High Plasma Estradiol-17\(\beta\) Levels in Dogs with Benign Prostatic Hyperplasia and Azoospermia

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ABSTRACT. The semen quality of 22 dogs (4 to 7 years old) with benign prostatic hyperplasia (BPH) was examined at the hospital of our university, and 4 of the 22 BPH dogs were diagnosed as azoospermic. The mean peripheral plasma estradiol-17\(\beta\) (E\(_2\)) level (17.3 ng/ml) of the 18 BPH dogs with spermatogenic function was higher than that of 5 normal male dogs and their mean T level (1.7 ng/ml) was lower. The mean E\(_2\) level (27.3 ng/ml) of the 4 BPH dogs with azoospermia was significantly higher than the value in the BPH dogs with spermatogenic function (P<0.01), and the mean T level (1.1 ng/ml) was significantly lower (P<0.05). Five normal male dogs were given 10 intramuscular injections of estradiol benzoate (E\(_2B\)) 5 \(\mu\)g/kg, at 3-day intervals to investigate the relationship between high plasma E\(_2\) levels and the cause of the BPH and azoospermia. Their testes and prostates were measured and biopsied both before and 30 days after the start of E\(_2B\) injections. At 30 days after the start of the E\(_2B\) injections, the mean peripheral plasma T levels had decreased by half, and the mean testicular volume had decreased to 88% of original volume. The numbers of spermatoocytes, spermatoïds, and spermatids in the seminiferous tubes of all of the dogs were significantly lower (P<0.05, 0.01). In addition, the mean prostatic volume increased to 130%, the mean height of the glandular epithelium decreased, and the glandular lumen became increased in diameter. These findings indicate that both BPH and serious spermatogenic dysfunction may be simultaneously induced by protracted high plasma E\(_2\) levels in dogs.

KEY WORDS: azoospermia, benign prostatic hyperplasia, canine, estradiol-17\(\beta\).

Canine benign prostatic hyperplasia (BPH) in dogs is known to occur chiefly in animals more than 6 years old [6], and its occurrence is presumed to be associated with abnormal estradiol-17\(\beta\) (E\(_2\)) and testosterone (T) secretion by the testes associated with advanced age [5, 19]. T is converted to 5\(\alpha\)-dihydrotestosterone (DHT) in the canine prostate, and prostatic function is maintained by the androgen effects of DHT [10, 11]. DHT concentration in the prostates of dogs with BPH has been reported to be higher than in normal dogs [8, 14]. Accumulation of DHT in the prostate is accelerated by high plasma E\(_2\) levels and is thought to cause BPH [8, 14].

Testicular and plasma E\(_2\) are produced by both Leydig cells and Sertoli cells [23]. Infertility in a few human patients has been attributed to elevated testicular E\(_2\) concentrations [13], and the plasma E\(_2\) levels of some azoospermic men have been found to be higher than those of normal men [9, 29]. Long term E\(_2\) administration has been shown to inhibit T secretion by the testis in rats [28], to cause the disappearance of spermatids and spermatozoa from the seminiferous tubules of bull testes [21], and to induce the testicular atrophy of dogs [15]. However, in many cases the cause of spermatogenic arrest in dogs is unknown [7, 24]. In the present study, the peripheral plasma E\(_2\) and T levels of BPH dogs with azoospermia and of BPH dogs with spermatogenic function were examined together with the changes in the volume and histological findings of the testes and the prostates of normal dogs after estradiol benzoate (E\(_2B\)) administration to determine the cause of both BPH and azoospermia in dogs.

MATERIALS AND METHODS

Animals: The subjects of this study were 22 dogs (4 to 7 years old) diagnosed with BPH at the teaching hospital of our university between April 1992 and March 1995. Blood samples were collected from them to determine their plasma hormone levels, and semen samples were collected 2 or 3 times weekly to evaluate semen quality. Each semen sample was examined for the volume and pH, and then sperm concentration in the semen was determined by hemacytometer counts. The percentage of motile sperm was determined by counting actively motile sperm on a warmed glass slide. Four (4 to 6 years old; one Shetland Sheepdog, two Beagles, and one Maltese) of the 22 dogs with BPH were diagnosed as having azoospermia by reason of no sperm in their semen. The reproductive tracts in the 4 BPH dogs were found to be not obstructed because of observation of a few germ cells in the semen. The semen quality of the other 18 BPH dogs was almost normal. The diagnosis of BPH was based on prostatic size measured by radiography and ultrasonography and clinical signs, e.g., hematuria, dysuria, or rectal obstruction [22].

Five normal male beagles (3 to 5 years old) cared for in our university were used to assess changes in the volume of the testes and prostates and their histological appearance. Another 3 normal male beagles were used to assess the changes in plasma E\(_2\) levels after a single E\(_2B\) injection.
Hormone assays: Plasma E\(_2\) and T levels in the blood samples were measured by radioimmunoassay [20, 30]. Rabbit antiserum to E\(_2\) -6-CMO-BSA and T-11\(\alpha\)-succinate-BSA were used. The intra-assay and inter-assay coefficients of variation were 7.3% and 12.6% for E\(_2\), respectively, and 3.0% and 9.6% for T, respectively.

E\(_2\)B injection: The 5 normal beagles were given 10 intramuscular injections of E\(_2\)B (Gyandol; Sankyo Zoki, Inc., Tokyo), 5 µg/kg, at 3-day intervals. The other 3 normal male beagles were given a single 5 µg/kg intramuscular injection of E\(_2\)B.

Peripheral blood collection to measure plasma T levels: Peripheral blood samples were collected from the 5 normal beagles before and 30 days after the start of E\(_2\)B injection to examine their plasma T levels.

Testicular and prostatic biopsies and histological examination: Before and 30 days after the start of E\(_2\)B injection, under halothane inhalation anesthesia, the sizes of the right and left testes were measured in the scrotum, and the right testis was biopsied with a scalpel. Laparotomy was performed to measure the exact size of the prostate and to perform a prostatic biopsy.

Testicular and prostatic volume were calculated according to the formula (4/3\(\pi\)abc/a, length; b, width; c, thickness) [27]. The biopsied testicular tissue was embedded in paraffin, sectioned at 3 µm, and stained with PAS-hematoylin. Round transverse sections of 5 seminiferous tubules of the testis and 5 glandular alveoli of the prostate were examined histologically. The diameter of the seminiferous tubules and the height of the epithelium of the alveoli were measured with a micrometer. The numbers of type A spermatagonia, pachytene primary spermatocytes, round spermatids, and spermatooza according to the classification of Ibach et al. [12] were counted in 5 seminiferous tubules at stage No. 9 of the seminiferous epithelial cycle by the method described previously [18]. The crude germ cell counts were corrected by the method of Abercrombie [1].

Statistical analysis: Statistical significance was tested by the unpaired Student’s t test. The data are summarized as mean values ± standard error (S. E.).

RESULTS

Plasma E\(_2\) and T levels in dogs with BPH: The mean (± S. E.) plasma E\(_2\) levels in both the 4 BPH dogs with azoospermia and the 18 BPH dogs with spermatogenesis were higher than the values in the 5 normal dogs (Table 1), and there was a significant difference between the mean plasma E\(_2\) level of the BPH dogs with azoospermia and the normal dogs (P<0.01).

The mean plasma T levels in both the BPH dogs with azoospermia and the BPH dogs with spermatogenesis were lower than the values in the normal dogs (Table 1). There was a significant difference between the mean plasma T level of the BPH dogs with azoospermia and the normal dogs (P<0.05).

Plasma E\(_2\) levels in normal dogs after a single E\(_2\)B injection: The mean (± S. E.) peripheral plasma E\(_2\) levels of the 3 normal dogs peaked (50 ± 3 pg/ml) 2 hr after the single intramuscular injection of 5 µg/kg E\(_2\)B (Fig. 1). The mean E\(_2\) level (17 ± 3 pg/ml) 72 hr after E\(_2\)B injection was still higher than the pretreatment value (11 ± 1 pg/ml).

Plasma T levels in normal dogs after E\(_2\)B injection: The mean plasma T level (0.9 ± 0.2 ng/ml) in the 5 normal dogs 30 days after the start of the E\(_2\)B injections was significantly lower than the pretreatment value (2.1 ± 0.3 ng/ml).

Volume of the testes and prostates of normal dogs after E\(_2\)B injections: The mean testicular volume of the normal dogs 30 days after the start of the E\(_2\)B injections decreased significantly to 88% of the pretreatment value (P<0.01) (Table 2). The mean prostatic volume, however, increased significantly to 130% of the pretreatment value (P<0.05) (Table 2).

Histology of the testes and prostates of normal dogs after E\(_2\)B injections: Large numbers of the various types of germ cells and spermatooza were seen in all seminiferous tubules in the testes before treatment (Fig. 2-a). The well-developed secretory epithelium of the glandular alveoli was composed of tall columnar cells, and the shape of the glandular lumen was irregular because of the marked projection of the glandular epithelium into the lumen (Fig. 3-a). The mean diameter of the glandular epithelium into the lumen (Fig. 3-a). The mean diameter of the seminiferous tubules 30 days after the start of the E\(_2\)B injections, however, was significantly lower than the pretreatment value (P<0.01) (Table 3), and the numbers of primary spermatocytes, round spermatids and spermatooza were significantly lower than before the injections (P<0.05, 0.01) (Fig. 2-b and Table 3). The mean height of the glandular epithelium 30 days after the start of the E\(_2\)B injections was significantly lower than the pretreatment value (P<0.01) (Table 3), and the glandular lumen became wider because of reduction of the epithelial projec-

<p>| Table 1. Mean (± S.E.) peripheral plasma estradiol-17β (E(_2)) and testosterone (T) levels in 4 dogs with benign prostatic hyperplasia (BPH) and azoospermia (AZ dogs), 18 dogs with BPH and spermatogenic function (SP dogs) and 5 normal dogs (NO dogs) |
|---|---|---|---|---|---|---|
| | E(_2) (pg/ml) | | T (ng/ml) | | |</p>
<table>
<thead>
<tr>
<th></th>
<th>AZ dogs</th>
<th>SP dogs</th>
<th>NO dogs</th>
<th>AZ dogs</th>
<th>SP dogs</th>
<th>NO dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>27.3 ± 3.3**</td>
<td>17.3 ± 0.8</td>
<td>14.8 ± 1.2</td>
<td>1.1 ± 0.1*</td>
<td>1.7 ± 0.1</td>
<td>2.1 ± 0.3</td>
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</table>

** P<0.01 and * P<0.05, in comparison with both SP dogs and NO dogs.
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DISCUSSION
Abnormally elevated E₂ and reduced T secretion by the testes has been reported to be a cause of both human [26] and canine [5, 19] BPH, and it is necessary to administer E₂ for a long period as well as androgen to artificially induce

Fig. 1. Changes in the mean (± S. E.) peripheral plasma E₂ levels of 3 normal dogs after a single intramuscular injection of E₂B, 5 µg/kg.

Table 2. Mean (± S.E.) volume (cm³) of the testes and prostates of 5 normal dogs before and 30 days after the start of estradiol benzoate injections

<table>
<thead>
<tr>
<th></th>
<th>Testes</th>
<th>Prostates</th>
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<tbody>
<tr>
<td>Before</td>
<td>7.0 ± 1.8 (100%)</td>
<td>6.2 ± 1.7 (100%)</td>
</tr>
<tr>
<td>After</td>
<td>6.2 ± 0.1** (88%)</td>
<td>8.1 ± 0.6* (130%)</td>
</tr>
</tbody>
</table>

** P<0.01 and P<0.05*, in comparison with before.

Fig. 2. Histological changes in the testes of a normal dog after E₂B injections (PAS-hematoxylin stain, × 200). (a) Testis before E₂B injection. Normal spermatogenesis is observed in all of the seminiferous tubules. (b) Testis 30 days after the start of E₂B injection. Seminiferous tubules diameter and the number of germ cells in the tubules have decreased markedly.
BPH in orchidectomized dogs [2, 6]. The peripheral plasma E2 levels of normal dogs 1–3 days after an intramuscular injection of 5 µg/kg of E2B were similar to the values (20–40 pg/ml) reported by us in azoospermic dogs with elevated plasma E2 levels [17], and thus the dose of E2B administered to the normal dogs in the present study was concluded to have been appropriate. In this study, the mean plasma E2 level in the dogs with spontaneous BPH was higher than the value in normal dogs, and E2 treatment induced expansion of the glandular lumen by reducing epithelial projections into the lumen. The volume of the stromal tissue in the canine prostate has been shown to be increased by E2 treatment [4, 25], and thus it is assumed that spontaneous and artificial BPH in dogs is the result of both expansion of the glandular lumen and an increase in stromal tissue caused by high plasma E2 levels. Protracted high plasma E2 levels in the dog were found to induce accumulation of DHT in the prostate and consequently cause BPH [8, 14]. Therefore, elevated plasma E2 levels are thought to be more important than reduced plasma T levels for the occurrence of BPH.

Some cases of azoospermic human patients have been reported in which the spermatogenic function in the testes was affected by increased plasma E2 levels and decreased T levels [9, 29]. Increased E2 in the blood plasma of some azoospermic men has been found to be due to increased secretion by the testes [9], and E2 treatment to male animals for long periods is well known to inhibit androgen production [16] and spermatogenesis in the testes [16, 21], and to cause testicular atrophy [15]. In the present study, the mean plasma E2 level of the BPH dogs with azoospermia was higher than in both the BPH dogs with spermatogenic function and the normal dogs. Therefore, BPH dogs with excessive plasma E2 levels are assumed to have a tendency to suffer from azoospermia. Decreases in the numbers of the

Table 3. Mean (± S.E.) diameter of the seminiferous tubules, number of germ cells in the testes and epithelial cell height of the prostatic alveoli of 5 normal dogs before and 30 days after the start of estradiol benzoate injections

<table>
<thead>
<tr>
<th>Testes</th>
<th>Prostates</th>
<th>Seminiferous tubule diameter (µm)</th>
<th>Type A (round spermatids)</th>
<th>Pachytene primary spermatocytes</th>
<th>Round spermatids</th>
<th>Spermatocytes</th>
<th>Epithelial cell height (µm)</th>
</tr>
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<tr>
<td>Before</td>
<td>195 ± 3</td>
<td>0.7 ± 0.1</td>
<td>7.2 ± 0.9</td>
<td>18.7 ± 1.2</td>
<td>15.3 ± 1.2</td>
<td>15.8 ± 1.4</td>
<td>11.0 ± 0.4</td>
</tr>
<tr>
<td>After</td>
<td>166 ± 8**</td>
<td>0.6 ± 0.1</td>
<td>4.1 ± 0.5*</td>
<td>11.7 ± 0.9**</td>
<td>6.4 ± 0.8**</td>
<td>11.0 ± 0.4**</td>
<td>11.0 ± 0.4</td>
</tr>
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</table>

*a) Germ cells were counted in stage 9 of the seminiferous epithelial cycle by the classification of Ibach et al. [12].

** P<0.01 and * P<0.05, in comparison with before.

Fig. 3. Histological changes in the prostate of a normal dog after E2B injections (PAS-Hematoxylin stain, × 200). (a) Prostate before E2B injection. Tall columnar epithelial cells and marked projections of the epithelium into the glandular lumen are observed. (b) Prostate 30 days after the start of E2B injection. Low epithelial cell height and reduction of epithelial projections into the lumen are observed.
various type of germ cells were observed in all the seminiferous tubules of the normal dogs after E2B treatment. The reductions of seminiferous tubule diameter and testicular volume in the normal dogs were presumably caused by a decrease in the number of germ cells after E2B treatment. Greater numbers of E2B injections may induce the disappearance of spermatzoa in the seminiferous tubules of normal dogs.

As BPH chiefly occurs in dogs more than 6 years old [3], aging is a factor in the abnormal steroid hormone secretion by the testes of dogs with BPH. However, not only the age of the dog, but genetic defects, nutrition and environment are also presumably related to the development of BPH and azoospermia, and it seems necessary to thoroughly investigate the other possible causes of abnormal hormone secretion by canine testes.

In conclusion, the results of this study demonstrated that an elevated E2 secretory function of the testes may simultaneously induce both BPH and serious spermatogenic dysfunction in dogs. Especially, BPH dogs with high plasma E2 levels are assumed to have a tendency to suffer from azoospermia. This finding suggests that inhibitors of E2 synthesis in the testes may be useful in treating canine BPH and spermatogenic dysfunction.

REFERENCES


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