Superoxide Dismutase and Catalase Activities in the Seminal Plasma of Normozoospermic and Asthenozoospermic Beagles

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ABSTRACT. We measured the blood plasma testosterone (T) levels and superoxide dismutase (SOD) and catalase activities in the seminal plasma of the ejaculates of 5 normal (2–5 years old) and 5 asthenozoospermic (AZ–) (3–5 years old) Beagles. Sperm ejaculated by AZ-dogs was incubated for 3 hr in Eagle's MEM only (controls) or Eagle's MEM containing 100 units/ml of SOD or catalase. Sperm motility was examined during incubation. The mean ($\pm$ SE) plasma T level of the AZ-dogs (1.2 ± 0.2 ng/ml) was significantly lower than in the normal dogs (2.5 ± 0.2 ng/ml) ($P < 0.005$). The mean ($\pm$ SE) seminal plasma SOD and catalase activities (18.8 ± 1.9 and 0.5 ± 0.1 unit/g protein, respectively) were significantly lower in the AZ-dogs than in the normal dog (43.3 ± 2.5 and 2.2 ± 0.4 unit/g protein, respectively) ($P < 0.001$ and 0.01 , respectively). The motility of sperm incubated in Eagle's MEM containing SOD or catalase was significantly higher than that of control sperm incubated in only Eagle's MEM after 2 or 3 hr of incubation ($P < 0.05$). The results of this study indicate that poor T secretion by the testes and low antioxidant enzyme activities are related to AZ in the dog.

KEY WORDS: canine, catalase, seminal plasma, SOD.

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Seminal plasma is known to contain reactive oxygen species (ROS) produced by testicular tissue [18] and sperm [2, 20], and elevated seminal plasma ROS concentrations in both humans [1, 16] and dogs [22] have been reported to be a cause of oligozoospermia, asthenozoospermia, and teratozoospermia. Low sperm motility and morphologically abnormal sperm occur as a result of sperm plasma membrane dysfunction caused by ROS [7].

Superoxide dismutase (SOD) [2, 6, 9] and catalase [6, 19, 24] are the main antioxidant enzymes in seminal plasma that prevent increases in ROS concentration in seminal plasma and protect the sperm against damage and oxidative stress caused by ROS. The SOD [6, 8, 18] and catalase [17, 24] in seminal plasma are produced by the testis, epididymis, accessory reproductive organs, and sperm, and they are able to maintain sperm motility for a long time [6, 8]. Seminal plasma SOD has been reported to have the same effect on canine sperm [5]. However, the cause of spermatogenic arrest in the dog is unknown [12]. In the present study, we examined the interaction between peripheral blood plasma testosterone (T) levels and the activities of seminal plasma SOD and catalase in the dog. Based on the results of this study, we suggested some causes for spermatogenic dysfunction in the dog.

MATERIALS AND METHODS

Animals: Ten male Beagles aged 2–5 years were used in this study. They were cared for in our university and housed in pens with ample runs. Commercial dry dog food was provided twice a day, and the dogs were given free access to water. All animals were maintained according to the guidelines of the Animal Care and Use Committee of the Nippon

Veterinary and Life Science University.

The semen quality of the dogs was examined 3 times at one-week intervals. Five of the dogs (3–5 years old) were diagnosed with AZ based on their semen quality (percentage of actively motile sperm: less than 50%).

Collection of ejaculated sperm and evaluation of semen quality: Semen specimens from the 5 normal and 5 AZ-Beagles were collected once weekly for 4 weeks by digital manipulation without a teaser bitch to measure, the SOD and catalase activities in the seminal plasma. Each semen specimen was examined for total semen volume, sperm concentration, and percentages of actively motile sperm and morphologically abnormal sperm by methods described previously [13]. Briefly, the sperm concentration in the semen was determined by hematocytometer counts, and the percentage of actively and progressively motile sperm on glass slides was estimated by examining 500 sperm using a warm-plate and a light microscope. Sperm morphology was assessed after Rose Bengal staining (3 g of Rose Bengal and 1 ml of formalin in 99 ml distilled water).

Assay of SOD and catalase activities in seminal plasma: The sperm-rich fraction of the semen specimens collected from all dogs was centrifuged at $1,500 \times g$ for 15 min. The supernatant (seminal plasma) was collected, and the SOD and catalase activities in the seminal plasma were measured by enzyme analysis reactions using an SOD Assay Kit (Trevigen, Inc., MD, U.S.A.), Catalase Assay Kit (Cayman Chemical Company, MI, U.S.A.) and a spectrophotometer (UV-160 A, Shimadzu Corporation, Tokyo, Japan) at an absorbance of 550 nm for both enzymes. Total protein concentrations in the seminal plasma were determined by the method of Bradford [4].

Blood sampling and blood plasma T assay: Heparinized

peripheral blood samples were collected from superficial leg veins of all dogs on each day of semen collection. Blood samples were collected at 4 different times during the day (09:00, 12:00, 15:00, and 18:00) because of diurnal fluctuation of the plasma T levels in the dog [10, 23]. The plasma was then isolated and stored at -20°C until assay.

Blood plasma T levels were measured by radioimmunoassay as described by Makino *et al.* [14]. Rabbit antiserum to T-11 α -succinate-BSA was used. The intra- and interassay coefficients of variation for T were 3.0% and 9.6%, respectively.

Sperm incubation: The sperm ejaculated by the AZ-dogs was washed twice by centrifugation at $300 \times g$ for 5 min in 5 ml of Eagle's minimal essential medium (MEM; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 1 mg/ml BSA at 38°C . The final sperm pellet was diluted to a concentration of 1×10^7 sperm/ml in MEM (control) and MEM supplemented with 100 units/ml SOD derived from bovine erythrocytes (Calbiochem Inc., Darmstadt, Germany) or 100 units/ml catalase derived from bovine liver (Wako Pure Chemical Industries Inc., Osaka, Japan). The sperm were incubated in loosely capped 120×15 mm glass test tubes for 3 hr at 38°C under an atmosphere of 5% CO_2 in air.

Statistical analysis: All semen and endocrine data were averaged for each dog, and the data for the normal and AZ-group, were summarized as mean values $\pm$ standard error (SE). Differences between means were analyzed for statistical significance by Student's *t* test. *P* values <0.05 were regarded as significant.

RESULTS

Semen quality and plasma T levels: The semen qualities of the normal and AZ-Beagles are shown in Table 1. The mean total number of sperm and mean percentages of actively motile sperm and morphologically abnormal sperm in semen ejaculated by the AZ-dogs were significantly lower than the mean values of the normal dogs ($P<0.001$). The very low sperm motility of the AZ-dogs was especially noteworthy.

The peripheral blood plasma T levels of the normal and AZ-dogs are shown in Table 1. The mean blood plasma T level of the AZ-dogs was significantly lower than in the normal dogs ($P<0.005$).

Seminal plasma SOD and catalase activities: The seminal plasma SOD and catalase activities of the normal and AZ-dogs are shown in Table 2. The mean SOD and catalase activities in the seminal plasma of the sperm-rich fraction of the AZ-dogs were significantly lower than the mean activities of the normal dogs ($P<0.001$ and 0.01 , respectively).

Sperm motility in the medium containing SOD or catalase: The changes in the mean percentages of actively motile sperm of the AZ-dogs in MEM containing SOD or catalase and in the control MEM during incubation are shown in Fig. 1. The mean percentages of motile sperm in MEM containing SOD and catalase were significantly

Table 1. Mean ($\pm$ SE) semen quality values and plasma testosterone levels in specimens collected once a week for four weeks from 5 normal (2–5 years old) and 5 asthenozoospermic (AZ–) (3–5 years old) Beagles

	Normal dogs	AZ–dogs
Total volume of semen (ml)	11.2 ± 4.2	9.4 ± 3.8
Total number of sperm ($\times 10^6$)	558.5 ± 72.4	$127.3 \pm 47.2^{**}$
Actively motile sperm (%)	82.3 ± 9.1	$19.9 \pm 3.6^{**}$
Abnormal sperm (%)	3.6 ± 1.5	$16.2 \pm 1.6^{**}$
Testosterone (ng/ml)	2.54 ± 0.17	$1.22 \pm 0.24^*$

$^{**}P<0.001$, compared with the normal dogs.

$^*P<0.005$, compared with the normal dogs.

Table 2. Mean ($\pm$ SE) superoxide dismutase (SOD) and catalase activities (units/g protein) in the seminal plasma of specimens collected once a week for four weeks from 5 normal and 5 asthenozoospermic (AZ–) Beagles

	Normal dogs	AZ–dogs
SOD	43.34 ± 2.47	$18.82 \pm 1.85^{**}$
Catalase	2.18 ± 0.38	$0.48 \pm 0.11^*$

$^{**}P<0.001$, compared with the normal dogs.

$^*P<0.01$, compared with the normal dogs.

higher than for the controls in MEM only after 2 or 3 hr of incubation ($P<0.05$).

DISCUSSION

SOD [6, 8] and catalase [19, 24] are very important antioxidant enzymes in seminal plasma [9, 11]. An increase in the seminal plasma ROS concentration has been reported to cause poor semen quality in humans [1, 16] and dogs [22], and low SOD [2] and catalase [24] activities in seminal plasma cause an increase in the ROS concentration. Seminal plasma SOD has been found to protect canine sperm from damage by oxidative stress caused by ROS [5]. We assume that the low SOD and catalase activities in the seminal plasma of the AZ-dogs in the present study were closely related to the low motility of the ejaculated sperm. The seminal plasma ROS concentration of the AZ-dogs may be higher than in normozoospermic dogs.

The mean blood plasma T level of the AZ-dogs in this study was lower than in the normal dogs. As a result of the low T secretory function of canine testes, epididymal and prostatic function declines and abnormal seminal pH and osmotic pressure values develop [12]. The low motility of canine sperm is caused by abnormal pH and osmotic pressure of the seminal plasma [12]. Other factors responsible for low sperm motility are thought to be low SOD [21] and catalase [24] activities and high ROS concentrations [1, 3] in seminal plasma. Since the epididymis and prostate produce antioxidant enzymes [6, 15], epididymal and prostatic dysfunction caused by poor T secretion by the testes is

Fig. 1. Changes in the mean ($\pm$ SE) percentages of actively motile sperm in 5 asthenozoospermic dogs after 1, 2, and 3 hr of incubation in MEM with 100 units/ml superoxide dismutase (●—●) or 100 units/ml catalase (○---○) and in MEM without the enzymes (▲-----▲). * $P < 0.05$, compared with MEM without the enzymes.

assumed to be responsible for the low SOD and catalase activities in the seminal plasma.

The results of the present study demonstrate that canine sperm motility can be maintained by the effects of SOD and catalase. Given that the mean seminal plasma SOD activity was higher than the catalase activity in the normal dogs and that the percentage of actively motile sperm was higher in MEM containing SOD than in MEM containing catalase, we believe that SOD is a more effective and important antioxidant enzyme than catalase for maintenance of canine sperm motility.

We therefore concluded that the low sperm motility of the AZ-dogs in this study was attributable to both low blood plasma T levels and low seminal plasma SOD and catalase activities.

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