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## **Occurrence of Prostatic Adenocarcinoma in Castrated Dogs and Prostatic Superoxide Dismutase Activity of Healthy Dogs Before and After Castration**

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### **ABSTRACT**

Superoxide dismutase (SOD) has a key role in protecting cells from Reactive Oxygen Species (ROS). Cancer cells produce more ROS involved in proliferation and survival of cancer cells. We, therefore, investigated the percentage of castrated dogs with Prostatic Adenocarcinoma (PA) and the relation between the occurrence of PA in the dog and the prostatic SOD activity after castration. Between 2005 and 2012, diagnosis of Prostatic Adenocarcinoma (PA) was made in 24 dogs at the Veterinary Teaching Hospital of Nippon Veterinary and Life Science University. The percentage of castrated dogs in the 24 dogs with PA and the relation between the occurrence of PA in the dog and the prostatic superoxide dismutase (SOD) activity after castration were examined. Prostatic parenchyma specimens were collected from 6 healthy dogs just before castration and 6 months after castration to measure prostatic SOD activity with a SOD assay kit. Nineteen (79.2%) of the 24 dogs diagnosed with PA had been castrated at 1-6 ( $2.7 \pm 1.7$ ) years of age. Their prostatic SOD activity was significantly lower after castration than before castration ( $p < 0.01$ ). The results of this study indicated that castration could not prevent PA from occurring and the reduction of prostatic SOD activity after castration might lead to oncogenesis in PA of the dog.

**Key words:** Adenocarcinoma, castration, dog, prostate, superoxide dismutase

### **INTRODUCTION**

Reactive Oxygen Species (ROS) generated during metabolic processes is the chemical species that are formed upon incomplete reduction of oxygen and includes hydroxyl radical, superoxides and peroxides (D'Autreaux and Toledano, 2007). Cancer cells generated more ROS compared with normal cells (Szatrowski and Nathan, 1991) and ROS promoted tumor progression, metastasis and survival (Mahalingaiah and Singh, 2014; Sotgia *et al.*, 2011; Verschoor *et al.*, 2010). Superoxide dismutase (SOD) is the main antioxidant enzyme in seminal plasma that prevents ROS from increasing and it protects spermatozoa from damage caused by ROS (Alkan *et al.*, 1997; Chen *et al.*, 2003; Gavella *et al.*, 1996). It has been suggested that a high ROS concentration (Zini and Schlegel, 1997) and low SOD activity (Kawakami *et al.*, 2007a) in the testis may induce testicular tumor. We have previously reported that low SOD activity in the testis (Kawakami *et al.*, 2007b) and seminal plasma (Kawakami *et al.*, 2007c) of the dog is caused by testicular testosterone secretory dysfunction.

It has been reported that castration does not prevent Prostatic Adenocarcinoma (PA) in the dog and that the rate of occurrence of PA in castrated dogs is very high (Bryan *et al.*, 2007; Evans *et al.*, 1985; Obradovich *et al.*, 1987; Waters and Bostwick, 1997; Williams *et al.*, 1999). There have been a few reports that the occurrence of PA in human males is related to low SOD activity in the blood and/or prostate (Kotrikadze *et al.*, 2008; Mikhak *et al.*, 2008). In the present study, we investigated the percentage of castrated dogs with PA and the relation between the occurrence of PA in the dog and the prostatic SOD activity after castration.

## MATERIALS AND METHODS

**Animals:** We investigated the percentage of castration among the dogs diagnosed with PA at the Veterinary Teaching Hospital of Nippon Veterinary and Life Science University between April 2005 and March 2012 and the age of which they had been castrated. Reliable diagnosis of PA was performed by prostatic biopsy under inhalation anesthesia and then pathological examination of the prostatic tissue specimen. All of the dogs diagnosed with PA were examined for metastasis by radiography, ultrasonography and palpation.

**Prostatic SOD assay:** Six healthy dogs (4-8 years old; body weight 10-13 kg) consisting of 2 Beagle dogs and 4 mongrel dogs, that had been cared for at our university were castrated under inhalation anesthesia. The dogs were maintained according to the guidelines of the Animal Care and Use Committee of Nippon Veterinary and Life Science University. Prostatic biopsy was performed on these 6 dogs by laparotomy immediately before the castration and 6 months after the castration. The prostatic parenchyma tissue collected by each biopsy was homogenized and the suspension obtained was used to measure SOD activity with an SOD assay kit (Trevigen Inc., MD, U.S.A.) and spectrophotometer at an absorbance of 550 nm, as described previously (Kawakami *et al.*, 2007a). In addition, SOD activity in the prostatic tissue collected by the biopsy from 5 dogs with PA was measured.

The data is summarized as Mean±Standard Deviation (SD). Differences between means were analyzed for statistical significance by Student's t-test.

## RESULTS

The 24 dogs were diagnosed with PA from 2005-2012 at the Veterinary Teaching Hospital of Nippon Veterinary and Life Science University. The ages of the 24 dogs, the number of dogs that had been castrated, their ages when castrated and the number of dogs with metastasis are shown in Table 1. The metastasis of the PA was detected to pelvic bone, femur, bladder and/or lung. Superoxide dismutase (SOD) activity in the prostatic parenchyma tissue collected from the 6 health

Table 1: Number, age and metastasis of castrated dogs and non-castrated dogs among the 24 dogs diagnosed with prostatic adenocarcinoma in the Veterinary Teaching Hospital between April 2005 and March 2012

Parameters	Castrated dogs	Non-castrated dogs
No. of dogs	19 (79.2% of the 24 dogs)	5 (20.8% of the 24 dogs)
Age (Mean±SD)	8-15 (10.8±2.2)	10-12 (11.4±0.8)
Age when castrated (Mean±SD)	1-6 (2.7±1.7)	-
No. of dogs with metastasis	14 (73.4% of the 19 dogs)	3 (60.0% of the 5 dogs)

Table 2: Superoxide dismutase (SOD) activity ( $\text{U mg}^{-1}$  protein) in prostatic parenchyma tissue collected from the 6 healthy dogs before and 6 months after castration and from the 5 dogs with Prostatic Adenocarcinoma (PA)

Before castration	6 months after castration	Dogs with PA
16.8±1.3	6.0±1.4**	5.3±1.2**

\*\* $p < 0.01$ , in comparison with before. Values are Mean±SD

dogs before and 6 months after castration and from the 5 dogs with PA is shown in Table 2. The mean SOD activity 6 months after castration was significantly lower than before castration ( $p < 0.01$ ). The mean SOD activity of the dogs with PA was also significantly lower than the value of the health dogs before castration ( $p < 0.01$ ).

## DISCUSSION

It has been reported that the most common age of occurrence of canine PA is approximately 10 years old (Krawiec and Heflin, 1992; Lee-Parritz and Lamb, 1988; Teske *et al.*, 2002; Williams *et al.*, 1999) and that the most frequent sites of metastasis by PA in the dog are bones in the vicinity of the prostate, bladder and lung (Krawiec and Heflin, 1992; Lee-Parritz and Lamb, 1988; LeRoy and Northrup, 2009; Weaver, 1981). These reports are consistent with the results obtained in the present study.

Based on the rate of occurrence of PA among the castrated dog in this study, we concluded that castration does not prevent the occurrence of PA and may be one of risk factors for PA in the dog, the same as other reports (Evans *et al.*, 1985; Obradovich *et al.*, 1987). The blood plasma and prostatic SOD activity of human males with PA have been found to be lower than in healthy men (Kotrikadze *et al.*, 2008; Mikhak *et al.*, 2008) and there is a report that PA was prevented in mice by administration of antioxidant materials (Venkateswaran *et al.*, 2004). We have reported that low SOD activity in canine testis may be a risk factor for testicular tumor (Kawakami *et al.*, 2007b) and that low testosterone secretory function of the canine testis is associated with decreased SOD activity in ejaculated semen (Kawakami *et al.*, 2007c). In the present study, the prostatic SOD activity of the healthy dogs was clearly lower 6 months after castration than it was before castration. The prostatic SOD activity of the dogs with PA was also lower than before castration of the healthy dogs. Since the decreased prostatic SOD activity in the dog may be one of risk factors for PA the same as human males (Kotrikadze *et al.*, 2008; Mikhak *et al.*, 2008), it is considered that low prostatic SOD activity caused by loss of the effects of testicular testosterone after castration may be related to the occurrence of PA in the dog.

## CONCLUSION

The study showed that castration might increase the occurrence of PA and the reduction of prostatic SOD activity after castration may be related to the tumorigenesis of PA in the dog.

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