Finasteride-induced prostatic involution by apoptosis in dogs with benign prostatic hypertrophy

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Objective—To determine the effect of finasteride on programmed cell death (apoptosis) of prostatic cells during prostatic involution in dogs with benign prostatic hypertrophy (BPH).

Animals—9 dogs with BPH.

Procedure—Dogs were randomly assigned to treatment or control groups. Treatment dogs (n = 5) were administered finasteride (0.1 to 0.5 mg/kg, PO, q 24 h) for 16 weeks, whereas the 4 control dogs were administered an inert compound. Prostatic cells from the prostatic fluid portion of the ejaculate of treatment and control dogs were obtained before and 1, 2, 3, 4, 8, and 16 weeks after initiation of treatment. Cells were concentrated by use of centrifugation. Prostatic cells were examined for indications of apoptosis by use of a terminal deoxynucleotidyl transferase-mediated deoxyuracil triphosphate nick-end labeling technique. After receiving the inert compound for 16 weeks, the 4 control dogs were administered finasteride for 16 weeks, and evaluations were repeated.

Results—Percentage of apoptotic prostatic cells in ejaculated prostatic fluid of treatment dogs increased significantly (from 9% before treatment to 33, 31, 26, and 27% after 1, 2, 3, 4, 8, and 16 weeks of treatment, respectively). There was no significant change in percentage of apoptotic prostatic cells in the ejaculated prostatic fluid of control dogs.


Programmed cell death (apoptosis) is a morphologically distinct form of cell death that plays a major role during development, in homeostasis, and in many diseases. Apoptosis results through the activation of a cell-intrinsic suicide program, resulting in controlled autodigestion of the cell, cytoskeleton disruption, chromatin aggregation, nuclear and cytoplasmic condensation, cell shrinkage, membrane blebbing, and phagocytosis by neighboring phagocytic cells, macrophages, or adjacent epithelial cells. Apoptotic cell death can be distinguished from necrotic cell death, which results from acute cellular injury and is typified by rapid swelling and lysis of the cell, spilling of cellular contents into the extracellular space, and induction of an inflammatory response in the adjacent tissues. Contents of apoptotic cells do not leak into the extracellular space, and there is no local inflammatory process. Apoptosis can be induced by exogenous agents such as radiation, chemicals, and viruses or by changes in concentrations of endogenous compounds such as hormones or growth factors, which then trigger an increase in intracellular calcium concentrations that cause the subsequent process of apoptosis.

The terminal deoxynucleotidyl transferase-mediated deoxyuracil triphosphate nick-end labeling (TUNEL) technique is a sensitive and convenient method for detecting apoptotic cells. The principle of the TUNEL technique is to detect DNA fragments, using terminal deoxynucleotidyl transferase to add digoxigenin-nucleotides to the free 3' hydroxyl end of DNA fragments. Antidigoxigenin peroxidase antibodies and 5' diaminobenzidine are incubated with tissues or cells to enable detection of digoxigenin-labeled DNA fragments. Apoptotic cells have hyperchromatic dense nuclear bodies with a halo of extracellular space surrounding them.

Benign prostatic hypertrophy (BPH) is a common, naturally developing, age-related condition in human beings and dogs. More than 80% of sexually intact male dogs > 5 years old have gross or microscopic evidence of BPH. The pathogenesis of BPH is not completely known; however, dihydrotestosterone (DHT) is a key hormone involved in enlargement of the prostate by enhancement of growth in stromal and glandular components. Estradiol and other growth factors stimulate proliferation of the prostatic cells by paracrine actions. Dihydrotestosterone is metabolized from testosterone by the enzyme 5α-reductase. Finasteride, a 5α-reductase inhibitor, administered at a dosage of 0.1 to 0.5 mg/kg, PO, q 24 h for 16 weeks can decrease serum concentrations of DHT in dogs by 58%...
and prostatic volume by 43%, and it has been used successfully in a clinical trial to treat dogs with BPH.20 The mechanism (apoptosis or necrosis) for this prostatic involution in dogs has not been reported.

The purpose of the study reported here was to determine whether finasteride-induced DHT deprivation and prostatic involution in dogs with BPH is the result of apoptosis. The null hypothesis was that finasteride-induced DHT deprivation did not have an effect on the number of apoptotic prostatic cells in dogs with BPH.

**Materials and Methods**

**Animals**—Nine client-owned sexually intact male dogs with naturally developing BPH were used in the study. All dogs were ≥ 2.7 years old, had an enlarged prostate (detected radiographically and ultrasonographically), did not have evidence of bacterial or mycoplasmal infection in the semen, and had clinical signs of BPH such as constipation or blood in the semen. Recruitment of dogs into the study and the effects of finasteride on prostatic size (mean decrease in prostatic volume was 43%, and mean decrease in serum concentration of DHT was 58%) have been reported elsewhere.21

Dogs were randomly assigned to treatment and control groups. The 5 dogs assigned to the treatment group ranged from 6.9 to 11 years of age (mean, 8.7 ± 1.7 years), weighed between 10.7 and 43.2 kg (mean, 28.5 ± 13.9 kg), and included 5 breeds (Australian Shepherd Dog, Bernese Mountain Dog, Doberman Pinscher, Labrador Retriever, and Shetland Sheepdog). The 4 dogs assigned to the control group ranged from 2.7 to 9.5 years old (mean ± SD, 7.1 ± 2.7 years), weighed between 10.7 and 43.2 kg (mean, 28.5 ± 13.9 kg), and included 4 breeds (German Shepherd Dog, Labrador Retriever, English Setter, and Shetland Sheepdog). Ages and weights of treatment and control dogs were not significantly different.

**Experimental design**—We examined the effect of finasteride on dogs with BPH in a double-blind controlled clinical trial. Dogs in the treatment group were administered a single 5-mg finasteride capsule21 (equivalent to a dosage of 0.1 to 0.5 mg/kg), PO, q 24 h, for 16 weeks. Dogs in the control group were administered 5 mg of powdered sugar in a gelatin capsule, PO, q 24 h, for 16 weeks.20,22 After the initial 16 weeks of the study, dogs in the control group were administered 5 mg of finasteride, PO, q 24 h, for the next 16 weeks. Results for these 4 dogs while receiving finasteride were not significantly different from results for the original 5 dogs in the treatment group; therefore, data for all 9 dogs while receiving finasteride were combined. Prostatic cells were collected from the prostatic fluid fraction of the ejaculate before and 1, 2, 3, 4, 8, and 16 weeks after initiation of treatment. Results were considered significant for values of P ≤ 0.05.

**Results**

Mean ± SD percentage of apoptotic ejaculated prostatic cells before administration of finasteride in treatment and control dogs was 9 ± 6% (range, 1 to 19%) and 9 ± 8% (range, 4 to 21%), respectively, and these percentages were not significantly different (Fig 3).

**Collection of prostatic cells**—Using an artificial vagina, dogs were manually stimulated, and semen was collected as described elsewhere.23,24 For each dog, the first (presperm) and second (sperm-rich) fractions of semen were collected together in a calibrated 15-mL centrifugation tube attached to the artificial vagina.22 The first tube was then removed from the artificial vagina, and a second tube was attached to enable collection of 1 mL of prostatic fluid, the last fraction of the ejaculate.21

Within 30 minutes after collection of the ejaculate, prostatic cells from each sample of prostatic fluid were applied to 4 microscope slides, using a commercial centrifugation technique.2 An aliquot (100 µL) of each sample of prostatic fluid was pipetted into each cytofunnel, which was centrifuged at 250 × g for 4 minutes. Prostatic cells were concentrated around a 6-mm-diameter circle at the center of each slide. Prostatic cells on each slide were fixed in neutral-buffered 4% formalin for 10 minutes, washed twice in neutral PBS solution (3 min/wash), and then dehydrated in a series of ethanol solutions. Slides were stored at –20 C until analyzed for apoptosis by use of the TUNEL technique. All slides were examined within 6 months after sample collection.

**Detection of apoptotic cells**—Slides containing prostatic cells were examined for apoptosis, using a commercially available detection kit, as reported elsewhere.23 Prostatic cells from each dog were examined at the same time as prostatic cells from the clinically normal sexually intact male adult dog (negative-control sample). Prostatic cells were digested with proteinase K (20 µg/ml) for 10 minutes at 25 C. Hydrogen peroxide was used to neutralize endogenous peroxidase. Prostatic cells were subjected to enzymatic incorporation of digoxigenin-labeled nucleotides, using terminal deoxynucleotidyl transferase, and then washed and incubated with antidigoxigenin peroxidase. After washing, prostatic cells were incubated with diaminobenzidine and counterstained with hematoxylin. On each slide, 100 prostatic cells were examined at 1,000X magnification, and the number of cells that had positive staining for apoptosis was recorded (Fig 1 and 2). Results were expressed as the number of apoptotic nuclei per 100 prostatic cells.

**Statistical analysis**—A Kruskal-Wallis ANOVA23 was used to compare the percentage of apoptotic prostatic cells in treatment and control dogs before and 1, 2, 3, 4, 8, and 16 weeks after initiation of treatment. Results were considered significant for values of P ≤ 0.05.
Mean percentage of apoptotic prostatic cells in treatment dogs 1, 2, 3, 4, 8, and 16 weeks after initiation of finasteride treatment was 33 ± 6.8 (range, 29 to 41), 31 ± 6.6 (range, 26 to 40), 26 ± 8.7 (range, 18 to 35), 24 ± 15.9 (range, 7 to 50), 27 ± 16.5 (range, 14 to 44), and 18 ± 14 (range, 5 to 35)%, respectively (Fig 3). There was a significant increase in the percentage of apoptotic prostatic cells in ejaculated prostatic fluid of finasteride-treated dogs 1 (P = 0.017), 2 (P = 0.008), 3 (P = 0.030), and 8 (P = 0.015) weeks after initiation of treatment, compared with values for these dogs before treatment or with values for the control group. Mean percentage of apoptotic prostatic cells in dogs 4 and 16 weeks after initiation of treatment was increased, compared with the percentage of apoptotic prostatic cells before treatment, but this increase did not differ significantly.

**Discussion**

Estrogen and androgen inhibit apoptosis. In the study reported here, the number of apoptotic prostatic cells in treated dogs increased significantly following treatment with finasteride, a 5α-reductase inhibitor, suggesting that the drug decreased serum DHT and resulted in apoptosis of prostatic cells. In castrated rats, serum testosterone concentration decreases significantly between days 1 and 3 after castration, with an increased number of apoptotic prostatic cells between days 1 and 4 after castration. In those rats, the prostate was significantly smaller by 7 days after castration, compared with size of the prostate in control rats. Male rats given finasteride (40 mg/kg, SC, q 24 h) had a significant decrease in intraprostatic DHT content on day 4 of treatment. Number of apoptotic prostatic cells in finasteride-treated rats increased to 6.4% on day 4, and significantly increased to 16% on day 9, compared with values for the control group (3.6%). By days 14 and 21 of finasteride treatment, the number of apoptotic prostatic cells was not significantly different from those of the control group.

Those results are similar to results obtained in our study in which we documented that finasteride treatment resulted in an increased percentage of apoptotic prostatic cells by day 7 of treatment. However, percentage of apoptotic prostatic cells in treated dogs of our study also increased on days 14, 21, and 56 of treatment. A possible explanation is that the dosage used in our experiment (0.1 to 0.5 mg/kg) was much lower than that used in the study that involved rats (40 mg/kg), resulting in a slower decrease in serum DHT concentration and slower increase in the rate of apoptosis of prostatic cells.

Rittmaster et al reported that men treated with finasteride (5 mg, PO, q 24 h for 3 months) had an increase in the number of apoptotic prostatic cells in specimens obtained during prostatectomy, compared with values for control patients. In the finasteride-treated men, number of apoptotic prostatic cells before (baseline) and 6 to 18, 23 to 73, and 90 days after onset of treatment was 0.4, 2.8, 1.7, and 0.7 apoptotic cells/mm³, respectively. Prostatic involution attributable to atrophy of prostatic cells has been reported in rats and men treated with finasteride. Cell atrophy is a mechanism in which the cell responds to a change in cellular environment by a decrease in cell size. Atrophic cells are alive, but they are smaller than normal cells and are distinct from apoptotic or necrotic cells, which are dead. Atrophic prostatic cells can be detected in paraffin-embedded stained tissue by measuring the area of a strip of epithelial cells surrounding a prostatic duct and determining the size of prostatic cells, using the ratio of cell width to cell length. In our study, atrophic cells in prostatic tissue could not be detected because of our use of centrifugation of prostatic fluid and examination of individual exfoliated prostatic cells.
To our knowledge, this is the first report of detection of apoptotic prostatic cells in the prostatic fluid of the ejaculate in any species. Reports of apoptotic prostatic cells in finasteride-treated rats and men were derived from histologic examination of specimens obtained by prostatic excision (rats) or prostatectomy (men). Ejaculated prostatic fluid may contain dying exfoliated prostatic cells and a percentage of apoptotic cells that is higher than that obtained by histologic evaluation of prostatic tissues. Mean number of apoptotic prostatic cells detected in our study by use of the TUNEL technique was 9% on day 0 in dogs with BPH, compared with a baseline percentage of 0.2% in rats and 0.4/mm² in men that were detected by histologic examination of tissue sections.

References