. The above modeling methods prepare an animal model of autoimmune prostatitis starting from different mechanisms (Table 1). In practice, an appropriate test method can be selected in combination with the purpose of the experiment and the display conditions. However, the etiology and pathogenesis of prostatitis are very complex. The above methods still have many deficiencies. Therefore, we need to make continuous efforts to explore more suitable models for the etiology, pathogenesis and clinical manifestations of prostatitis.

Conflict of interest

All authors declare that there is no conflict of interest regarding the content of this article.

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References

[1] M. Pontari, L. Giusto, New developments in the diagnosis and treatment of chronic prostatitis/chronic pelvic pain syndrome, Curr. Opin. Urol. 23 (6) (2013) 565–569.
[2] M.M. Collins, R.S. Stafford, M.P. O'Leary, M.J. Barry, How common is prostatitis? A national survey of physician visits, Urology 159 (1998) 1224–1228.
[3] T.D. Moon, L. Hagen, Dennis M. Heisey, Urinary symptomatology in younger men, Urology 50 (1997) 700–703.
[4] P.Y. Cheah, H.L. Liong, K.H. Yuen, C.L. Teh, T. Khor, J.R. Yang, Chronic prostatitis: symptom survey with follow-up clinical evaluation, Urology 61 (2003) 60–64.
[5] Z.Z. Weihua Fu, Shijian Liu, Qianwei Li, Jiwei Yao, Weibing Li, Junan Yan, The effect of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) on semen parameters in human males: a systematic review and meta-analysis, PLoS One 9 (4) (2014).
[6] Y. Hu, X. Niu, G. Wang, J. Huang, M. Liu, B. Peng, Chronic prostatitis/chronic pelvic pain syndrome impairs erectile function through increased endothelial dysfunction, oxidative stress, apoptosis, and corporal fibrosis in a rat model, Andrology 4 (6) (2016) 1209–1216.
[7] J.D. Done, C.N. Rudick, M.L. Quick, A.J. Schaeffer, P. Thumbikat, Role of mast cells in male chronic pelvic pain, J. Urol. 187 (4) (2012) 1473–1482.
[8] F. Zeng, H. Chen, J. Yang, L. Wang, Y. Cui, X. Guan, Z. Wang, J. Niu, X. Zu, L. Qi, X. Zhang, Z. Tang, L. Liu, Development and validation of an animal model of prostate inflammation-induced chronic pelvic pain: evaluating from inflammation of the prostate to pain behavioral modifications, PLoS One 9 (5) (2014) e96824.
[9] R. Radhakrishnan, R.S. Nallu, Development and characterisation of a novel animal model of prostate inflammation-induced chronic pelvic pain, Inflammopharmacology 17 (1) (2009) 23–28.
[10] J.G. Copeland, R.G. Smith, F.A. Arabia, P.E. Nolan, G.K. Sethi, P.H. Tsau, D. Mcclellan, M.J. Slepian, Cardiac replacement with a total artificial heart as a bridge to transplantation, ACC Curr. J. Rev. 351 (9) (2004) 859.
[11] A.J. Schaeffer, W. Weidner, G.A. Barbalias, H. Botto, T.E.B. Johansen, W.W. Hochreiter, J.N. Krieger, B. Lobel, K.G. Naber, J.C. Nickel, J.M. Potts, P. Tenke, C. Hart, Summary consensus statement: diagnosis and management of chronic prostatitis/chronic pelvic pain syndrome, Eur. Urol. Suppl. 2 (2) (2003) 1–4.
[12] P. Tyagi, M. Kashyap, S. Pore, Z. Wang, N. Yoshimura, Mp25-07 prostatic inflammation evokes upregulation of neurotrophins in sensory ganglia: possible contribution to dysfunctional voiding, J. Urol. 193 (4) (2015) e287.
[13] G. Lee, Chronic prostatitis: a possible cause of hematospermia, World J. Mens Health 33 (2) (2015) 103–108.
[14] B.P.R.M. Depiante-Depaoli, S. Britos, Experimental autoimmune damage to rat male accessory glands. I. Transfer of autoimmune response by spleen cells, Reprod. Immunol. 5 (9) (1984) 14.
[15] A.C.D.A.M. Depiante-Depaoli, Inflammatory cells and MHC class II antigens expression in prostate during time-course experimental autoimmune prostatitis development, Clin. Immunol. Immunopathol. 85 (2) (1997) 158–165.
[16] 周晓辉, 韩蕾, 周智恒, 刘忠德, 杨吉相, 吕延伟, 尤春来, 免疫性慢性非细菌性前列腺炎大鼠模型的形态学与分子生物学特性, 中华男科学杂志 (04) (2005) 290-295.
[17] X. Qi, L. Han, X. Liu, J. Zhi, B. Zhao, D. Chen, F. Yu, X. Zhou, Prostate extract with aluminum hydroxide injection as a novel animal model for chronic prostatitis/chronic pelvic pain syndrome, Urology 80 (6) (2012) 1389 e9-15.
[18] X. Wang, S. Zhong, T. Xu, L. Xia, X. Zhang, Z. Zhu, M. Zhang, Z. Shen, Histopathological classification criteria of rat model of chronic prostatitis/chronic pelvic pain syndrome, Int. Urol. Nephrol. 47 (2) (2015) 307–316.
[19] D.W. Keetch, P. Humphrey, T.L. Ratliff, Development of a mouse model for nonbacterial prostatitis, Urology 152 (1994) 247–250.
[20] C.M. Jackson, D.B. Flies, C.A. Mosse, A. Parwani, E.L. Hipkiss, C.G. Drake, Strain-specific induction of experimental autoimmune prostatitis (EAP) in mice, Prostate 73 (6) (2013) 651–656.
[21] A.U. Ihsan, F.U. Khan, W. Nawaz, M.Z. Khan, M. Yang, X. Zhou, Establishment of a rat model of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) induced by immunization with a novel peptide T2, Biomed. Pharmacother. 91 (2017) 687–692.
[22] F.U. Khan, A.U. Ihsan, W. Nawaz, M.Z. Khan, M. Yang, G. Wang, X. Liao, L. Han, X. Zhou, A novel mouse model of chronic prostatitis/chronic pelvic pain syndrome induced by immunization of special peptide fragment with aluminum hydroxide adjuvant, Immunol. Lett. 187 (2017) 61–67.
[23] L. Zhang, A.U. Ihsan, Y. Cao, F.U. Khan, Y. Cheng, L. Han, X. Zhou, An immunogenic peptide, T2 induces interstitial cystitis/painful bladder syndrome: an autoimmune mouse model for interstitial cystitis/painful bladder syndrome, Inflammation 40 (6) (2017) 2033–2041.
[24] L. Zhang, A.U. Ihsan, Y. Cao, Y. Cheng, X. Zhou, Establishment of experimental autoimmune prostatitis model by T2 peptide in aluminium hydroxide adjuvant, Andrologia 50 (3) (2018).
[25] R.B. Alexander, Sathibalan Ponniah, Jeffrey Hasday, J.R. Hebel, Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome, Urology 52 (5) (1998) 744–749.
[26] V.E. Rivero, C. Cailleau, Mirtha Depiante-Depaoli, Clelia M. Riera, Claude Carnaud, Non-obese diabetic (NOD) mice are genetically susceptible to experimental autoimmune prostatitis (EAP), Autoimmunity 11 (1998) 603–610.
[27] F.D. Cengiz, Z. Altuntas, Elias Veizi, Kenan Izgi, Fuat Bicer, Ahmet Ozer, Kerry O. Grimberg, Bakytzhan Bakhautdin, Cagri Sakalar, Cemal Tasdemir, A novel murine model of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) induced by immunization with a spermine binding protein (p25)peptide, Physiol. Regul. Integr. Comp. Physiol. 304 (2013) 415–422.
[28] B.H. Rolf Lundgren, Margareta Hesselvik, Jonas Muntzing, Treatment of prostatitis in the rat, Prostate 5 (1984) 277–284.
[29] I.M. Keith, J. Jin, D. Neal Jr., Brian D. Teunissen, TimothyD. Moon, Cell relationship in a wistar rat model of spontaneous prostatitis, Urology 166 (2001) 323–328.
[30] E.V. Vykhovanets, M.I. Resnick, S.R. Marengo, The healthy rat prostate contains high levels of natural killer-like cells and unique subsets of CD4+ helper-inducer T cells: implications for prostatitis, J. Urol. 173 (3) (2005) 1004–1010.
[31] E.V. Vykhovanets, M.I. Resnick, G.T. MacLennan, S. Gupta, Experimental rodent models of prostatitis: limitations and potential, Prostate Cancer Prostatic Dis. 10 (1) (2007) 15–29.
[32] S.A. Giuseppe Penna, Chiara Cossetti, Francesca Aquilano, Roberto Mariani, Nadia Giarratana, Elena De Carli, Benedetta Fibbi, Luciano Adorini, Spontaneous and prostatic steroid binding protein peptide-induced autoimmune prostatitis in the nonobese diabetic mouse, Immunology 179 (2007) 1559–1567.
[33] V. Rivero, C. Carnaud, C.M. Riera, Prostatein or steroid binding protein (PSBP) induces experimental autoimmune prostatitis (EAP) in NOD mice, Clin. Immunol. 105 (2) (2002) 176–184.
[34] I.A. Ludwig, J. Bravo, M.P.D. Peña, C. Cid, Effect of sugar addition (torrefacto)

during roasting process on antioxidant capacity and phenolics of coffee, Lwt - Food Sci. Technol. 51 (2) (2013) 553–559.
[35] E.J. Birks, P.D. Tansley, J. Hardy, R.S. George, C.T. Bowles, M. Burke, N.R. Banner, A. Khaghani, M.H. Yacoub, Left ventricular assist device and drug therapy for the reversal of heart failure, 新英格兰医药杂志 355(18) (2006) 1873.
[36] M. Mitka, Academic alert. Midwest trials of heart-assist device, Jama J. Am. Med. Assoc. 286 (21) (2001) 2661.
[37] C.L. Robinette, Sex-hormone-induced inflammation and fibromuscular proliferation in the rat lateral prostate, Prostate 12 (1988) 271–286.
[38] T. Pakarainen, F.P. Zhang, S. Makela, M. Poutanen, I. Huhtaniemi, Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice, Endocrinology 146 (2) (2005) 596–606.
[39] S.J. Ellem, H. Wang, M. Poutanen, G.P. Risbridger, Increased endogenous estrogen synthesis leads to the sequential induction of prostatic inflammation (prostatitis) and prostatic pre-malignancy, Am. J. Pathol. 175 (3) (2009) 1187–1199.
[40] b. Tammy E. Stokera, C. Lee Ro binette b, Ralph L Coopera, Perinatal exposure to estrogenic compounds and the su bsequent effects on the prostate of the adult rat evaluation of inflammation in the ventral and lateral lo bes, Reproductive Toxicology 13 (1999) 463–472.
[41] C.S. Chen, P.J. Chang, W.Y. Lin, Y.C. Huang, D.R. Ho, Evidences of the inflammasome pathway in chronic prostatitis and chronic pelvic pain syndrome in an animal model, Prostate 73 (4) (2013) 391–397.
[42] Z. Li, A.U. Ihsan, Y. Cao, F.U. Khan, Y. Cheng, H. Lei, X. Zhou, An immunogenic peptide, T2 induces interstitial cystitis/painful bladder syndrome: an autoimmune mouse model for interstitial cystitis/painful bladder syndrome, Inflammation 40 (6) (2017) 2033–2041.
[43] T.R. Huang, W. Li, B. Peng, Correlation of inflammatory mediators in prostatic secretion with chronic prostatitis and chronic pelvic pain syndrome, Andrologia 50 (2) (2018).
[44] E.S. Schwartz, J.H. La, E.E. Young, B. Feng, S. Joyce, G.F. Gebhart, Chronic prostatitis induces bladder hypersensitivity and sensitizes bladder afferents in the mouse, J. Urol. 196 (3) (2016) 892–901.
[45] M.L. Breser, F.C. Salazar, V.E. Rivero, R.D. Motrich, Immunological mechanisms underlying chronic pelvic pain and prostate inflammation in chronic pelvic pain syndrome, Front. Immunol. 8 (2017) 898.
[46] S.F. Murphy, A.J. Schaeffer, J. Done, L. Wong, A. Bell-Cohn, K. Roman, J. Cashy, M. Ohlhausen, P. Thumbikat, IL17 mediates pelvic pain in experimental autoimmune prostatitis (EAP), PLoS One 10 (5) (2015) e0125623.