Age-Dependent Expression of 5α-Reductase and Androgen Receptors mRNA by the Canine Prostate

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Summary

The growth of the prostate gland is androgen-dependent in which testosterone is converted to the most potent dihydrotestosterone (DHT) by 5α-reductase within the prostate. Androgen interacts with its androgen receptors (AR) to regulate normal growth of the prostate and has been also implicated in both the progression of benign prostate hyperplasia and prostate cancer. This study was conducted to compare the mRNA expression of AR and 5α-reductase by the prostate gland from three age categories: immature, young-mature and old dogs. Quantitative gene expression was assessed by the real-time PCR and the results were expressed as a relative mRNA expression of the target gene. This study revealed that there was no significant ($P > 0.05$) difference in the mRNA expression of the AR by the prostate gland of immature, young and old dogs. In contrast to mRNA expression of AR gene, there is a highly significant ($P<0.001$) down-regulation in 5α-reductase gene by the prostate of young and old dogs as compared with immature dogs. However, there is no significant ($P>0.05$) difference in mRNA expression of the 5α-reductase gene by the prostate gland from young and old dogs. This differential expression of AR and 5α-reductase genes, which are involved in the regulation of androgen effect on prostate gland, might reflect an age-dependent growth requirement of the gland for androgens.

Key words

Prostate, androgen receptors, 5α-reductase, canine

Continuous supply of androgen is a prerequisite for driving the prostate gland growth and for maintaining its steady-state growth. Androgen has also been implicated in the progression of prostatic diseases, such as benign prostate hyperplasia (BPH) and prostate cancer. Although testosterone is the prevalent circulating androgen, dihydrotestosterone (DHT) is the most active androgen involved in the regulation of the prostate gland. The conversion of testosterone to its active metabolite is achieved through the activity of 5α-reductase, which occurs in two isozymes, type I and type II. While the type II predominantly is expressed by prostatic cells, type I is expressed by other tissues, such as skin and liver. Deficiency of type II but not type I severely impeded the prostate gland development in human and to a lesser extent in rats (Imperato-Mcginley and Zhu 2002, Carson and Rittmaster 2003, Mahendroo *et al.* 2001). Therefore, inhibitors of 5α-rductase, which were adapted as therapeutic agents for the treatment of BPH, resulted in a significant reduction in the prostate gland size (Tarter and Vaughan 2006, Cohen *et al*. 1995, Laroque *et al*. 1995).

Androgen exerts its effect on prostate gland development through the interaction with androgen receptor (AR). AR acts as a transcription factor that regulates the expression of androgen response genes that are involved in many cellular activities that ranges from proliferation to programmed cell death (So *et al*. 2003). AR expression level has been shown to increase in close association with the continuous growth of the dorsal and lateral lobes of the prostate gland in some rat strains which develop an age-dependent spontaneous hyperplasia (Banerjee *et al.* 2001). In contrast, AR expression level has been shown to decrease in the ventral lobe of the gland, which exhibited age-dependent

growth senescence (Banerjee *et al*. 2001). Androgen ablation studies also revealed in rats a rapid regression of the ventral lobe of the prostate gland by inducing epithelial cell apoptosis (Perlman *et al*. 1999, Banerjee *et al*. 2000, Banerjee *et al*. 2002) but not in the dorsolateral lobes (Banerjee *et al*. 2002). In addition, AR expression has been shown to be associated with cell proliferation and survival of prostate cancer and it might therefore, contribute to prostate cancer progression (Amirghofran *et al*. 2004). These results demonstrate clearly the importance of androgen receptor expression level in regulating the rates of prostate gland growth and senescence.

Dogs are known to develop an age-dependent spontaneous prostate hyperplasia (Brendler *et al*. 1983). Although this process is androgen-dependent, the expression of genes that are involved in the regulation of androgen action, such as AR receptor and 5α-reductase, are not well established. Therefore, this study was designed to compare the mRNA expression levels of AR and 5α-reductase (Type II) genes by the prostate gland in immature, young and aged dogs.

To conduct this experiment, twelve male dogs were divided into three age groups: immature, young-adult and old-adult dogs. Each group had 4 dogs. The immature dogs were about 1 month of age, the young dogs were about two years old, and the old dogs were between 6-8 years of age. After the dogs were euthanized by intravenous injection of 10% thiopentone sodium, the prostate glands were removed and stored in liquid nitrogen for subsequent RT-PCR analysis. The protocol of animal handling and

74 euthanasia were approved by the Jordan University of Science and Technology Animal
75 Care and Use Committee (JUST-ACUC).
76
77 Total RNA was extracted from the frozen prostate gland using an SV Total RNA
78 isolation kit (Promega Corporation, Madison, WI, USA). The RNA concentration was
79 determined by measuring the absorbance at 260 nm using SmartSpec™ Plus
80 spectrophotometer (Bio-Rad, Hercules, CA, USA). 0.5 μg of total RNA was used to
81 synthesize a complementary DNA (cDNA) using the reverse transcription kit (Promega
82 Corporation, Madison, WI, USA). The RT reaction was carried out at 25 ˚C for 5 min
83 followed by 42 ˚C for 60 min and then at 95 ˚C for 5 min. The samples were then placed
84 on ice for 5 minutes and stored at -20 ˚C for PCR amplification.
85
86 Real time PCR analysis was performed using a commercial PCR kit containing
87 sybergreen florescent dye (QuantiTect™ SYBR® Green; Qiagen, Valencia, CA, USA) in
88 the presence of 2 μM of specific primers. The primers were designed to be specific for
89 the canine sequence using the web-based QuantiProb Design software (QuantiTec
90 Cuatom Assays, www.qiagen.com). The forward sequence for the primer used for
91 androgen receptors was GAG GTA GTA TCA GAA GGT AG and the reverse primer
92 was CTG TCC GAG ATG GTC GAA. The forward primer for 5α-reductase type II was
93 ACT CAT TGC TCA CTA GAG G and the reverse primer was CTC AGC GCA GTA
94 AAT CAG A. The forward sequence for glycerlaldehyde-3-phosphate dehydrogenase
95 (GAPDH) primer was CTG GAG AAA GCC AAA and the reverse primer was TGT
96 TGA CAC AGG AGA. The PCR amplification reactions were started with an initial

denaturation at 95 ˚C for 15 min, followed by 45 cycles each composed of denaturation at 95 ˚C for 30 sec, annealing at 55 ˚C for 30 sec and extension at 72 ˚C for 30 sec. At the end of the 45 cycles the melting curve for the reactions was performed at temperatures ranging from 72˚C to 95˚C.

Relative mRNA gene expression was determined using the $2^{-\Delta\Delta C}$T method and normalized to GAPDH expression (Livak and Schmittgen, 2001). One-way analysis of variance (ANOVA) of mRNA expression was performed using Minitab software (Minitab Inc.,State College, PA, USA). The results were presented as the mean ± SEM. A probability of less than 0.05 ($P< 0.05$) was considered statistically significant. The mRNA expression of each gene was twice analyzed from different pools of total RNA.

The results of this study revealed a differential mRNA expression of AR and 5α-reductase type II genes by the prostate gland obtained from immature, young and old dogs. As shown in figure 1, there is a highly ($P < 0.001$) significant reduction in 5α-reductase mRNA expression by the prostate gland from young and old dogs as compared with its expression in prostates of immature dogs. However, there is no significant difference in 5α-reductase mRNA expression between young and old prostate glands. It has been shown that inhibition of 5α-reductase resulted in a significant decrease in prostate gland volume probably by inducing prostatic cell apoptosis (Laroque *et al*. 1995, Steers 2001). This reduction in the prostate gland size correlated well with a decrease in DHT and the concomitant increase in testosterone level (Cohen *et al*. 1995), indicating that DHT rather than testosterone play a key permissive role in the prostate gland growth

and development. It has been shown that the physiological concentration of testosterone is higher in adults as compared to immature dogs (Brendler *et al*. 1983, Mialot *et al*. 1988). Therefore, the present results emphasized the importance of 5α-reductase as a rate limiting step for the reduction of the effective concentration of testosterone within the prostate gland in young- and old-adult dogs and thus should reduce the prostate gland growth. Surprisingly, the canine prostate gland continued its post-maturation growth (Brendler *et al*. 1983) despite this highly significant inhibition of 5α-reductase. One possible explanation for this discrepancy might be due to the fact that DHT is an immediate precursor for 3βAdiol which interact with estrogen receptor β (ERβ) and acts as a negative regulator of prostate gland growth. Therefore, the inhibition of 5α-reductase would also prevent the formation of 3βAdiol and, by doing so, would remove the growth-limiting effect of ERβ (Weihua *et al*. 2002).

In contrast to the expression of 5α-reductase, there is no significant difference in AR mRNA expression by prostate gland of immature, young and old dogs (Figure 2). Androgen receptor expression has been shown to vary in an age- and species-dependent manner. Although AR expression by the ventral lobes of rats prostate shows an age-dependent decrease (Banerjee *et al*. 2001, Prins *et al*. 1996), its expression level by young adult and old dogs remained either unchanged (Prins *et al*. 1996) or increased with age (Niu *et al*. 2003). This age-dependent and species-specific variation in AR receptor expression appeared to parallel the inherent differential character of age-dependent growth and senescence of the prostates in rat and dogs. While the rat prostate gland growth ceases after maturity, the dog prostate continues to grow as the animal gets older.

However, in some rat strains when there is a tendency of the dorsal and lateral lobes of the rat prostate gland to continue its development beyond the mature state, there is a parallel increase in AR expression (Banerjee *et al*. 2001). Interestingly, the spontaneous and age-dependent development of prostate hyperplasia has been shown to occur despite the decrease of serum testosterone level (Banerjee *et al*. 1998). Therefore, the increment of AR expression might act to enhance the responsiveness of prostatic cells to androgen stimulation and to compensate for its decline. As the dogs are known to develop an age-dependent benign prostate hyperplasia, then the consistent expression level of AR by the dog prostate gland from different ages, might be at least in part, involved in its continuous growth.

In conclusion, the results presented herein demonstrated that the expression level of AR is consistent with the expected age-dependent continuous growth of the prostate gland. The transition of the prostate gland from immature to mature required a dramatic down-regulation in 5α-reductase expression; nevertheless, a steady-state of its expression is required for the post-maturation growth.

Acknowledgments

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References

AMIRGHOFRAN Z, MONABATI A, GHOLIJAN*I* N: Androgen receptor expression in relation to apoptosis and expression of cell cycle related protein in prostate cancer. *Pathol Oncol Res* **10**: 37-41, 2004.

BANERJEE PP, BANERJEE S, BROWN TR: Increased androgen receptor expression correlates with development of age-dependent, lobe-specific spontaneous hyperplasia of Brown Norway rat prostate. *Endocrinology* **142**: 4066-4075, 2001.

BANERJEE PP, BANERJEE S, BROWN TR: Bcl-2 protein expression correlates with cell survival and androgen independence in rat prostatic lobes. *Endocrinology* **143**: 1825-1832, 2002.

BANERJEE S, BANERJEE PP, BROWN TR: Castration-induced apoptotic cell death in the brown Norway rat prostate decreases as function of age. *Endocrinology* **142**: 821-832, 2000.

BANERJEE PP, BANERJEE S, LAI JM, STRANDBERG JD, ZIRKIN BR, BROWN TR: Age-dependent and lobe-specific spontaneous hyperplasia in the brown Norway rat prostate. *Biol Reprod* **59**: 1163-1170, 1998.

BRENDLER CB, BERRY SJ, EWING LL, MCCULLOUGH AR, COCHRAN RC, STRANDBERG JD, ZIRKIN BR, COFFEY DS, WHEATON LG, HILER ML, BORDY MJ, NISWENDER GD, SCOTT WW, WALSH PC: Spontaneous benign prostatic hyperplasia in the beagle. Age-associated changes in serum hormone levels, and the morphology and secretory function of the canine prostate. *J Clin Invest* **71:** 1114-1123, 1983.

CARSON C, RITTMASTER R: The role of dihydrotestosterone in benign prostatic hyperplasia. Urology **61** (suppl 4A): 2-7 2003.

COHEN S M, WERRMANN JG, RASMUSSON GH, TANAKA WK, MALATESTA PF, PRAHALADA S, JACOBS JG, HARRIS G, NETT TM: Comparison of the effect of new specific azasteroid inhibitors of steroid 5 alpha-reductase on canine hyperplastic prostate: suppression of prostatic DHT correlated with prostate regression. *Prostate* **26:** 55-71, 1995.

IMPERATO-MCGINLEY J, ZHU Y-S: Androgen and male physiology the syndrome of 5α-reductase-2 deficiency. *Mol Cell Endocrinol* **98:** 51-59, 2002.

LAROQUE PA, PRAHALADA S, MOLON-NOBLOT S, COHEN SM, SOPER K, DUPRAT P, PETER CP, VAN ZWIETEN MJ: Quantitative evaluation of glandular and stromal compartment in hyperplastic dog prostate: effect of 5-alpha reductase inhibitors. *Prostate* **27:** 121-128, 1995.

LIVAK KJ, SCHMITTGEN TH: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}$T. *Methods* **25**: 402-408, 2001.

MAHENDROO MS, CALA KM, HESS DL, RUSSELL DW: Unexpected virilization in male mice lacking steroid 5a-reductase enzymes. *Endocrinology* **142:** 4652-4662, 2001.

MIALOT JP, THIBIER M, TOUBLANC JE, CASTANIER M, SCHOLLER R: Plasma concentration of luteinizig hormone, testosterone, dehydroepiandrosterone, androstenedione between birth and one year in the male dog: longitudinal study and hCG stimulation. *Andrologia* **20**: 145-154, 1988.

NIU YJ, MA TX, ZHANG J, XU Y, HAN R.F, SUN G: Androgen and prostatic stroma. *Asian J Androl* **5:** 19-26, 2003.

PERLMAN H, ZHANG X, CHEN MW, WALSH K, BUTTYAN R: An elevated bax/bcl-2 ratio corresponds with the onset of prostate epithelial cell apoptosis. *Cell Death Differ* **6:** 48-54, 1999.

PRINS GS, JUNG MH, VELLANOWETH RL, CHATTERJEE B, ROY AK: Age-dependent expression of the androgen receptor gene in the prostate and its implication in glandular differentiation and hyperplasia. *Dev Genet* **18:** 99-106, 1996.

SO AI, HURTADO-COLL A, GLEAVE ME: Androgen and prostate cancer. *World J Urol* **21:** 325-337, 2003.

STEERS WD: 5alpha-reductase activity in the prostate. *Urology* **58:** 17-24, 2001.

TARTER TH, VAUGHAN EDJR: Inhibitors of 5alpha-reductase in the treatment of benign prostatic hyperplasia. *Curr Pharm Des* **12:** 775-783, 2006.

WEIHUA Z, LATHE R, WARNER M, GUSTAFSSON J-A: An endocrine pathway in the prostate, ERβ, AR, 5α-androstane-3β,17β-diol, and CYP7B1, regulates prostate growth. *PNAS* **99:** 13589-13594.

Figure 1: Relative mRNA expression of 5α-Reductase type II by the prostate gland from immature, young-adult dogs and old-adult dogs. Each bar represents the mean ± SEM of 4 dogs. Bars with different letters represent means that are significantly different ($P < 0.001$).

Figure 2: Relative mRNA expression of androgen receptor by the prostate gland from immature, young-adult and old-adult dogs. Each bar represents the mean ± SEM of 4 dogs. There were no significant statistical differences between groups ($P > 0.05$).

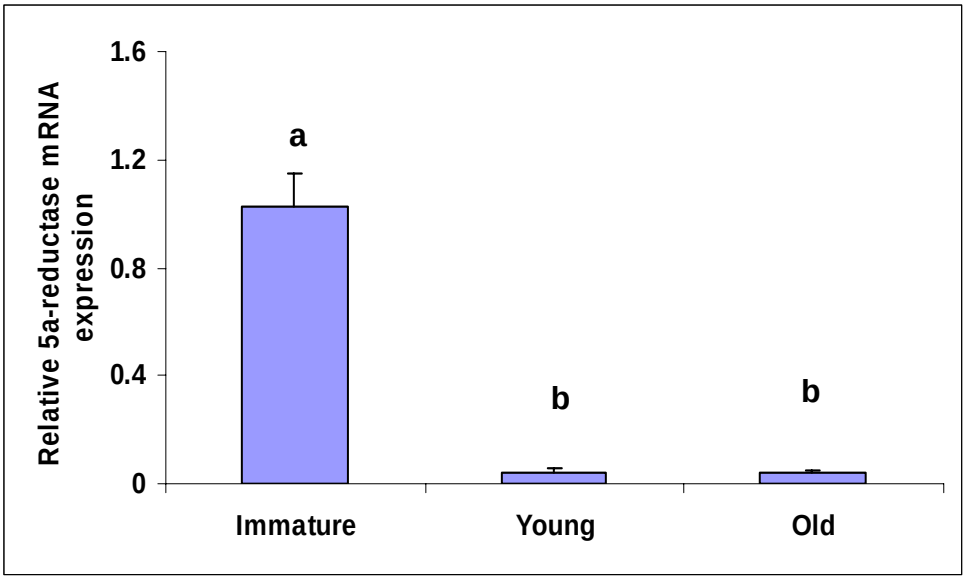
Relative 5a-reductase mRNA expression
1.6
1.2
0.8
0.4
0
a
b
b
Immature
Young
Old

Figure 1

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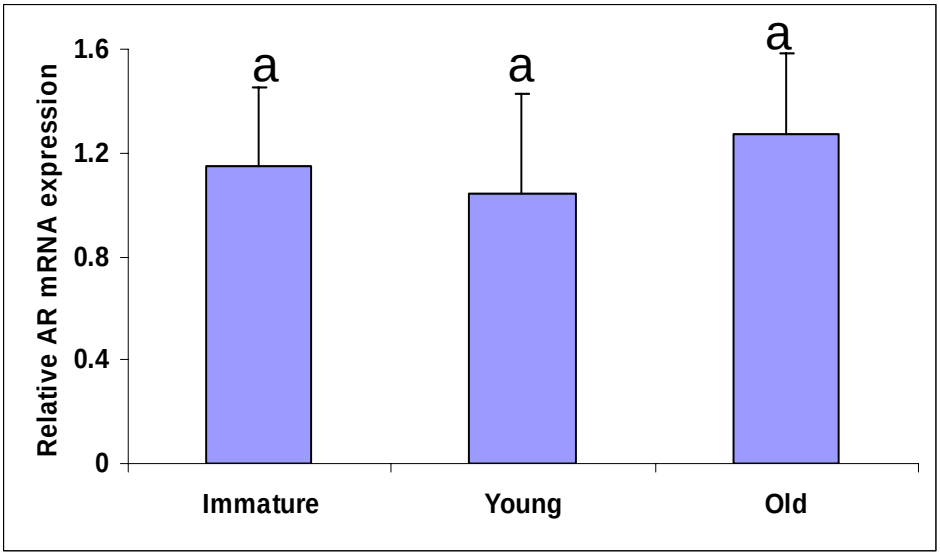
Relative AR mRNA expression
1.6
1.2
0.8
0.4
0
a
a
a
Immature
Young
Old

Figure-2

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