

**Nonbacterial Prostatitis in Kunming Mouse Induced by Long-Term
Stimuli of Passive Sexual Abstinence**

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ABSTRACT

In this study, a state of sexual abstinence had been imitated in Kunming male mice. Further assessment on the body and prostate weight, and histomorphometric changes of the prostate showed that nonbacterial prostatitis in Kunming mice could be induced by long-term stimuli of passive sexual abstinence, and the normal physiological state of the mice could be kept in the process of induction. The analysis of the result suggests that copulation might be a protective factor in the process of non-bacterial prostatitis. The underlying mechanisms of this model may include immune response on prostate antigen, chronic pelvic congestion, and disturbance of hormone regulation and others, while requires confirmation from further research.

KEY WORDS

Nonbacterial Prostatitis, Sexual Abstinence, Kunming Mice,

INTRODUCTION

Prostatitis is a commonly diagnosed disease in males, with 2 million office visits recorded annually in the United States [Collins et al., 1998]. More recent studies have shown the incidences of prostatitis ranging from 2.2% to 16% in North America, Europe and Asia, about 50% of whom have had repeated episodes, suggesting that prostatitis is an important health problem world-wide [Nickel et al., 2001; Mehik et al., 2000; Kunishima et al., 2002; Cheah et al., 2003]. Unlike benign prostate hyperplasia and prostate cancer, prostatitis affects adult males of all ages and is generally considered as the most common outpatient condition in urologic practice among males less than 50 years old [Collins et al., 1998].

Uropathogens such as *Escherichia coli*, *Enterococcus* spp, *Klebsiella* spp, *Proteus* spp and *Pseudomonas aeruginosa*, have been implicated present in about 5–40% of cases of prostatitis [Rosette et al., 1993; Badalyan et al., 2003]. Other clinic prostatitis without evidence of pathogen can be classified into category III, the chronic prostatitis/chronic pelvic pain syndrome, and may involve several pathogeneses, including hormonal imbalance [Nickel et al., 2004], neurological dysfunction [Miller et al., 2002], α -adrenergic system abnormalities [Nickel, 2004], urinary reflux into the prostate [Kirby et al 1982], inappropriate cytokine release [Jang et al., 2003], and autoimmune response [Batstone et al., 2002; John et al, 2003; Klyushnenkova et al, 2005].

Chronic prostate inflammation may play a role in the development of prostate cancer. MacLennan GT et al made an analysis on prostate needle biopsies from 177 patients with clinical parameters suspicious for malignancy, which revealed a strong association between chronic

prostate inflammation and premalignant or malignant changes in prostate epithelium [MacLennan et al., 2006].

During the last three decades, considerable attention has been focused on creating novel reliable animal models of prostate inflammation, in hope of uncovering the etiology and pathogenesis. Apart from infective agents, noninfectious factors such as endogenous chemicals, immunologic factors, obstruction, trauma and autoimmune factors have been considered as possible causes of prostatitis in rat or mouse [Meares et al., 1993].

Current data regarding the nonbacterial prostatitis in mice is limited, though many rat models of nonbacterial prostatitis have been successfully developed. In this study, a state of sexual abstinence had been imitated in Kunming male mice. Further assessment on the body and prostate weight, and histomorphometric changes of the prostate were made in order to determine whether nonbacterial prostatitis of mice can be induced by long-term passive sexual abstinence. The potential mechanisms were discussed in order to suggest potential solution for the treatment of nonbacterial prostatitis.

MATERIALS AND METHODS

1. Animals and Diet

Forty Kunming male mice and forty Kunming female mice, 8 weeks old, were randomly grouped into 3 experimental groups and 1 control group. The mice were fed with nutritional cooperated pelletized feeds. The mice were cared for in a specific pathogen-free environment. A controlled temperature (22℃) and humidity (55%) were maintained. The water and diet were provided ad-libitum. The care and use of laboratory animals in this experiment adhered to the guidelines and regulations for use and care of animals in Animal Laboratory of the Second Xiangya Hospital, Central South University.

2. Treatment

In Experiment Group A, each of the 10 male mice was cared for with another female mouse in the same cage for 6 weeks, and the 2 mice were separated by a steel net to ensure that the male mouse could be under the sexual stimuli from the female mouse but without copulation. In Experiment Group B, each of the 10 male mice was cared for with another female mouse in the same cage together without steel net for 3 weeks, and then the 2 mice were separated by a steel

net for another 3 weeks. In Experiment Group C, each of the 10 male mice was cared for with another female mouse in the same cage but separated by a steel net for 3 weeks, and then cared for together without steel net for another 3 weeks. In Control Group, each of the 10 male mice was cared for with another female mouse in the same cage together without steel net for 6 weeks. All animals tolerated the procedure well and did not show any notable adverse reaction throughout the period of this experiment.

The male mice were weighed at the initial of treatment and once each week thereafter. At the end of 6 weeks of treatment, the male mice were euthanized. A lower abdominal incision was used for the collection of prostate. The prostate were carefully dissected, removed and fixed in 10% formalin at 20°C for 1 day, then embedded in paraffin and stored at -20°C until analysis. At necropsy, the prostate was separated from the adjacent tissues and weighed.

3. Histology and Morphometric Analysis

The mice prostate specimens embedded in paraffin were cut at 5µm thickness. The slides were stained with hematoxylin and eosin, examined and scored by 2 experienced pathologists separately. Each prostate specimen had 4 qualified slides (satisfactorily stained, enough tissue for estimation). Each pathologist scored every slide separately based on the 3 aspects: infiltration of inflammatory cells, proliferation of epithelium and stroma, and inflammatory secretion within the lumens. Every aspect was divided into 4 degrees: severe, moderate, mild and normal, which were scored as 3, 2, 1 and 0 separately. Every given slide had 2 scores, and the mean of the 2 scores is the final score for this slide. The mean of the 4 slide's scores in a given prostate is the final score for this prostate. All slides were assessed under 100× microscope magnification.

4. Statistic Analysis

SPSS 17.0 was applied for statistic analysis in this study. Means and standard deviations were calculated for all groups. The ANOVA of Tukey-HSD Test was used to inspect the significant difference of the interval body weight changes, the prostate weight, and the histomorphometric scores in all groups. P value <0.05 was considered significant.

RESULTS

1. Changes in Body and Prostate Weight

The mean initial body weight in the Experiment Group A, B, C and Control Group were 30.59 ± 1.66 g, 30.39 ± 1.36 g, 30.67 ± 1.91 g and 30.51 ± 1.91 g, respectively. After 3 and 6 weeks of treatment with or without stimuli of passive sexual abstinence, the mean body weights in all groups have increased during the experiment (Table 1). The ANOVA of Tukey-HSD Test did not manifest significant statistic differences among all groups at 0, 3 and 6 weeks respectively ($P > 0.05$) (Table 2). After 6 weeks of the treatment, the mean prostate weights in the Experiment Group A, B, C and Control Group were 41.24 ± 2.89 mg, 40.42 ± 2.61 mg, 41.24 ± 2.12 mg and 41.22 ± 3.42 mg, respectively (Table 1). The ANOVA of Tukey-HSD Test neither manifest significant statistic differences among prostate weights of all groups nor between any two groups ($P > 0.05$) (Table 2).

2. Histologic and Morphometric Changes

Histologically, both the ventral and dorsolateral prostates from the experiment groups have shown more microscopic inflammatory changes in some degree, comparing with control group. The mean histomorphometric scores of the Experiment Group A, B, C and Control Group were 6.90 ± 2.38 , 4.35 ± 3.22 , 5.25 ± 3.21 , and 1.35 ± 1.51 respectively. The ANOVA Test manifested that the histomorphometric scores of Experiment Group A, B and C had significant statistic differences, comparing with the Control Group ($P < 0.05$) (Table 3). Further ANOVA of Tukey-HSD Test within experiment groups failed to showed significant statistic differences of histomorphometric scores between Experiment Group A and B ($P = 0.164$, $P > 0.05$), Group A and C ($P = 0.522$, $P > 0.05$), or Group B and C ($P = 0.876$, $P > 0.05$) (Table 4). The ANOVA of Tukey-HSD Test confirmed significant statistic differences of histomorphometric scores between Experiment Group A, C and the Control Group ($P < 0.05$, and $P = 0.013$ respectively), while comparison between Group B and the Control Group had a P value close to test criterion ($P = 0.076$), which could be caused by the small sample size of this study. Moderate to severe focal inflammatory changes were observed in slides of a score over 4. The glandular cavity lost its normal morphous, with foci of both epithelial and stromal hyperplasia. Infiltration of inflammatory cells, and prostatic concretion could be observed in the lumens atypically and locally (Fig 1-3). 73% of prostate specimens from the experiment groups were scored over 4.

DISCUSSION

A great many of rodent models for prostatitis have been developed, including spontaneous prostatitis models, infectious prostatitis models, immune-induced prostatitis models, hormone-associated prostatitis models and some other miscellaneous prostatitis models. Significant progress in unraveling the underlying mechanisms of prostatitis has been made since the time of Young HH in 1906 [Young et al., 1906]. The prostatitis in most of the current rodent models were induced by different agents that administrated subcutaneously [Keetch et al., 1994; Rivero et al., 2002], intraventrally [Kaplan et al., 1983], transurethrally [Nickel et al., 1990] or orally [Kwon et al., 2001]. In this research, a state of sexual abstinence was imitated to induce the prostatitis in mice; meanwhile the mice were kept in their normal physiological state as much as possible. There was no significant statistic difference of body weights of mice between the experiment groups and the control group at 0 week ($0.986, >0.05$), 3 week ($P=0.336, >0.05$) and 6 week ($P=0.089, >0.05$), and the comparison between subgroups within the experiment groups did not show any differences in the aspect of body weight either, suggesting that the stimuli in the experiment groups did not have significant effect in the development of mice, and excluding that the prostatitis in the experiment groups was induced by effect of strong stress or irritation, such as starvation, low surrounding temperature or small space [Aronsson et al., 1988].

The histology and morphometric analysis of this research showed microscopic inflammatory changes in the prostate of mice. The principal change is the focal hyperplasia in epithelium and stroma, while infiltration of inflammatory cells in epithelium and stroma was not commonly observed (Fig 1-3). The mean weights of prostate in experiment groups had no difference from the control group ($P=0.888, >0.05$). The result suggests that stimuli of passive sexual abstinence do not induce significant changes of benign prostatic hyperplasia but chronic inflammation in Kunming mice. The histomorphometric scores of experiment groups is significant different from the control group ($P<0.05$), and Group A has a higher mean score (6.90 ± 2.38) than the other two experiment groups (4.35 ± 3.22 and 5.25 ± 3.21 , respectively). This result consists with our design for the experiment, in which Group A have the strongest stimulus. However, in the comparison within experiment groups in aspect of pathological scores, this research failed to manifest the difference between Group A and B, Group A and C, and Group B and C--the time-effect of copulation is still unclear in this research and requires some further work.

Many efforts have been made to develop reliable experimental animal models of prostatitis for the discovery of molecular pathways, genetic influences, environmental factors, and successful management strategies for the treatment of prostatitis in humans. For the discussion of the underlying mechanism of our current prostatitis model, there is lack of further research of serum biochemical or immunohistochemical test on mice under the stimuli of passive sexual abstinence, but basing on the revealed mechanisms of the current non-bacterial prostatitis rodent models, we presume that the following factors may play important roles in the induction process of prostatitis in Kunming mice of this research.

1. Immune Response on Prostate Antigen

In current immune-induced prostatitis models, single or multiple immunization of homogenate of rat male sex accessory glands [Rivero et al., 1998], rat prostate tissue [Rivero et al., 2002], mouse prostate homogenate [Keetch et al., 1994], purified mouse or rat prostate steroid binding protein (PSBP) [Rivero et al., 2002; Penna et al., 2006], and synthetic mouse PSBP peptides [Rivero et al., 2002; Penna et al., 2006] with complete Freund's adjuvant (CFA) induced different incidence (30%-100%) of mononuclear cell prostatitis. Accumulation of circulating auto-antibodies to prostate antigens has been observed in these models, and the auto-antibodies are high cell adaptively transferable.

In our research, the mice in experiment groups under the stimuli of passive sexual abstinence failed to have regular drainage of prostate glands, resulting in the disturbance of metabolism of the prostate and the accumulation of prostate antigen like PSBP in the rodent prostate [Heyns et al., 1977], which presented as the accumulation of inflammatory prostatic concretion in glandular cavity of the mice prostate from our experiment groups. The local or systemic immune response on prostate antigen may induce the non-bacterial prostatitis consequently, however this need to be confirmed by further immunological research.

2. Chronic Pelvic Congestion

A survey of 2,554 urologic outpatients showed that there was correlation between chronic prostatitis syndrome and pelvic venous disease [Pavone et al., 2000]. Distention of venous plexus of prostate peripheric zone or chronic congestion of pelvic cavity caused by prolonged sitting or inadequate sexual life can be observed in a certain number of prostatitis patients, suggesting that the chronic prostatitis syndrome correlates with chronic pelvic congestion [Pavone et al., 2000].

In the process of a sexual impulse in human, the pelvic congestion will regress in about 15-30 minutes after an orgasm, or may last longer without an orgasm. In this research, the mice in experiment groups may be in the long-term repeated state of pelvic congestion, which results in the over-congestion of prostate and helps to accelerate the process of prostatitis.

3. Disturbance of Hormone Regulation

The prostate gland is an androgen-dependent organ in males. The androgen plays a key role in the regulation of prostatic growth, function and disease. Supra-physiological doses of exogenous testosterone do not stimulate additional prostate growth [Naslund et al., 1986], while estrogen administered subcutaneously is a classic induction method of prostatitis in rodents [Robinette et al., 1988; Naslund et al., 1988]. Research on periodic changes in human serum testosterone levels after ejaculation in men showed that a peak of serum testosterone appeared on day 7 of sexual abstinence, which would have negative feedback on the release of LH [Catt et al., 1980; Finkelstein et al., 1991], and result in the decrease of the release of testosterone, but the changes of estrogen is not clear in such situation [Keenan et al., 1998; Pierro et al., 1999]. It has been demonstrated that testosterone alone could block 17 β -estradiol-induced prostate inflammation [Pakarainen et al., 2005], thus testosterone may be a protective factor in the pathogenesis of prostatitis. Mice under the stimuli of passive sexual abstinence may have the similar changes in serum testosterone levels as humans and may lose the protection from certain level of testosterone, and the disturbance of hormone regulation may help in the process of prostatitis, which need to be confirmed by further serum biochemical research work.

CONCLUSIONS

Our results have shown the feasibility of inducing nonbacterial prostatitis in Kunming mice by long-term stimuli of passive sexual abstinence. Meanwhile the normal physiological state of the mice has been kept in the process of induction. The analysis of the result suggests that copulation might be a protective factor in the process of non-bacterial prostatitis. This mouse model may be used for additional studies to discover molecular pathways, genetic influences, environmental factors, and management strategies for nonbacterial prostatitis. The underlying mechanisms of this model may include immune response on prostate antigen, chronic pelvic congestion, and disturbance of hormone regulation and others, while requires confirmation from further research.

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FIGURE LEGENDS

Histologic and Morphometric Changes of Prostate from Mice in Experimental Groups and Control Group.

Figure 1, 2. (100× microscope magnification) The glandular cavity lost its normal morphous, with foci of both epithelial and stromal hyperplasia. Infiltration of inflammatory cells, inflammatory prostatic concretion could be observed in the lumens (Slide's Score 7-9).

Figure 3. (100× microscope magnification) Focal hyperplasia in prostate epithelial with relatively normal stroma and normal prostatic concretion in the lumens (Slide's Score 4-6).

Figure 4. (100× microscope magnification) Normal prostate tissue without hyperplasia or infiltration of inflammatory cells (Slide's Score 0-3).

TABLES

Table 1. Changes of Body and Prostate Weight in Adult Male Kunming Mice

Group	No. Mice	Body Weight (g)						Prostate Weight (mg)	P
		0 Week	P	3 Week	P	6 Week	P		
A	10	30.59±1.66		34.25±2.45		38.04±3.08		41.24±2.89	
B	10	30.39±1.36	0.986,	34.76±1.27	0.336,	39.88±1.56	0.089,	40.42±2.61	0.888,
C	10	30.67±1.91	>0.05	35.63±1.77	>0.05	40.01±1.64	>0.05	41.24±2.12	>0.05
Control	10	30.51±1.91		35.30±1.36		40.37±1.99		41.22±3.42	

Table 2. ANOVA of Tukey-HSD Test on Changes of Body and Prostate Weight in Adult Male Kunming Mice

Groups	P of Body Weight			P of Prostate Weight
	0 Week	3 Week	6 Week	
A, B	0.994	0.918	0.242	0.913
A, C	1.000	0.320	0.191	1.000
A, Control	1.000	0.556	0.092	1.000
B, C	0.983	0.695	0.999	0.913
B, Control	0.999	0.904	0.957	0.919
C, Control	0.997	0.976	0.982	1.000

Table 3. Histomorphometric Scores of Prostate in Adult Male Kunming Mice

Group	No. Mice	Mean	SD	F	P
A	10	6.90	2.38		
B	10	4.35	3.22	7.547	<0.05
C	10	5.25	3.21		
Control	10	3.21	1.51		

Table 4. ANOVA of Tukey-HSD Test on Histomorphometric Scores of Prostate in Adult Male Kunming Mice

Groups	Mean Difference	SE	P
A, B	2.55		0.164, >0.05
A, C	1.65		0.522, >0.05
A, Control	5.55	1.198	<0.05
B, C	0.90		0.876, >0.05
B, Control	3.00		0.076<0.05
C, Control	3.90		0.013, <0.05

FIGURES

Figure 1

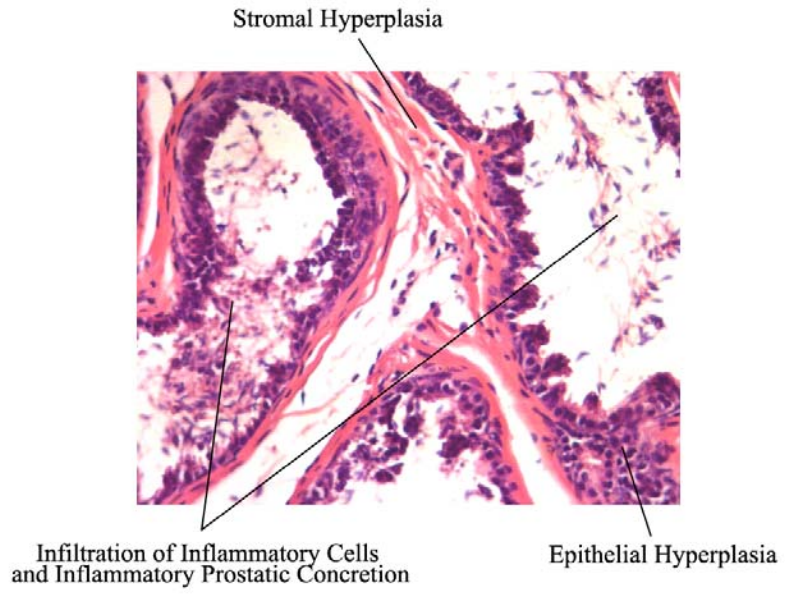
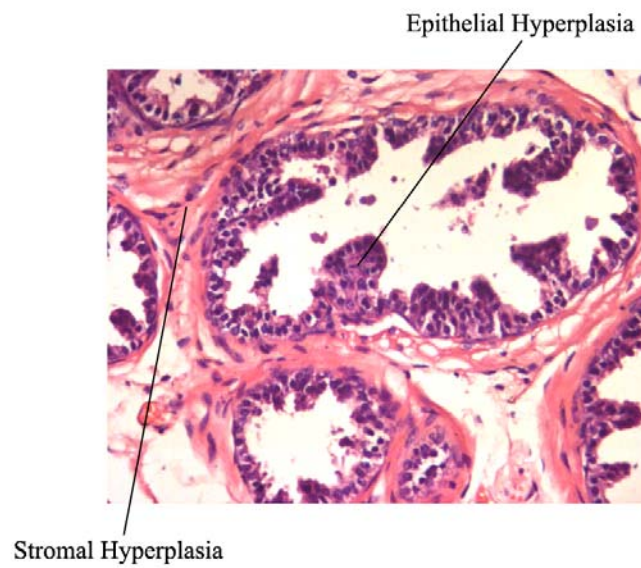


Figure 2



FIGURES

Figure 3

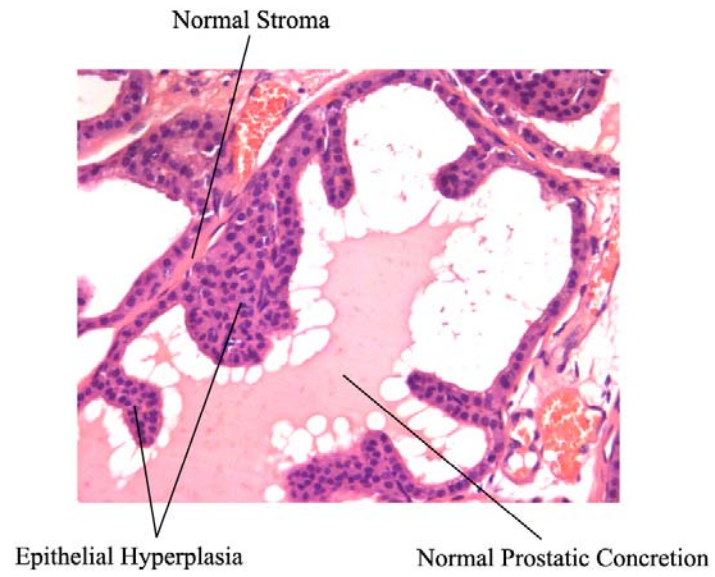


Figure 4

