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Male Genital Tract Antioxidant Enzymes: Their Source, Function in the Female, and Ability to Preserve Sperm DNA Integrity in the Golden Hamster

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ABSTRACT: Recently, we reported that male accessory sex gland (ASG) secretions protect sperm genomic integrity by demonstrating that DNA damage was more extensive in sperm not exposed to the secretions. The present study was conducted to find out if ASGs secrete the main antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx or GSH-Px), and catalase (CAT) and if the most abundant one, SOD, can protect those sperm that were not exposed to ASG secretions against NADPH-induced oxidative stress. Four experimental groups of male golden hamsters were used: intact animals with proven fertility, animals with all major ASGs removed (TX), animals that were bilaterally vasectomized, and sham-operated controls. SOD, CAT, and GPx activities were measured in secretions from all 5 ASGs and sperm-free uterine flushing from virgin females and those mated with the experimental males. The alkaline comet assay was used to analyze DNA integrity of the TX group sperm after incubation in a medium containing 50 U/mL of SOD along with 0 to 20 mmol/L NADPH. The main antioxidant enzyme in ASGs was SOD from coagulating glands ($P < .05$) and GPx together with CAT from ampullary glands ($P < .05$). Uterine flushing of ejaculates that contained ASG secretions had more SOD and CAT activities than those with epididymal secretions alone ($P < .05$ and $P < .001$, respectively), whereas activity of GPx was the same ($P > .05$). Addition of SOD in vitro dose dependently decreased the incidence of single-strand DNA damage in sperm not exposed to ASG secretions incubated in the presence of 0 to 20 mmol/L NADPH ($P < .001$). These results indicated that, in terms of abundance, SOD was the main antioxidant enzyme secreted by male ASGs, whereas CAT was the second one. The GPx activity came from both epididymis and ASGs. We conclude that ASG secretions play a significant role in protecting sperm against oxidative stress.

Key words: Superoxide dismutase, glutathione peroxidase, catalase, sperm DNA damage, oxidative stress, Syrian hamster.

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DNA damage in ejaculated human sperm can be attributed to underprotamination during spermiogenesis, abortive apoptosis during spermatogenesis (Sakkas et al, 1999), and oxidative stress (Alvarez et al, 1987; Aitken and Fisher, 1994). The latter is mainly due to reactive oxygen species (ROS) associated with excess residual cytoplasm and depletion of antioxidants (Aitken and Krausz, 2001). Currently, environmental factors such as low-level radiation, heat stress, and smoking are widely recognized as agents that can induce sperm DNA damage (Sailer et al, 1995, 1997). ROS such as superoxide anions ($O^{\cdot -}$), hydroxyl radicals ($OH^{\cdot -}$), and hydrogen peroxide (H_2O_2) may influence the structural integrity and function of sperm, such as motility, capacitation, and sperm-oocyte fusion (Jones and Mann, 1976; Aitken, 1995; de Lamirande and Gagnon, 1995a,b; Griveau et al, 1995). Spermatozoa are particularly vulnerable to oxidative stress because their plasma membrane is rich in polyunsaturated fatty acids (PUFAs) and membrane-bound NADPH oxidase. An optimal amount of ROS is maintained by balancing the ROS generated during sperm maturation in the epididymis and antioxidants in secretions of the male reproductive system. Sperm experience oxidative stress if the balance is upset. ROS have been shown to correlate with reduced male fertility (Iwasaki and Gagnon, 1992), cause peroxidative damage to the sperm plasma membrane (Hughes et al, 1996), and induce both DNA strand breakages and oxidative base damage in human sperm (Kodama et al, 1997). Epididymal secretory glutathione peroxidase (GPx5), superoxide dismutase (SOD), and antioxidant factors from seminal plasma have been reported to play important parts in counteracting oxidative stress on sperm (Perry et al, 1993; Vernet et al, 1996; Twigg et al, 1998; van Overveld et al, 2000). Albumin in human seminal plasma protects sperm against peroxidative damage by absorbing lipid peroxides from

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sperm plasma membrane (Twigg et al, 1998). In addition, a decrease in total antioxidant capacity of seminal plasma has been correlated with a reduction in sperm quality, such as concentration, motility, and morphology (Lewis et al, 1994; Palan and Naz, 1996; Smith et al, 1996; Sharma et al, 1999).

Our previous investigations demonstrated that removal of all or some of the male accessory sex glands (ASGs) in the golden hamster lowered fertility and increased embryonic wastage during implantation and the postimplantation period (Chow et al, 1986; O et al, 1988), structural abnormalities in implanted embryos were more extensive (Chan et al, 2001; Jiang et al, 2001), whereas sperm chromatin decondensation and DNA replication in the first cell cycle were delayed (Ying et al, 1998, 1999). These could be attributed to sperm DNA damage. Recently, we demonstrated that DNA damage was more extensive in sperm not exposed to male ASG secretions. Mature epididymal spermatozoa were less susceptible to NADPH treatment and male ASG secretions could protect uterine sperm DNA from breakage after NADPH treatment, indicating that male ASG secretions might have a role in preserving sperm genomic integrity in the female genital tract (Chen et al, 2002). In the golden hamster, the major male ASGs include ampullary glands, ventral prostate, dorsolateral prostates, coagulating glands, and seminal vesicles. Their secretions make up the bulk of seminal plasma. In humans, seminal plasma has been reported to contain antioxidant enzymes, such as SOD, catalase (CAT), and glutathione peroxidase (GPx), and free radical scavengers, such as vitamins C and E, hypotaurine, taurine, uric acid, and albumin (Lewis et al, 1997; Yeung et al, 1998; Zini et al, 2002). Rodent epididymis also secretes antioxidant enzymes and free radical scavengers (Aumüller et al, 1990).

In this study, we address 3 questions: 1) Do male ASGs of golden hamster secrete antioxidant enzymes, such as SOD, GPx, and CAT? 2) What are the main antioxidant enzymes present in postcoital uterine fluid? and 3) Can SOD protect sperm that are not exposed to male ASG secretions against NADPH-induced oxidative stress? Comet assay, also known as single-cell gel electrophoresis, is used to assess sperm DNA integrity. It is rapid, simple, visual, and sensitive for detecting alkali labile sites and DNA strand breaks in individual mammalian cells (Ostling and Johanson, 1984; Singh et al, 1988; Olive et al, 1990). The introduction of alkaline (pH >13) condition to unwind, denature, and separate DNA by electrophoresis makes the revelation of single-strand DNA (ssDNA) breakage more obvious.

Materials and Methods

All chemicals were purchased from Sigma Chemical Company (St Louis, Mo) unless otherwise mentioned. Tyrode albumin lactate pyruvate medium (TALP, pH 7.4 [pH 7.7 before gassing], 285–290 mOsm) was prepared with Milli-Q water and contained 114 mmol/L NaCl (BDH, Poole, England); 3.2 mmol/L KCl, 2.0 mmol/L $CaCl_2 \cdot 2H_2O$, 0.4 mmol/L $NaH_2PO_4 \cdot H_2O$, 5.0 mmol/L D-glucose (E. Merck, Darmstadt, Germany); 0.5 mmol/L $MgCl_2 \cdot 6H_2O$, 25 mmol/L $NaHCO_3$, 10 mmol/L sodium lactate, 0.1 mmol/L sodium pyruvate, 0.1 mmol/L sodium penicillin G, 0.01 mg/mL phenol red, and 3 mg/mL bovine serum albumin (BSA). Modified TALP (m-TALP) medium for capacitation contained 0.5 mmol/L taurine, 0.05 mmol/L adrenaline (CALBIOCHEM, San Diego, Calif), and 15 mg/mL BSA. Phosphate-buffered saline (PBS; 10× PBS, pH 7.4), which was made up of 80.1 g of NaCl, 2 g of KCl, 11.5 g of Na_2HPO_4 (Fisher, Fairlawn, NJ), and 2 g of KH_2PO_4 (WAKO, Osaka, Japan) in 1 L of Milli-Q water, was diluted 10-fold just before use.

Animal Model

Animals were maintained and handled in compliance with a protocol approved by the Committee on the Use of Life Animals for Teaching and Research of The University of Hong Kong. Randomly bred Syrian hamsters (*Mesocricetus auratus*) were supplied by and housed in the Laboratory Animal Unit of the Faculty of Medicine, The University of Hong Kong, under a 14 hours light–10 hours dark lighting cycle, with lights on from 1100 to 0100 hours. Temperature was maintained at 22°C, and food and tap water were available ad libitum. Vaginal secretions of 6- to 8-week-old female hamsters were checked daily for at least 2 normal consecutive cycles before mating.

Under general anesthesia with a 2:1 mixture of 10% ketamine and 2% xylazine (0.2 mL/100 g of body weight, intraperitoneal) (Alfasan, Woerden, Holland), major male ASGs were surgically ablated from 6- to 8-week-old male hamsters according to procedures described by Chow et al (1986) and placed in the following 2 groups: sham-operated control hamster (SH; n = 8) and hamsters that underwent bilateral excision of ampullary gland, ventral prostate, dorsolateral prostate, coagulating gland, and seminal vesicle (TX; n = 10). VX stood for bilaterally vasectomized hamsters (n = 5). The animals were allowed to recuperate for 1 month and success of surgery was confirmed post mortem.

Sample Preparation

Collection of ASG Secretion—Fourteen- to 20-week-old intact male hamsters of proven fertility were killed with an overdose of sodium pentobarbital injected intraperitoneally (Boehringer Ingelheim, Artamon, New South Wales, Australia). Blood was withdrawn by cardiac puncture. Male ASGs were individually removed and cleared of connective tissues and blood. Secretions were drained from the seminal vesicles and coagulating glands. Cuts were made on the dorsolateral prostate, ventral prostate, and ampullary gland to release secretions. Secretions were pooled, dispensed into microcentrifuge tubes, kept in ice, and centrifuged at 200 × *g* for 20 minutes. The supernatant was collected and kept at 4°C for immediate assay of enzyme activity and protein content.

Collection of Postejaculatory Uterine Fluid—Each normally cycling female hamster was mated with one operated-on male for 15 minutes on the day of estrus and killed with an overdose

of sodium pentobarbital (Boehringer Ingelheim) within 30 minutes after mating. Uterine horns were removed, cleared of blood and connective tissues, and flushed with 1 mL of 1× PBS (pH 7.4). Debris and cells were removed by 2 rounds of centrifugation at 10 036 × *g* for 5 minutes. The supernatant was collected and stored at −70°C for enzyme activity measurement and protein assay later. Uterine content from virgin females at estrus was collected in the same manner.

Collection of Uterine Sperm—Sperm was collected by flushing the uterine horns with TALP medium with or without SOD at 50 U/mL (Roche, Mannheim, Germany). Samples from 2 females were pooled, washed, and centrifuged twice at 300 × *g* for 10 minutes. The pellet was resuspended in m-TALP medium with or without SOD at 50 U/mL followed by NADPH (Roche) treatment.

Treatment of Sperm With β-NADPH

Uterine sperm (1×10^6 cells/mL) ejaculated by SH and TX males was incubated for 2 hours at 37°C under 5% CO_2 in media containing 0, 1.25, 2.5, 5, 10, or 20 mmol/L NADPH with or without 50 U/mL of SOD. After incubation, the treated sperm were pelleted twice at 300 × *g* for 10 minutes and resuspended in fresh medium for comet assay.

Assessment of Sperm DNA Damage by Comet Assay

Comet assay for sperm was adapted from Shen and Ong (2000) and was performed in darkness. Sperm were resuspended in PBS at 6 to 8×10^5 cells/mL. Ten microliters of sperm suspension was thoroughly mixed with 75 μL of 0.5% (wt/vol) agarose 3:1 (Ameresco, Solon, Ohio). Fifty microliters of the sperm suspension was pipetted onto a CometSlide (Trevigen, Gaithersburg, Md) and allowed to gel. The slides were carefully submersed in cold lysis buffer made up of 2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris-HCl, 10% (vol/vol) DMSO, and 1% (vol/vol) Triton-X 100, pH 10, for 1 hour at 4°C. The slides were then transferred to another buffer (2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris-HCl, pH 7.4) containing DNase-free proteinase K (10 μg/mL; Amresco) and incubated at 37°C overnight.

After enzyme treatment, the slides were transferred into an electrophoresis chamber (Pharmacia Biotech, San Francisco, Calif) filled with alkaline buffer made up of 300 mmol/L NaOH (E. Merck), 1 mmol/L EDTA, 0.2% (vol/vol) DMSO, and 0.1% (wt/vol) 8-hydroxyquinoline (pH >13). The slides were left side by side in alkaline buffer for 20 minutes to allow unwinding of the DNA before being electrophoresed at 0.96 V/cm, 250 mA for 20 minutes at room temperature. After electrophoresis, the slides were equilibrated with 0.4 mol/L Tris-HCl (pH 7.4) for 15 minutes followed by fixation in 100% (vol/vol) ethanol for 15 minutes and air-dried. The dried slides were immersed in 10 mmol/L NaH_2PO_4 and 5% (wt/vol) sucrose for 10 minutes and finally stained with 0.25 μmol/L YOYO-1 (Molecular Probes, Eugene, Oregon) in 5% (vol/vol) DMSO and 5% (wt/vol) sucrose for 10 minutes.

CometSlides were examined at ×250 with a fluorescence microscope (Carl Zeiss, Oberkochen, Germany) fitted with a cooled CCD camera (KX Series Imaging System, Apogee Instruments Inc, Tucson, Ariz). The incidence of comet formation was scored for all the sperm on each circle of the CometSlide (2 circles) and expressed as the number of comets per 10 000 sperm.

Enzyme Activity Assays

Activities of antioxidant enzymes in individual ASG secretions, expressed per milligram of secretory protein, were assayed using Shimadzu UV-Visible Recording Spectrophotometer UV 1601 (Shimadzu Company, Tokyo, Japan). Total SOD activity was determined by the nitroblue tetrazolium (NBT) assay (Oberley and Spitz, 1985). One unit of activity was defined as the quantity of SOD required to produce 50% reduction of NBT. Total GPx activity was detected by the standard indirect coupled method using cumene hydroperoxide (Glunzler and Flohe, 1985), and one unit of activity was defined as 1 μmol NADPH oxidized at 37°C per minute using $[GSH]_0$ equal to 1 mmol/L. Catalase activity assay based on rate of peroxidation of titanium oxysulfate as described by Sun et al (1975) was used. Enzyme activity in red blood cells was determined in the same way. Their contribution to activity of each type of enzyme in the samples was found to be negligible (data not shown).

The SOD, GPx, and CAT activities in postcoital sperm-free uterine flushing were measured and expressed per milliliter of uterine flushing. Spectrophotometer equipped with Cary WinUV software (Cary 300 Bio UV-Visible Spectrophotometer, Varian Australia Pty Ltd, Victoria, Australia) was used to measure SOD and GPx activity. SOD activity assessment was based on NADH oxidation (Paoletti and Mocali, 1990), and one unit was defined as the amount of enzyme that inhibited the NADH oxidation of the control by 50%. Total GPx activity was estimated by the same method used for ASG secretions. CAT activity was determined by the decrease in concentration of H_2O_2 after incubation with test samples. The horseradish peroxidase–dependent oxidation of phenol red to a blue derivative (absorbance, 630 nm) was used to estimate H_2O_2 (Yeung et al, 1996). One unit of activity was defined as 1 μmol H_2O_2 removed at 37°C per minute.

Protein Assay

The BCA (bicinchoninic acid) Kit (Pierce, Rockford, Ill) was used to determine protein concentration. Manufacturer's instruction was followed and absorbance was read with a SPECTRAmax 340 Microplate Reader (Molecular Devices Corporation, Sunnyvale, Calif).

Statistical Analysis

All results were presented as mean ± SEM. The incidence of DNA damage was arcsine transformed before applying statistical analyses. All results were analyzed by 1-way analysis of variance (ANOVA) followed by Dunnett's posttest. Two-way ANOVA was also applied to analyze the response of uterine sperm to NADPH incubation. $P < .05$ was considered statistically significant (Prism software version 3.0, GraphPad, San Diego, Calif). Each experiment was repeated at least 5 times.

Results

Antioxidant Enzymes in Male ASG Secretions

SOD was the main antioxidant enzyme in male ASG secretions, and most of it came from the coagulating glands

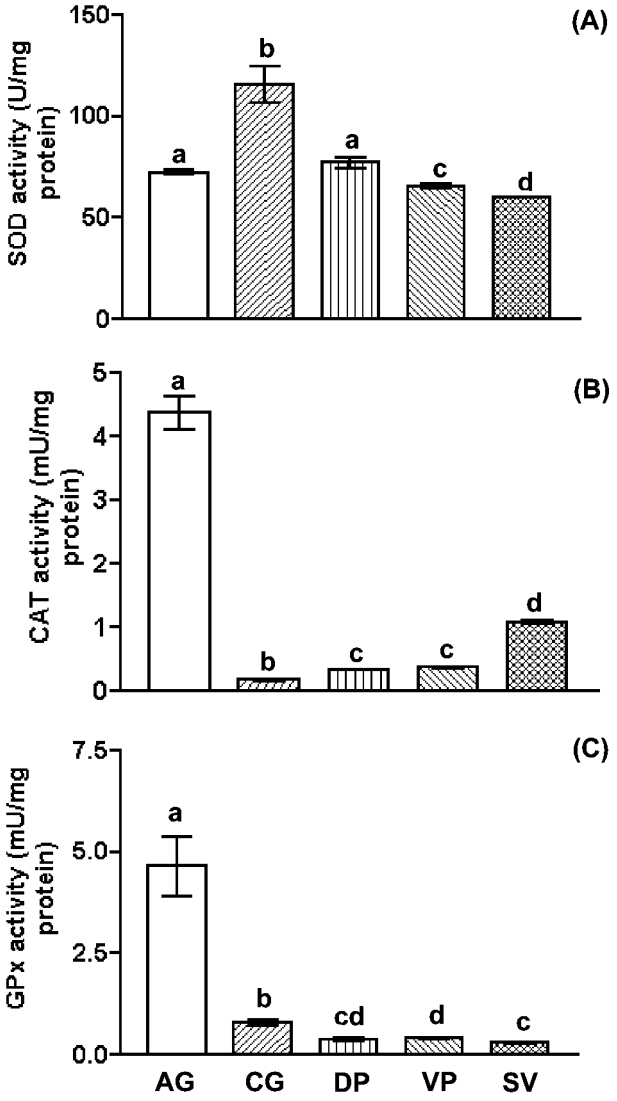

Figure 1. Specific activity of antioxidant enzymes in male accessory sex gland secretions. AG indicates ampullary gland; CG, coagulating gland; DP, dorsolateral prostate, VP, ventral prostate; and SV, seminal vesicle. (A) Superoxide dismutase activity (units per milligram of protein); (B) catalase activity (milliunits per milligram of protein); (C) glutathione peroxidase activity (milliunits per milligram of protein). Different superscripts in each panel represent significant difference ($P < .05$).

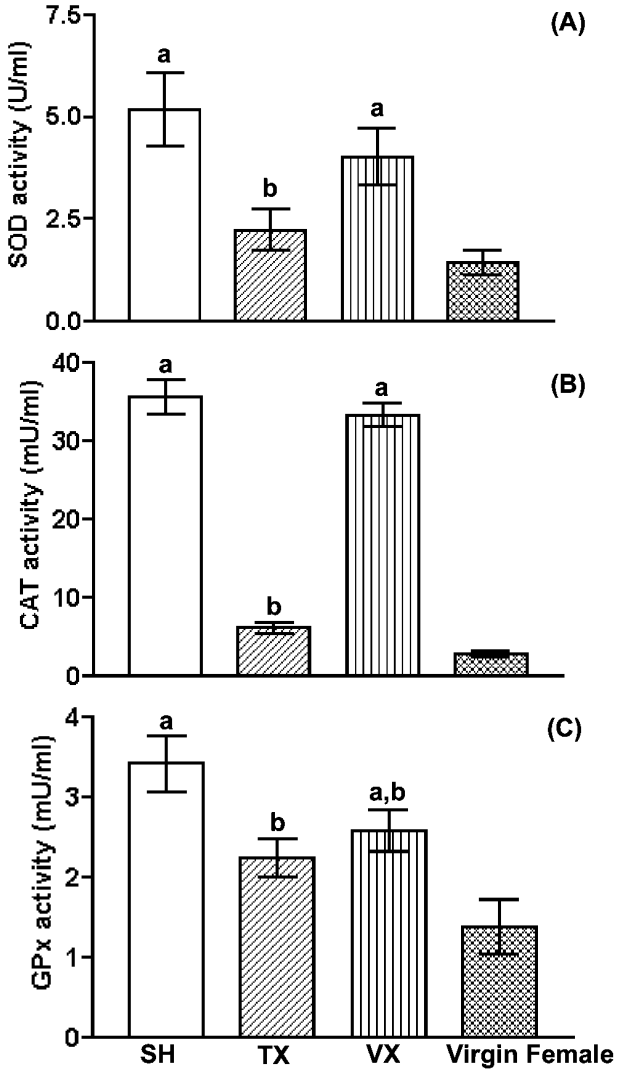

Figure 2. Total antioxidant enzyme activity in uterine flushing after mating with an operated-on male hamster. SH indicates sham-operated control; TX, males with all major male accessory sex glands removed; and VX, vasectomized male. (A) Superoxide dismutase activity (units per milliliter); (B) catalase activity (milliunits per milliliter); (C) glutathione peroxidase activity (milliunits per milliliter). Different superscripts in each panel represent significant difference ($P < .05$).

($P < .05$). GPx and CAT mainly came from ampullary glands ($P < .05$) (Figure 1A through C).

Antioxidant Enzymes in Postcoital Uterine Flushing

Activities of antioxidant enzymes in uterine flushing collected immediately after mating with operated-on male hamsters are shown in Figure 2A through C. SOD activity (units per milliliter of uterine flushing) in samples from males with all ASGs removed was higher than the control ($P < .01$, TX vs SH) and vasectomized groups ($P < .05$, TX vs VX). Values for virgin female were used as baseline. Similarly, CAT activity (milliunits per milliliter of uterine flushing) from males with all ASGs removed was also significantly lower compared with the control ($P < .001$, TX vs SH) and vasectomized hamsters ($P < .001$, TX vs VX). However, total GPx activity (milliunits per milliliter of uterine flushing) was statistically lower only when compared with the control ($P < .05$, TX vs SH) but not the vasectomized group ($P > .05$, TX vs VX).

Protective Effects of Antioxidant Enzymes In Vitro

The results are shown in Figure 3. Without SOD treatment, sperm not exposed to ASG secretions (TX) presented a very significant dose-dependent increase in the incidence of ssDNA damage compared with those from the SH group when incubated with increasing dose of

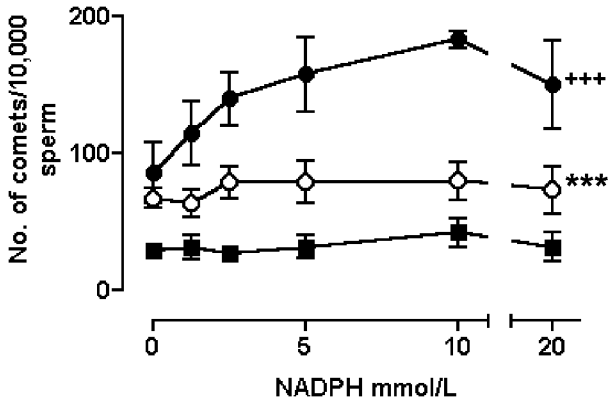

Figure 3. Protective effects of superoxide dismutase (SOD) against NADPH-induced oxidative stress. Uterine sperm ejaculated by males with all major accessory sex glands removed (●; TX without SOD addition in vitro) are more vulnerable to single-strand DNA (ssDNA) damage when incubated with NADPH ($^{+++}P < .001$) compared with those from the sham-operated control (■). Addition of SOD in the flushing and incubation media significantly decreased the incidence of NADPH-induced ssDNA damage in uterine sperm ejaculated by males with all major accessory sex glands removed (○; TX with SOD addition in vitro) compared with those without SOD (●; TX without SOD addition in vitro) in a dose-dependent manner ($^{***}P < .001$).

NADPH ($P < .001$, TX vs SH). However, addition of SOD at 50 U/mL into the flushing medium when sperm were collected, followed by incubation with NADPH added to 50 U/mL of SOD before performing the comet assay, induced a significant and reversible dose-dependent decrease in incidence of ssDNA damage in sperm from hamsters with all ASGs removed ($P < .001$, TX with SOD vs TX).

Discussion

We have demonstrated that SOD was the main antioxidant enzyme secreted by male ASGs in the golden hamster. A comparison of samples from the TX and VX hamsters shows that in postcoital uterine flushing, SOD and CAT activities are significantly higher in samples with contribution from male ASGs. Male ASGs also have GPx activity, but the main source is the epididymis. Thus, male ASGs contribute toward protecting sperm from oxidative stress in the uterine environment.

Antioxidant Enzyme Activity

ROS consists mainly of superoxide anions ($O^{\cdot -}$), hydroxyl radicals ($OH^{\cdot -}$), and hydrogen peroxide (H_2O_2), and they are toxic to sperm. Their harmful effect is reduced by serial antioxidant actions of SOD, which catalyses the rapid removal of $O^{\cdot -}$ to form H_2O_2. If not removed, H_2O_2 in turn forms highly toxic hydrogen radicals ($OH^{\cdot -}$) that has been reported to induce DNA damage and cause a rapid loss of sperm fertilizing potential by promoting lipid peroxidation and loss of adenosine triphosphate (de Lamirande and Gagnon, 1992; Aitken et al, 1993). CAT and/or GPx rapidly convert the H_2O_2 to H_2O. In the present study, we have found that antioxidant enzymes such as SOD, GPx, and CAT are present in the male reproductive tract of golden hamsters. GPx is abundant in the epididymis, whereas SOD and CAT are secreted mainly by male ASGs. This may be related to protecting the maturing sperm before ejaculation. GPx messenger RNA (mRNA), including glutathione peroxidase (GPx3), phospholipid hydroperoxide glutathione peroxidase (PH-GPx or GPx4), and secretory epididymal glutathione peroxidase (E-GPx or GPx5), is highly expressed in epididymis (Zini and Schlegel, 1997; Jervis and Robaire, 2001), whereas CAT mRNA expression is lower (Zini and Schlegel, 1996). GPx, especially GPx4, is thought to regulate the redox status of mammalian sperm during completion of sperm chromatin compaction in the epididymis (Godeas et al, 1997). Moreover, together with CuZn-SOD, it constitutes the core antioxidant system of the epididymis defense. The relative paucity of intracellular antioxidant enzymes and the high concentration of unsaturated fatty acids in epididymal spermatozoa membrane (Awano et al, 1993; Aitken, 1999) make sperm extremely vulnerable to oxidative stress (de Lamirande et al, 1997). On reaching the cauda epididymis, sperm are provided with a microenvironment full of antioxidant enzyme and scavengers that keep the ROS at a low and defensible level. These include antioxidant systems, such as glutathione synthetase, GPx5, PH-GPx or GPx4, thioredoxin peroxidase, CuZu-SOD, and glutathione S-transferases (Jervis and Robaire, 2001). A low intraluminal pH also helps to suppress NADPH-oxidase activity (Breton et al, 1996; Aitken et al, 1998). Epididymis can also synthesize lactoferrin to bind free ion (Jin et al, 1997), secrete albumin-like protein to sequester toxic lipid peroxides (Carles et al, 1992), and produce small-molecular-weight free radical scavengers such as vitamin C, glutathione, and polyamines (Khan et al, 1992; Muscari et al, 1995).

The postcoital uterine fluid of the hamster has SOD and CAT mainly contributed from male ASGs and GPx from accessory sex glands and epididymis. These enzymes are there to protect sperm. The uterine environment contains many elements that are harmful to sperm. They include nitric oxide (Norman and Cameron, 1996) and amino acids (Fahning et al, 1967). The latter have been reported to stimulate production of H_2O_2 in bovine sperm through the action of an amino acid oxidase (Lapointe and Sirard, 1998). Following mating, leukocytes (Thompson et al, 1992; Williams et al, 1993), mainly eosinophils (Perez et al, 1996), are stimulated by seminal plasma to produce large quantities of H_2O_2 (Hansen et al, 1987). Moreover, estrogen has prooxidant and antioxidant effects. At estrus, under the influence of estrogen, endo-

metrial epithelial NADPH oxidase produces NADPH (Moulton and Barker, 1971; Hilf et al, 1972; Swanson and Barker, 1983), superoxide anions (Laloraya et al, 1991; Jain et al, 1999, 2000), and hydrogen peroxide (Riley and Behrman, 1991). An estrogen-stimulated antioxidant system that maintains an optimal amount of these oxidants also regulates sperm capacitation. The estrogen-induced antioxidant system mainly includes SOD, peroxidase from eosinophils and endometrial epithelial cells (Hosoya and Saito, 1981; Anderson et al, 1986; Riley and Behrman, 1991), and glutathione peroxidase/reductase system (Ohwada et al, 1996; Diaz-Flores et al, 1999; Kaneko et al, 2001). Northern blot could only detect a low level of CAT mRNA in uterus (Lapointe et al, 1998). Taken together, GPx and SOD are key antioxidant enzymes for sperm maturation and postejaculatory function.

Protective Effects of SOD In Vitro

A mature sperm is particularly vulnerable to oxidative stress, because its membrane is rich in PUFAs and NADPH oxidase but its scanty cytoplasm has little antioxidant enzymes. During maturation, epididymal fluid provides them with many antioxidant enzymes and free radical scavengers. At ejaculation, male ASG secretions put in some more SOD, CAT, GPx, vitamins C and E, hypotaurine, taurine, uric acid, and albumin. We have previously demonstrated that in the total absence of ASG (TX) sperm, ssDNA damage is more extensive and more frequent (Chen et al, 2002). In the present study, we found high SOD activity in secretions of these glands. We proposed that SOD could counteract the NADPH-induced oxidative stress in sperm, and we confirmed that the incidence of ssDNA damage was significantly reduced in the presence of exogenous SOD. Other reports also suggested that antioxidants such as ascorbate (vitamin C), α-tocopherol (vitamin E), and urate can guard ejaculated human sperm against x-ray–induced and H_2O_2-induced DNA damage in vitro (Hughes et al, 1998; Donnelly et al, 1999).

In summary, we have demonstrated that SOD and CAT from the male ASGs are the main antioxidative enzymes in the postcoital uterine environment. GPx, equally contributed by ASGs and epididymis, is also present. SOD protects sperm against NADPH-induced DNA breakage in a dose-dependent manner.

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