A Study on Prostate Inflammation in SD Rats After Castration Under the Effect of Estrogen/androgen at Different Concentrations

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Research article

Keywords: estrogen, androgen, benign prostatic hyperplasia, prostatitis

Posted Date: January 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-150925/v1

Abstract

Background: To clarify the expression of histological inflammation and major inflammatory factors in prostate of castrated rats induced by different concentrations estrogen/ androgen.

Methods: Male Sprague-Dawley (SD) rats aged 3-4 months were randomly divided into the blank group (sham operation group, bilateral testicular specimens were retained), and the castration group (surgical removal of bilateral testes) and different concentrations of estrogen/androgen treatment after castration. Dihydrotestosterone (DHT) and estradiol (E) were administered daily by subcutaneous injection for one month, and the rats in each group were sacrificed by neck-broken method after one month. Obtained prostate specimens by surgery, and performed routine paraffin embedding and sectioning of prostate tissue. Observed the changes of prostate tissue structure and prostate inflammation under light microscope after Hematoxylin-eosin (HE) staining. Immunohistochemical method was used to detect the expression of TGF-β1, IL-6 and IL-8 in the rats prostate tissues.

Results: After castration, when the exogenous E concentration was constant, the exogenous DHT(0-0.15mg/kg) concentration of SD rats in each group increased gradually, and the anatomical position score of inflammatory cell infiltration in each group of rats gradually increased. Further, even if the DHT concentration increased again after the exogenous DHT concentration reached at 0.5mg/kg, the score did not increase but decreased insteadly. From the area of tissues involved in inflammatory cell infiltration and the density of typical inflammatory cells, the inflammation score of each group of rats increased gradually with the increase of DHT concentration. When the exogenous DHT concentration was constant, from the anatomical location and the area of tissues involved in inflammatory cell infiltration in each group of SD rats, the inflammation score of each group of rats increased gradually with the increase of exogenous E concentration. The results of the immunohistochemical reaction showed that the positive rates of TGF-β1, IL-6 and IL-8 in SD rats after castration were higher than those in the blank group, and the positive rate of TGF-β1 was statistically significant compared with the blank group (P<0.05). When the concentration of exogenous E was constant, the positive rates of TGF-β1 and IL-8 in each group of DHT0.015-0.5mg/kg increased with the increase of the concentration of exogenous DHT. However, even if the exogenous DHT concentration increased again after the exogenous DHT concentration exceeded 0.5mg/kg, the positive rates of TGF-β1 and IL-8 in the E0.05+DHT1.5 group did not increase with the further increase of exogenous DHT, but decreased to a certain extent. In addition, when the exogenous DHT concentration was constant, the exogenous E concentration was gradually increased, and the positive rates of TGF-β1, IL-6 and IL-8 in SD rats in each group increased to some extent with the increase of exogenous E concentration.

Conclusions: Sex hormone levels are involved in the regulation of prostate inflammation in SD rats. Different levels of estrogen and androgen have different levels of inflammatory response to prostate inflammation and the expression of TGF-β1, IL-6, IL-8 in castrated SD rats, and the positive expression of TGF-β1, IL-6, IL-8 can reflect the inflammation of prostate tissue in SD rats to a certain extent. In addition, there may be an inflection point between the ratio of estrogen/ androgen and prostate inflammation.

After crossing this inflection point, the inflammation of the prostate did not further deepen even if the concentration of exogenous androgens increased again. Of course, it needs to be confirmed by more systematic and comprehensive experiments in vivo and vitro.

Background

Benign Prostatic Hyperplasia(BPH) is one of the most common diseases in middle-aged and elderly males. Although the pathogenesis of BPH was not very clear at present, the role of sex hormone was a broad consensus in the academic community. While the breaking of the balance of estrogen/ androgen ratio was considered to be a key link in the pathogenesis of BPH. In addition, more and more scholars believed that BPH was essentially an immune inflammatory disease. It has been found that prostatic stromal cells were the main targets of inflammatory factors, and can also secrete a variety of inflammatory factors and increase the occurrence of inflammation. Under certain conditions, it can express and secrete inflammatory factors such as TGF-β, IL-6, IL-8, EGF, bFGF and IGF-1[1]. Moreover, those inflammatory cytokines can also react on prostate stromal cells and other cells to secrete a large amount of inflammatory cytokines and recruit inflammatory cells to invade prostate tissues, thus, inducing inflammatory response[2, 3]. Therefore, when the inflammation of the prostate tissues developed to a certain degree, the cells of the prostate tissues, the inflammatory cells and pro-inflammatory factors will promote each other to make the inflammatory response cycle enlarged continuously. A large number of inflammatory cells and inflammatory factors constituted the inflammatory microenvironment in the prostate tissues, and induced the dysfunction of prostate cells proliferation and apoptosis, which lead to the remodel of various cells and extracellular components in the prostate and initiated the occurrence of BPH.

At present, the relationship between sex hormones and inflammation of the prostate was still very subtle. However, there was no systematically research on the relationship between sex hormone levels and prostate inflammation. Castration combined with estrogen-induced rats prostatitis was a commonly used animal model of chronic non-bacterial prostatitis. In this method, castration combined with estrogen caused imbalance of hormone levels in animals, and the balance of estrogen/ androgen was destroyed, which made the prostate producing a non-bacterial inflammatory response[4]. In the previous studies, we found that estrogen can significantly promote the expression of inflammatory factors and collagen of prostate epithelial cells. In addition, we used the 2 μm thulium laser to resect part of the prostate of male beagle dogs[5]: 1. After castration, the inflammation of the beagle dogs' prostate wounds were relatively mild. 2. The inflammatory response of beagle dogs' prostate wounds increased significantly after the androgen treatment. The purpose of this study was to clarify the expression of histological inflammation and major inflammatory factors in prostate of castrated rats induced by different concentrations estrogen/ androgen. And it provided a new theoretical basis and method for systematically to reveal the relationship among sex hormone levels, prostate inflammation and the pathogenesis of BPH.

Methods

1.1 Experimental Animals

Fifty-three male SD rats, aged from 3 to 4 months and weighing 250 g-350 g, were provided by the Experimental Animal Center of Guizhou Medical University. License number: SCXK (Guizhou) 2012-0001. The disposal process of animals conformed with the "Opinions under the Guidance of Treating Experimental Animals". All animals (two rats in each cage) were kept in the light cycle from 08.00 to 20.00 at room temperature of 20 ~ 26°Cand relative humidity of 40–70%. During the feeding period, the animals can freely take in water and food. This experiment was approved by the Ethics Committee of Guizhou Provincial People's Hospital (No. 2018025).

1.2 Castration of male rats

Male SD rats aged 3–4 months were anesthetized by intraperitoneal injection of 0.2 ml/100 g sodium pentobarbital. After satisfactory anesthesia, the rats were fixed supine on the anatomical table, and the surgical field of the scrotum was disinfected with iodophor. The scrotal incision was about 1-2cm long, and the skin and flesh membrane were cut open layer by layer to the testicular sheath, one testicle was squeezed out from the incision, and the spermatic cord was ligated and then broken. The contralateral testis was removed in the same way, and each layer was closed in turn. Penicillin was injected intramuscularly at 50,000U/(kg/d) on the day of surgery and two consecutive days after surgery, and the wounds healed well one week after surgery.

1.3 The rats after castration were randomly divided into:

1.3.1 Four rats in the blank group (sham operation group, bilateral testis were kept) were injected with corn oil every day for one month.

1.3.2 In the castration group (surgical removal of bilateral testis), four rats were injected with corn oil every day for one month.

1.3.3 After castration (surgical removal of both testes), and there were four rats in different concentrations estrogen/androgen treatment groups, including: DHT0.15 + E(0-0.5) group and E0.05 + DHT(0-1.5) group.

1.4 Inflammatory response in prostate tissues of rats in each group

HE staining light microscopy was used to observe the morphology of prostate tissue in rats(Supplement Fig. 1 and Fig. 2), and the evaluation of histopathological inflammation of the prostate refers to the International Prostatitis Diagnosis and Grading Standard[6], including the anatomical location of inflammatory cell infiltration(Anatomical location), the tissue area involved by inflammatory cell infiltration (Extent) and the density of typical inflammatory cell infiltration (Morphological description).

1.5 Expression of TGF- 1, IL-6 and IL-8 in prostate tissue

The image analysis system of image-J software was used to detect and analyze the prostate tissue in each group. Five randomly selected fields in the 40×10 field of view were used for quantitative analysis of the positive rate of inflammatory factors in rats in each group.

1.6 Statistical Methods

The experimental data were statistically processed by SPSS24.0 software. The Kruskal Wallis Test was used to analysis the inflammation score of prostate histology, and one-way analysis of variance was used to analysis the positive rates expression of inflammatory factors. A value of $p < 0.05$ was considered significant.

Results

2.1 General situation of rats in each group

SD rats were generally in good condition during feeding in each group. There was no obvious abnormality in appearance, physical signs and behavioral activities. Food intake, drinking water, and bowel movements were normal. After the operation, the 4 rats in the sham operation group were generally in good condition. A total of 49 cases underwent castration operation, among which one rat died after operation. The autopsy revealed that the vascular ligature was loose during the operation and the postoperative hemorrhagic shock died. Except for that, there was no death or postoperative wound infection during the experiment.

2.2 Evaluation of histological inflammation of the prostate of rats in each group

With reference to the international prostatitis histological diagnosis and grading standards, and evaluated the anatomical location of the infiltration of prostatitis cells in each group of rats (Anatomical location Table 1). The infiltration scope of inflammatory cells in the blank group, the castration group, the E0.05 + DHT0 group and the DHT0.15 + E0 group were relatively limited, which mainly located in the duct/ glandular epithelium and/ or lumen. Inflammatory cells infiltration in the E0.05 + DHT0.015 group, the E0.05 + DHT0.05 group, the E0.05 + DHT1.5 group and the DHT0.15 + E0.015 group were mainly located in the matrix, with ducts/glands as the center, and close to duct/gland within 50 μm. Inflammatory cells were mainly located in the prostate stroma in the E0.05 + DHT0.5 group, the DHT0.15 + E0.05 group, the DHT0.15 + E0.15 group and the DHT0.15 + E0.5 group, while not in the prostate/catheter and the distance from them were ≥ 50 μm. After the rats were castrated, when the exogenous E concentration remained constant, the inflammation score of the rats in each group increased gradually with the increase of exogenous DHT concentration (0-0.15 mg/kg). However, even if the DHT concentration was increased again after the exogenous DHT concentration reached at 0.5 mg/kg, the score did not increase further but decreased. In addition, when the concentration of exogenous DHT was constant and the concentration of exogenous E increased accordingly, the inflammation scores of rats in each group increased gradually with the increase of E concentration. Among them, compared with blank group and castration group, the

DHT0.15 + E0.05 group, the DHT0.15 + E0.15 group and the DHT0.15 + E0.5 group were statistically significant ($P < 0.05$).

With reference to the international prostatitis histological diagnosis and grading standards, the tissue area involved by the infiltration of prostatitis cells in each group of rats were evaluated(Extent, Table 2). The tissue areas affected by inflammatory cells infiltration were less than 10% in the blank group, the castration group and the E0.05 + DHT0 group. In the E0.05 + DHT0.015 group, the E0.05 + DHT0.05 group, the DHT0.15 + E0.005 group, the DHT0.15 + E0.015 group and the DHT0.15 + E0.15 group, the tissue area affected by inflammatory cells infiltration were mainly between 10%-50%. The tissue areas affected by inflammatory cells infiltration were mainly > 50% in the E0.05 + DHT0.15 group, the E0.05 + DHT0.5 group, the E0.05 + DHT1.5 group, the DHT0.15 + E0.05 group and the DHT0.15 + E0.5 group. After the rats were castrated, when the exogenous E concentration remained constant (E 0.05), the inflammation score in each group of rats increased gradually with the increase of exogenous DHT concentration. Among them, compared with the blank group and the castration group, the tissue areas involving inflammatory cell infiltration were significantly increased in the E0.05 + DHT0.05 group, the E0.05 + DHT0.15 group, the E0.05 + DHT0.5 group and the E0.05 + DHT1.5 group($P < 0.05$). When the exogenous DHT concentration was constant (DHT 0.15), the tissue areas involved in inflammatory cell infiltration were significantly greater in the DHT0.15 + E0.05 group, the DHT0.15 + E0.15 group and the DHT0.15 + E0.5 group than the blank group and the castration group($P < 0.05$).

With reference to the international prostatitis histological diagnosis and grading standards, the density of typical inflammatory cells infiltration in the prostate of each group of rats were evaluated (Morphological description, Table 3). The blank group, the castration group and the E0.05 + DHT0 group were mainly showed low density(single inflammatory cells), most of which were separated by different interventional spaces (< 100). The DHT0.15 + E0 group was mainly showed moderate density(fusion of inflammatory cell sheets), and no tissue destruction or lymph node/ follicle formation (100–500). The E0.05 + DHT0.15 group, the E0.05 + DHT1.5 group, the DHT0.15 + E0.05 group and the DHT0.15 + E0.5 group were mainly showed high density, and the fused inflammatory cell sheet has organization destruction or nodule/hair follicle formation (> 500). The E0.05 + DHT0.015 group and the E0.05 + DHT0.05 group were mainly in low and medium density. And the E0.05 + DHT0.5 group and the DHT0.15 + E0.005 group were mainly in medium and high density. In addition, after the rats were castrated, when the concentration of exogenous E was constant (E 0.05), the density of typical inflammatory cells infiltration in the E0.05 + DHT0.15, the E0.05 + DHT0.5 and the E0.05 + DHT1.5 groups were significantly higher than in the blank group and the castration group($P < 0.05$). When the concentration of exogenous DHT was constant (DHT0.15), the density of typical inflammatory cells infiltration in theDHT0.15 + E0 group, the DHT0.15 + E0.005 group, the DHT0.15 + E0.05 group and the DHT0.15 + E0.5 group were significantly higher than the blank group and the castration group($P < 0.05$).

2.3 Expression of TGF-β1, IL-6 and IL-8 in prostate tissue of rats.

The positive rates of inflammatory factors in each group of SD rats were quantitatively analyzed, and the results were shown in Fig. 3 and Fig. 4. The positive rates of TGF-β1, IL-6, and IL-8 in SD rats after castration were higher than those in the blank group, and the positive rates of TGF-β1 were statistically significant compared with the blank group ($P < 0.05$). After castration of SD rats, when only exogenous E (0.05 mg/kg) was given, the positive rates of TGF-β1 in the E0.05 + DHT0 group were lower than the castration group ($P < 0.05$). However, the positive rate of IL-6 and IL-8 were slightly higher than the castration group ($P > 0.05$). In addition, when SD rats were castrated with only exogenous DHT (0.15 mg/kg) stimulation and no exogenous E supplementation, the positive rates of TGF-β1, IL-6 and IL-8 in the DHT0.15 E0 group decreased compared with the castrated group, and the positive rate of TGF-β1 was statistically significant compared with the castrated group ($P < 0.05$). When the concentration of exogenous E was constant, the positive rates of TGF-β1 and IL-8 in E0.05 + DHT0.015 group, the E0.05 + DHT0.05 group, the E0.05 + DHT0.15 group and the E0.05 + DHT0.5 group increased with the increase of exogenous DHT concentration. But when the exogenous DHT concentration exceeded 0.5 mg/kg, even if the exogenous DHT concentration increased again, and the positive rate of TGF-β1 and IL-8 in E0.05 + DHT1.5 group did not increase with the further increase of exogenous DHT. On the contrary, compared with the E0.05 + DHT0.5 group, the positive rates of TGF-β1 and IL-8 in the E0.05 + DHT1.5 group decreased to a certain extent. In addition, when the concentration of exogenous DHT was constant, the positive rates of TGF-β1, IL-6 and IL-8 in each group of SD rats increased to a certain extent with the increase of exogenous E concentration. Among them, the positive rates of TGF-β1, IL-6, and IL-8 in the DHT0.15 + E0.05 group and the DHT0.15 + E0.5 group were statistically significant compared with the blank group and the castration group ($P < 0.05$). In addition, the positive rates of TGF-β1 in the DHT0.15 + E0.15 group were statistically significant compared with the blank group and the castration group ($P < 0.05$), and the positive rates of IL-6 and IL-8 were statistically significant compared with the blank group ($P < 0.05$), but there was no statistical significance compared with the castration group ($P > 0.05$).

Discussion

BPH was a ubiquitous chronic progressive disease[7]. Aging and the presence of androgen are considered as essential links for the development of BPH, but the exact pathogenesis of BPH was still unclear[8, 9]. In recent years, the role of sex hormone level has been paid more and more attention to scholars. In particular, the synergy between estrogen and androgen played a prominent role in the pathogenesis of BPH. In vitro studies, King et al.[10] found that increasing the ratio of estrogen to androgen could promote the proliferation of prostate stromal cells and epithelial cells. Current research found that there was a balance mechanism between estrogen and androgen in the body. Maintaining a dynamic balance between estrogen and androgen levels contributes to the normal growth and development of the prostate and the maintenance of physiological functions, while breaking the original balance may be the cause of changes in prostate proliferation and apoptosis, leading to BPH[11].

Inflammation was an important defense response for the body to resist pathogen invasion and repair tissue damage. However, excessive or uncontrolled inflammatory response could also cause tissue

damage, becoming the basic pathological mechanism for the occurrence and development of diseases. Inflammation also played a very central role in the development of various prostate diseases including BPH[12]. Compared with normal prostate, almost all prostate biopsy and surgical tissues had a large number of inflammatory factors infiltrated[13]. The IPSS, the prostate volume and the serum PSA were relatively higher in patients with more severe inflammation[14], and the degree of chronic inflammation was also inseparable from the clinical progress of BPH[15]. It has been suggested that elevated levels of lymphocyte-derived cytokines, such as IL-2, IL-4, and IFN-γ, could be found in the resected BPH tissues. These inflammatory factors seem to be involved in the stimulation of prostate fibromuscular growth[16]. In addition, BPH and chronic prostatitis could mutually induce, promote, and aggravate disease progression, and formed a vicious circle[17].

The interaction between androgens and inflammation played an important role in the pathogenesis of many tumors. In animal models, androgens could decrease the concentration of circulating pro-inflammatory factors and increase the levels of cytokines with anti-inflammatory effects[18]. Preclinical studies have shown that the inflammation and tissue structure reconstruction in the prostate of males with hypogonadism were more serious, but this phenomenon disappeard with the addition of androgen[19]. In addition, compared with people with normal gonadal function, patients with hypogonadism were five times more likely to have inflammation in the prostate gland, and the degree of inflammation was often more severe. Naslund et al.[20]found that oral estrogen significantly increased the degree of non-bacterial inflammation of the prostate in rats. Therefore, the academic community gradually realized that some inflammatory response genes in the body were regulated by sex hormones, but how the level of sex hormones in the body affects the expression of these inflammatory response genes was still poorly understood.

In this study, we used castration combined with different ratios of estrogen/ androgen to successfully induce the inflammatory response in SD rats. At the same time, the inflammatory response in the prostate tissue of each group of SD rats were systematically evaluated according to the international prostatitis histological diagnosis and grading standards. We found that only part of the glands showed a little inflammatory cell infiltration in the blank group. By contrast, the number of inflammatory cells in the glands of the castrated group relatively increased. However, according to the international prostatitis histological diagnosis and grading standards, the blank group and the castration group were scored 1 point in three aspects: the anatomical location of inflammatory cell infiltration, the tissue area of inflammatory cell infiltration and the density of typical inflammatory cells. After the rats were castrated, when the concentration of exogenous E was constant (E0.05 mg/kg), increased the concentration of exogenous DHT (0-0.15 mg/kg) of SD rats in each group. From the perspective of the anatomical location involved in inflammatory cells infiltration, the inflammation scores of rats in each group increased gradually as well. However, when the exogenous DHT concentration reached at 0.5 mg/kg, even if the DHT concentration increased again, the inflammation score did not increase further but decreased. In addition, judging from the tissue area affected by inflammatory cells infiltration and the density of typical inflammatory cells, After the rats were castrated, the source of androgen was blocked.

When a constant dose of exogenous E was given to stimulate, and the exogenous DHT concentration increased gradually, we found that the inflammation score in each group of rats increased gradually with the increase of DHT concentration. Therefore, we believed that when SD rats were given a certain dose of exogenous E concentration stimulation after castration, and increased gradually the exogenous DHT concentration. When DHT was within a certain concentration range, whether it was viewed from the anatomical location of inflammatory cells infiltration, tissue areas affected by inflammatory cells infiltration and typical inflammatory cells density, which could induce the inflammation response in SD rats. However, judging from the performance of the E0.05 + DHT0.5 group and the E0.05 + DHT1.5 group in the anatomical locations involved in inflammatory cell infiltration, there may be an inflection point between the ratio of estrogen/ androgen and the inflammation of the prostate. After crossing this inflection point, the inflammation of the prostate did not deepen even if the concentration of exogenous androgen increased again. Of course, it needs to be confirmed by more systematic and comprehensive experiments in vivo and vitro.

In our clinical work, we have found that BPH usually occured in middle-aged and elderly patients whose androgen levels have been reduced and estrogen levels were relatively high, while rarely occured in young patients with high androgen levels and relatively low estrogen levels. In addition, according to the epidemiological survey data, the incidence of chronic prostatitis was higher in people aged 40–49 and over 60 years old[21, 22, 23, 24]. Therefore, we found that both BPH and chronic prostatitis were prone to occur in middle-aged and elderly patients whose androgen levels have decreased and estrogen levels were relatively increased. Seethalakshmi et al.[25] induced chronic non-bacterial prostatitis in rats by using 17- -estradiol and found that the pathological manifestations of estradiol-induced non-bacterial prostatitis in rats were similar to those of clinical chronic non-bacterial prostatitis. In addition, Kwon et al. [26]successfully induced chronic non-bacterial prostatitis by giving oral soy isoflavones to SD rats, which confirmed that oral estrogen could also cause prostatitis, and further proved that the significance of hormone level changes in the occurrence of prostatitis. However, for middle-aged and elderly patients with low androgen levels, the effects of changes in different levels of estrogen/ androgen on BPH and prostate inflammation were still unclear. In this study, we supplemented the castrated rats with a constant concentration of exogenous DHT(0.15 mg/kg) lower than the physiological dose, and used the different concentrations of exogenous E to stimulate SD rats. Establishing a rat model in which androgen levels were relatively low and estrogen was sequentially increased (that was also in line with the performance of hormone levels in middle-aged and elderly men). We found that when the concentration of exogenous DHT was constant and the concentration of exogenous E increased gradually. SD rats in each groups either from the perspective of the anatomical location of inflammatory cells infiltration involvement ratings, or from the perspective of the regional scale of inflammatory cells infiltration involvement. With the increase of exogenous E concentration, and the inflammatory score in each group showed a gradual increase trend. To a certain extent, the results of this study also reflected from the perspective of animal experiments why BPH and chronic prostatitis rarely occured in young patients with high androgen levels and relatively low estrogen levels. However, it usually occured in middle-aged and elderly patients with relatively low androgen levels and relatively high estrogen levels.

In addition, we used immunohistochemistry to detect the expression of TGF-β1, IL-6, and IL-8 in the prostate tissue of SD rats in each group. After quantitative analysis, we found that: Compared with the blank group, the positive rate of TGF-β1, IL-6, and IL-8 in the prostate tissue of SD rats increased after castration, which indicated that castration or decreased androgen levels could induce the inflammation response of the prostate tissue in SD rats and the expression of TGF-β1, IL-6 and IL-8 could increase to a certain extent. In addition, after the rats are castrated, when the exogenous E concentration was constant, the positive rates of TGF-β1 and IL-8 in the E0.05 + DHT0.015 group, the E0.05 + DHT0.05 group, the E0.05 + DHT0.15 group and the E0.05 + DHT0.5 group all increased with the increase of exogenous DHT concentration to a certain extent. However, when the concentration of exogenous DHT exceeded 0.5 mg/kg, even if the concentration of exogenous DHT increased again, the positive rates of TGF-β1 and IL-8 in the E0.05 + DHT1.5 group did not increase again with the increase of exogenous DHT. While the positive rates of TGF-β1 and IL-8 in the E0.05 + DHT1.5 group decreased to a certain extent compared with the E0.05 + DHT0.5 group. In addition, when the concentration of exogenous DHT was constant, the concentration of exogenous E increased gradually. The positive rates of TGF-β1, IL-6, and IL-8 in each group of SD rats increased to a certain extent with the concentration of exogenous E. Therefore, we believed that TGF-β1, IL-6, and IL-8 were involved in the regulation of prostate inflammation in SD rats, and their positive rate expression varied with the change in the levels of estrogen and androgen in SD rats, and the positive rates expression of TGF-β1, IL-6 and IL-8 could reflect the inflammation in the prostate tissues of SD rats to a certain extent.

Conclusions

In this study, we used castration combined with different ratios of estrogen/ androgen to successfully induce inflammation in SD rats. In addition, we systematically analyzed and evaluated the relationship between sex hormone levels and prostate inflammation and the expression of related inflammatory factors. It is confirmed that the changes of sex hormone levels are involved in the regulation of the inflammatory response in the prostate of SD rats. And TGF-β1, IL-6, and IL-8 were involved in the regulation of prostate inflammation in SD rats, and their positive rate expression varied with the change in the levels of estrogen and androgen in SD rats, and the positive rate expression of TGF-β1, IL-6 and IL-8 could reflect the inflammation in the prostate tissues of SD rats to a certain extent. In addition, there may be an inflection point between the ratio of estrogen and androgen and prostate inflammation. After crossing this inflection point, even increasing the concentration of exogenous androgens, the inflammation of the prostate did not further deepen. Of course, it needs to be confirmed by more systematic and comprehensive experiments in vivo and vitro.

Abbreviations

bFGF=B Fibroblast growth factor

BPH=Benign Prostatic Hyperplasia

DHT=Dihydrotestosterone

E=Estradiol

HE=Hematoxylin-eosin staining

IFN-γ=Interferon-γ

IGF-1=Insulin-like growth factors -1

IL-2=Interleukin 2

IL-4=Interleukin 4

IL-6=Interleukin 6

IL-8=Interleukin 8

PSA=Prostate specific antigen

TGF-β1=Transforming growth beta-1

Declarations

Ethics approval and consent to participate

This experiment was approved by the Ethics Committee of Guizhou Provincial People's Hospital (No. 2018025). The committee approved the requirement for verbal informed consent to be obtained from participants.

Consent for publication

Written informed consent was obtained from all participants..

Competing interests

The authors declare that they have no competing interest.

This study was funded by grants from the National Natural Science Foundation of China (81860141), the Foundation of Health Family Planning Commission of Guizhou Province (Gzwjkj2017-1-032) and QianKeHeChengGuo (2019-4431)

Authors' contributions

BW contributed to the study design, data collection, interpretation and manuscript writing. YT, YB, ZW, BY and DP contributed to data collection and interpretation. GHL and ZLS contributed to data analysis and

manuscript writing. All authors read and approved the final manuscript.

Acknowledgments

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Tables

Table 1 Anatomical locations involving inflammatory cell infiltration

Variable	Location-based grade			Ranks
	1	2	3	
Blank group	4	0	0	11.0
Castration group	4	0	0	11.0
E0.05+DHT				
0	4	0	0	11.0
0.015	1	3	0	25.25
0.05	1	3	0	25.25
0.15	0	0	4	47.5* **
0.5	0	1	3	43.13* **
1.5	0	4	0	30.0
DHT0.15+E				
0	4	0	0	11.0
0.005	2	2	0	20.5
0.015	1	3	0	25.25
0.05	0	0	4	47.5* **
0.15	0	1	3	43.13* **
0.5	0	0	4	47.5* **

Note: Compared with the blank group, *P<0.05. Compared with the castration group, **P<0.05.

Table 2 The extent of inflammatory cell infiltration

Variable	Extent-based grade			Ranks
	1	2	3	
Blank group	4	0	0	8.50
Castration group	4	0	0	8.50
E0.05+DHT				
0	3	1	0	13.25
0.015	1	3	0	22.75
0.05	0	4	0	27.50* **
0.15	0	1	3	42.50* **
0.5	0	1	3	42.50* **
1.5	0	0	4	47.50* **
DHT0.15+E				
0	2	2	0	18.0
0.005	1	3	0	22.75
0.015	1	3	0	22.75
0.05	0	1	3	42.50* **
0.15	0	3	1	32.50* **
0.5	0	0	4	47.50* **

Note: Compared with the blank group, *P<0.05. Compared with the castration group, **P<0.05.

Table 3 The density of infiltration of typical inflammatory cells

Variable	Density-based grade			Ranks
	1	2	3	
Blank group	4	0	0	8.50
Castration group	4	0	0	8.50
E0.05+DHT				
0	4	0	0	8.50
0.015	1	3	0	22.0
0.05	1	3	0	22.0
0.15	0	0	4	46.50* **
0.5	0	3	1	31.50* **
1.5	0	0	4	46.50* **
DHT0.15+E				
0	0	4	0	26.50* **
0.005	0	3	1	31.50* **
0.015	1	2	1	27.0
0.05	0	0	4	46.50* **
0.15	1	2	1	27.0
0.5	0	0	4	46.50* **

Note: Compared with the blank group, *P<0.05. Compared with the castration group, **P<0.05.

Figures

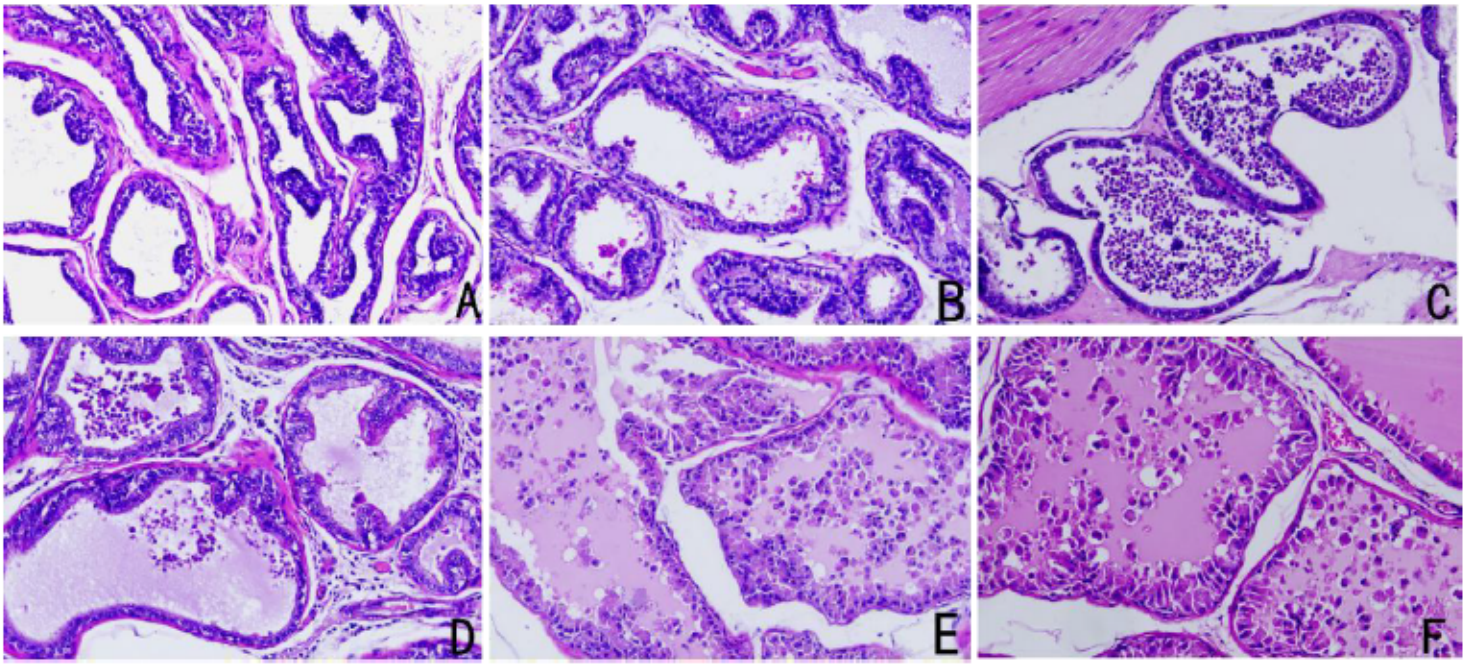

Figure 1

Exogenous estradiol concentration is constant, increasing dihydrotestosterone concentration. Note: A. E0.05+DHT0(×200) B. E0.05+DHT0.015(×200) C. E0.05+DHT0.05(×200) D. E0.05+DHT0.15(×200) E. E0.05+DHT0.5(×200) F. E0.05+DHT1.5(×200)

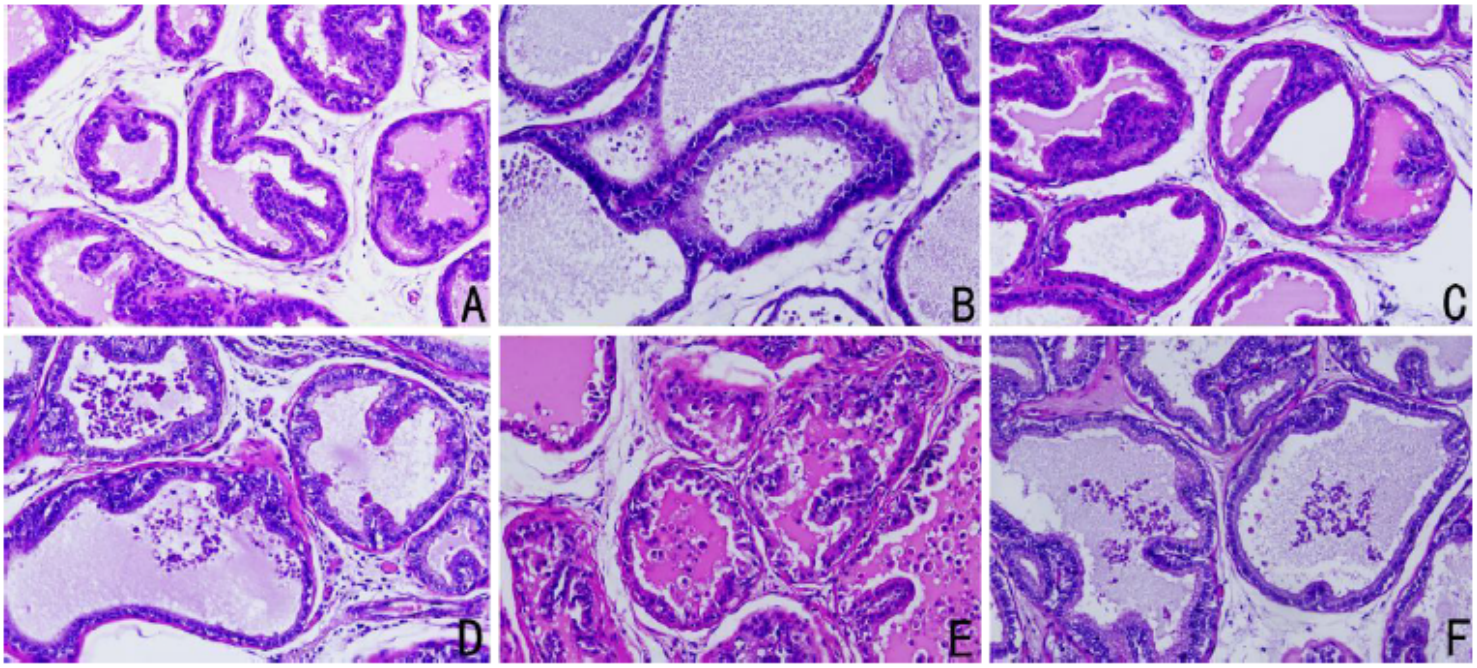

Figure 2

Exogenous dihydrotestosterone concentration is constant, increasing estradiol concentration. Note: A. DHT0.15+E0(×200) B. DHT0.15+E0.005(×200) C. DHT0.15+E0.015(×200) D. DHT0.15+E0.05(×200) E. DHT0.15+E0.15(×200) F. DHT0.15+E0.5(×200)

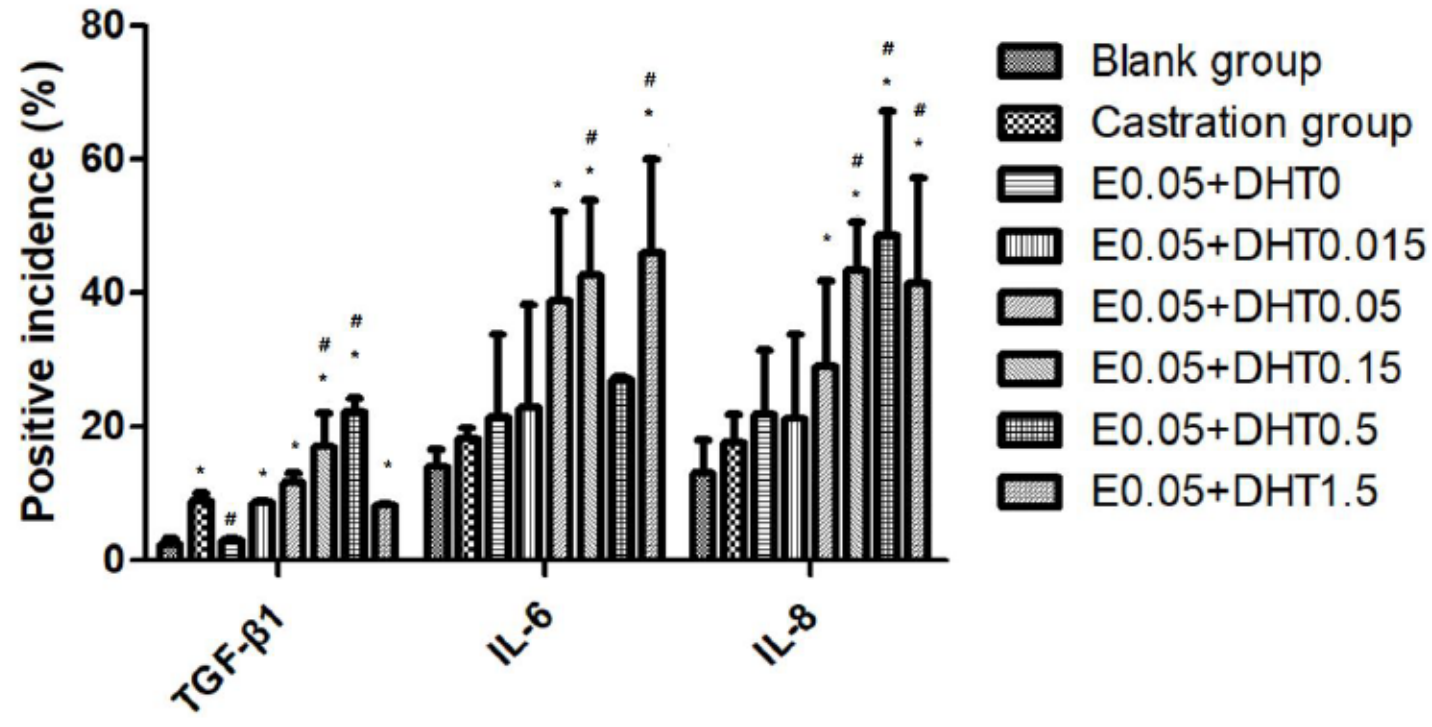

Figure 3

Exogenous estradiol concentration is constant, increasing dihydrotestosterone concentration. Note Compared with the blank group, *P<0.05. Compared with the castration group, #P<0.05.

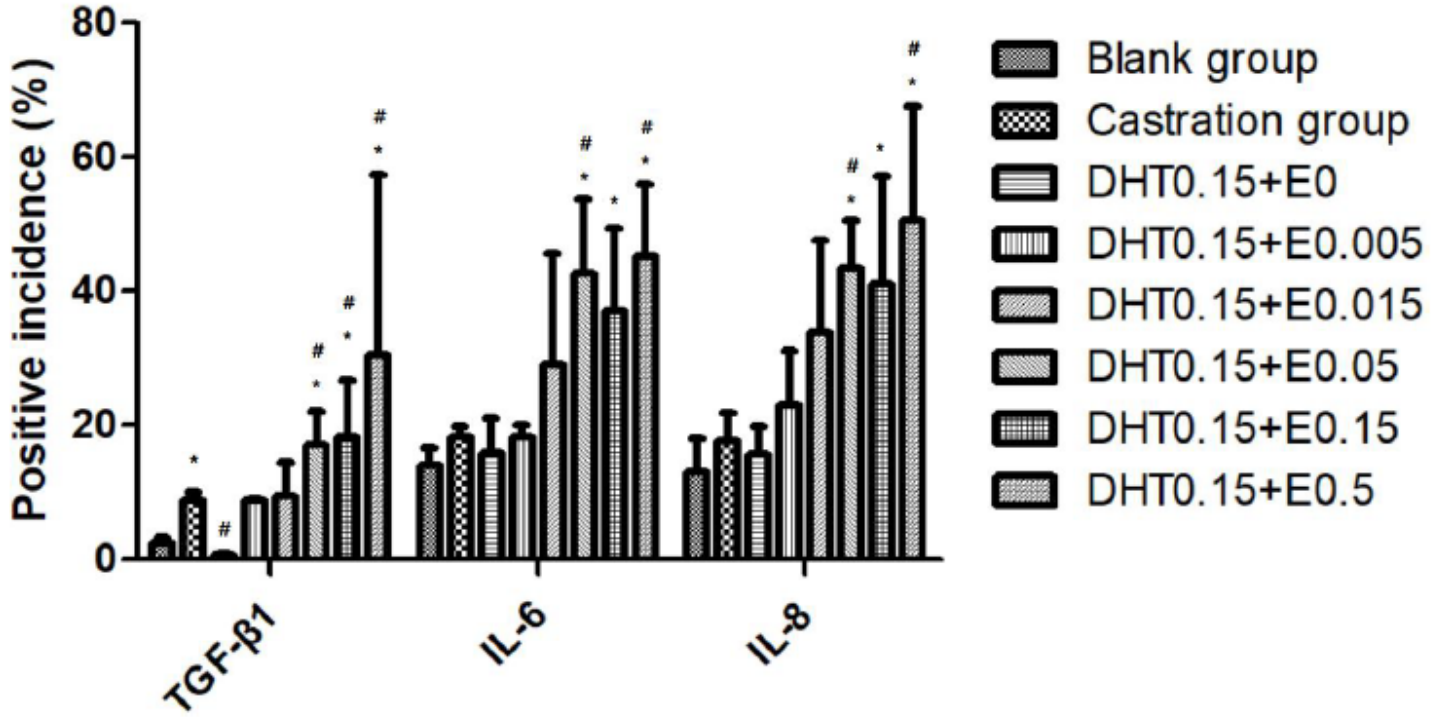

Figure 4

Exogenous dihydrotestosterone concentration is constant, increasing estradiol concentration. Note Compared with the blank group, *P<0.05. Compared with the castration group, #P<0.05.