Establishment of hormone-induced canine benign prostatic hyperplasia model: A prospective, controlled study

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ABSTRACT

Purpose: To evaluate the feasibility of the establishment of a more efficient hormone-induced canine benign prostatic hyperplasia (BPH) model.

Methods: This prospective pilot study included a total of 16 male beagle dogs who underwent dihydrotestosterone and β-estradiol treatment after castration. They were randomly divided into three groups; eight beagles in group A with 1.0 ml hormone treatment, four beagles in group B with 0.8 ml hormone treatment, and four beagles in group C with 1.2 ml hormone treatment, each according to the table of random digit. The size of the prostate was measured using magnetic resonance imaging before and 4, 8, and 12 weeks after intramuscular injection of hormone drugs. Prostate size larger than 18 g was regarded as BPH in dogs.

Results: Beagle dogs in all three groups were successfully modeled for BPH. The experimental group A (n = 8) was successfully modeled after 4 weeks of 1.0 ml hormone treatment, while the control group B (n = 4) was successfully modeled after 8 weeks of 0.8 ml hormone treatment, and the control group C (n = 4) was successfully modeled after 12 weeks of 1.2 ml hormone treatment.

Conclusions: Appropriate dose of hormone can significantly shorten the time of successful establishment of canine BPH model, and an increase in hormone dosage can inhibit prostatic hyperplasia.

1. Background

Benign prostatic hyperplasia (BPH) tends to occur in elderly men, and the incidence rate increases each year from 41% up to 90% [1]. At present, the treatment methods for BPH include drugs, open surgical resection, and transurethral resection of the prostate, but all of them have certain limitations, especially for the elderly patients with a variety of complications [2]. Recently, prostatic artery embolization (PAE), a promising alternative treatment for these patients, has been successfully applied to clinical practice. Although PAE has been widely recognized internationally for the treatment of BPH, a series of problems related to PAE, such as the selection of embolic materials, particle size, and technical scheme, have not been standardized. To explore these problems, basic experimental research is very important, thus establishing an efficient animal BPH model is the key. At present, the commonly used animal models of BPH are spontaneous BPH models (chimpanzee, canine), BPH-induction models (sex hormone-induced model, phenoxyphrine-induced model), and xenograft models [3, 4]. Each different animal model adapts to various experimental studies, but all of them have certain limitations. This study prospectively sets up control groups to explore the effects of different hormone doses on the establishment of a canine BPH model to identify a more efficient method.

2. Materials and methods

2.1. Animals

This prospective, comparative study was conducted in the animal experiment center of our hospital. The study was approved by the ethics review committee of the Chinese PLA General Hospital. Sixteen male beagle dogs (mean age 20 ± 2.8 months with a range of 13–34 months, mean weight 15.4 ± 3.7 kg with a range of 12–18.4 kg) were enrolled in this study and randomly divided into group A (n = 8), group B (n = 4),
and group C (n = 4). The technique of sample selection is according to the table of random digit, and the formula for calculating sample size is

\[ n_{ij} = \left( \frac{Z_{1-a/2} + Z_{1-\beta}}{\sigma_1 + \sigma_2} \right)^2 \]

The inclusion criteria were 1–3 years male beagle dogs with 10–20 kg. The exclusion criteria were beagle dogs with prostatitis or prostate cancer.

2.2. Induction of prostate hyperplasia

To rule out the effects of intrinsic sex hormones, the beagle dogs were castrated by a urologist. Then, the animals were administered Benzylpenicillin (50000 U/kg per day, intramuscular injection) for 3 days to prevent infection. All beagle dogs were treated with hormone drugs after 4 weeks. We dissolved 25 mg of dihydrotestosterone (Duma biotechnological Co., Ltd, Shanghai) and 0.25 mg of β-estradiol (Duma biotechnological Co., Ltd) in 1ml of triolein [5]. According to this ratio, we prepared three specifications of hormone drugs: 0.8 ml, 1 ml, and 1.2 ml. The hormones were injected into the subcutaneous tissue behind the neck three times per week. The doses of hormone drugs in experimental group A, control group B, and control group C were 1 ml, 0.8 ml, and 1.2 ml, respectively. All beagle dogs were given hormone therapy until the prostate size was larger than 18 g or otherwise for maximum 12 weeks.

2.3. MR imaging

After fasting for 24 h, beagle dogs were given an intramuscular injection of sedatives (Zoletil® 50, VIRBAC, France) in 10 mg/kg doses. The first Magnetic Resonance Imaging (MRI, Siemens, Skyra 3.0T) scan of the prostate was performed before the beagle dog was castrated. The subsequent MRI examinations were performed before and after every 4 weeks of the hormone treatment until the size of the prostate (Length * width * height * 0.52) measured was larger than 18 g (Figure 1). A prostate size larger than 18 g was regarded as a measure of BPH in dogs [6].

2.4. Angiography

When the hormone-induced BPH model was established, each beagle dog underwent transcatheter prostatic arteriography before it was euthanized. After anesthesia using the same method described above, the dog was fixed in the supine position, and the femoral artery was cannulated with a 4 Fr vascular sheath (Terumo, Japan) using Seldinger’s technique. Internal iliac arteriography was carried out with a 4 Fr Simmons catheter (Cordis, USA). The prostatic artery was identified and selectively catheterized with a 2.6 Fr microcatheter (Asahi Intecc Co., Japan). Prostatic artery angiography was carried out with manual injection of contrast medium (Figure 2 A-D).

2.5. Histopathologic study

After angiography, beagle dogs in the control groups were euthanized with an overdose of potassium chloride through the vascular sheath. The prostates were harvested and fixed with formalin, and subsequently, the specimens were observed grossly (sectioned axially) and microscopically (stained with hematoxylin and eosin).

2.6. Statistical analysis

The statistical analysis was conducted with SPSS software (version 25; SPSS, Armonk, NY, USA). The success rate of the establishment of a canine BPH model in the three groups at different time points was expressed as percentage. Prostate size was analyzed descriptively and expressed as mean ± standard deviation. The differences in the change in

![Flowchart of the experimental process.](image-url)
Figure 2. T2-weighted MR images and internal iliac arteriography of beagle dogs in group A. A.B. The images were obtained before and 4 weeks after hormone treatment. C.D. Angiography of the bilateral internal pudendal arteries demonstrated the prostatic arteries.

Table 1. Characteristics of dogs and prostate size (g).

| Group/Dog No. | Age (Months) | Weight (Kg) | Prostate Size (g) | Initial | before | 4 weeks | 8 weeks | 12 weeks |
|---------------|--------------|-------------|-------------------|---------|--------|---------|---------|----------|
| A1            | 18           | 12.8        | 15.3              | 7.0     | 21.2   | -       | -       |          |
| A2            | 24           | 15.8        | 24.7              | 6.7     | 28.1   | -       | -       |          |
| A3            | 18           | 17          | 10.1              | 3.4     | 28.0   | -       | -       |          |
| A4            | 13           | 12          | 3.8               | 1.5     | 34.1   | -       | -       |          |
| A5            | 14           | 12.5        | 4.9               | 1.7     | 23.1   | -       | -       |          |
| A6            | 24           | 18          | 5.6               | 2.3     | 18.5   | -       | -       |          |
| A7            | 17           | 13          | 6.4               | 1.9     | 22.3   | -       | -       |          |
| A8            | 20           | 15          | 12.1              | 4.7     | 24.5   | -       | -       |          |
| B1            | 34           | 18.3        | 10.3              | 6.2     | 13.8   | 20.4    | -       |          |
| B2            | 26           | 12.8        | 13.4              | 4.7     | 13.1   | 21.2    | -       |          |
| B3            | 20           | 17          | 7.1               | 3.2     | 12.3   | 19.8    | -       |          |
| B4            | 19           | 16          | 9.6               | 4.3     | 12.5   | 21.4    | -       |          |
| C1            | 26           | 13.6        | 5.0               | 2.2     | 10.7   | 17.7    | 29.4    |          |
| C2            | 28           | 18.4        | 6.8               | 2.9     | 5.2    | 9.1     | 20.2    |          |
| C3            | 18           | 17          | 11.5              | 5.1     | 8.3    | 14.7    | 22.5    |          |
| C4            | 22           | 18          | 7.9               | 3.5     | 6.8    | 11.2    | 19.4    |          |

* The first MRI scan was performed before castrated, the next MRI examinations at the time of before and every 4 weeks after the hormone treatment until the size of prostate >18g.
prostate size within groups and between multiple inter-groups were assessed by Kruskal-Wallis tests.

3. Results

The hormone-induced canine BPH model was successfully established in all beagle dogs. The experimental group A (n = 8, mean prostate size 27.7 ± 9.1 g, range: 18.5–34.1 g) was successfully modeled after 4 weeks of 1.0 ml hormone treatment, while the control group B (n = 4, mean prostate size 20.7 ± 0.7 g, range: 19.8–21.4 g) was successfully modeled 8 weeks after 0.8 ml hormone treatment and the control group C (n = 4, mean prostate size 22.9 ± 4.5 g, range: 19.4–29.4 g) was successfully modeled 12 weeks after 1.2 ml hormone treatment (Table 1). There were no statistically significant differences in baseline indicators (age, weight) among the groups (P > 0.05). However, a significant change in prostate size was observed from pre-hormone to post-hormone treatment (P < 0.01) and statistically significant difference was observed among the groups (P < 0.01) (Table 2).

Internal iliac arteriography of beagle dogs showed that the arterial supply to the prostate from the vesical arteries and to the bilateral prostates from the corresponding prostatic arteries did not communicate with each other in all the 16 dogs (100%). In 6 beagle dogs (37.5%), the prostatic artery branch communicated with the rectal artery. In 4 beagle dogs (25%), the prostatic artery branch communicated with the ductus deferential artery. In 2 beagle dogs (12.5%), the prostatic artery branch communicated with the urethra (Figure 3 A-D).

Upon macroscopic study, the prostate was found to be enlarged significantly, and nodular hyperplasia was seen on the surface in all beagle dogs. Upon microscopic examination, the prostate showed a variant of epithelial hyperplasia; the hyperproliferative glands were arranged in lobules and focal intraluminal papillary folds, and had cribiform architecture (Figure 4 A-C).

4. Discussion

Dogs have been widely used as animal models in the study of BPH due to their anatomic similarity to humans and adaptability to treatment [7, 8]. Canine spontaneous BPH models are used less frequently because of

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**Table 2. The differences in the change in prostate size between multiple inter-groups.**

| Groups      | p-value | Before hormone treatment | 4 weeks post hormone treatment | 8 weeks post hormone treatment |
|-------------|---------|--------------------------|-------------------------------|-------------------------------|
| A vs B vs C | 0.6576  | 0.584                    | 0.002                         | -                             |
| A vs B      | 0.6104  | 0.4439                   | 0.007                         | -                             |
| A vs C      | 0.8651  | 0.7341                   | 0.007                         | -                             |
| B vs C      | 0.2482  | 0.2482                   | 0.021                         | 0.021                         |

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![Figure 3. The prostatic branches. A. vesical artery (→). B. rectal artery (→). C. ductus deferential artery (→). D. urethral branch (→).](image)
the longer growth times (over 7 years old) and higher housing costs [9].

Previous studies have demonstrated that dihydrotestosterone plus β-estradiol-induced BPH models are more likely to cause BPH, similar to humans [10, 11]. To rule out the effects of intrinsic sex hormones, the beagle dogs were castrated. The prostate size decreased significantly (from 11.6 ± 5.2 g to 5.3 ± 2.5 g) 4 weeks after castration, which also confirmed that BPH was related to sex hormones. However, the time taken to establish an efficient model in previous studies was at least 3–6 months [4, 5]. Thus, this prospective, comparative study evaluated the feasibility of a simpler and time-saving method of establishing a sex hormone-induced canine BPH model in a total of 16 beagle dogs.

Beagle dogs in all three groups, i.e., group A, group B, and group C were successfully modeled 4, 8, and 12 weeks post hormone treatment, respectively. Based on some preliminary data, it is believed that treatment with an appropriate dose of hormone can significantly shorten the time of establishment of a canine BPH model, and high dose of hormone treatment can inhibit prostatic hyperplasia. However, these findings have been rarely reported; thus, further research in this area may clarify the pathogenesis of BPH.

Wakui et al. dissected 50 male dogs and summarized the branch types of the middle rectal artery from the prostatic artery, which starts from the internal pudendal artery [12]. In our study, the bilateral prostates were supplied by independent vascularization in all the 16 dogs (100%), that branched out to communicate with the rectal artery (37.5%), ductus deferential artery (25%), and the urethral branch (12.5%). Familiarity with the anatomy of the prostatic artery in beagle dogs can reduce the operation time of PAE and can predict if there will be complications after embolization, such as ischemic necrosis of the urinary bladder or rectal mucosa or fistula between prostate and urethra [13].

Upon macroscopic study, the prostate was found to be enlarged significantly in all directions, unlike in human patients with hyperplasia of the periurethral glands. T2-weighted MR images of BPH in dogs appeared as spokes, while in humans, they appear as hyperplasie micronodulaire. Upon microscopic examination, the prostatic hyperplasia was observed mainly as epithelial hyperplasia, followed by stromal hyperplasia, which was thought to be more suitable for mimicking BPH in humans [14, 15].

This study has some limitations. First, the sample size of beagle dogs was relatively small. Second, because of castration, the hormone-induced BPH model cannot be applied for the evaluation of sexual function and hormone-related treatment. In addition, the dose range of hormone treatment can be further subdivided, such as 0.9 ml and 1.1 ml. Finally, we did not observe the effect of hormone therapy on prostate size after the diagnosis of BPH.

5. Conclusions

The findings of the present study suggested that treatment with an appropriate dose of hormone can significantly shorten the time of successful establishment of a canine BPH model, and with an increase in hormone dosage, prostatic hyperplasia will be inhibited. We believe these results warrant further laboratory investigation.

Declarations

Author contribution statement

Bing Yuan: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Feng Duan and Mao-Qiang Wang: Conceived and designed the experiments.

Heng Zhang: Performed the experiments; Analyzed and interpreted the data.

Jin-long Zhang: Analyzed and interpreted the data.

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Data availability statement

The data that has been used is confidential.

Declaration of interest’s statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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