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Catalase-like and superoxide dismutase-like activities in human seminal plasma

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Abstract Human spermatozoa are highly susceptible to oxidative injury but are naturally protected from such injury by the antioxidant properties of seminal plasma. We measured catalase-like and superoxide dismutase (SOD)-like activities in the seminal plasma of fertile and vasectomized men in order to gain insight into the potential source(s) and function(s) of these antioxidants in semen. Semen samples were obtained from fertile men ($n=11$) and men post-vasectomy ($n=16$). Catalase-like activity was measured by the decrease in hydrogen peroxide concentration after incubation with seminal plasma. SOD-like activity was measured as the inhibition of nitroblue tetrazolium reduction due to superoxide anion generation by xanthine plus xanthine oxidase. Mean seminal catalase-like activity ($\pm$1SD) in the fertile group was not significantly different from that of the post-vasectomy group (389 ± 163 and 325 ± 119 U/ml, respectively). Similarly, mean seminal SOD-like activity in the fertile group was not significantly different from that of the post-vasectomy group (37 ± 10 and 36 ± 10 U/ml, respectively). Our data suggest that the testis and epididymis are not an important source of catalase-like and SOD-like activities in semen. These findings indicate that antioxidants in semen are primarily of post-testicular origin and probably serve to protect ejaculated spermatozoa from oxidative stress such as that which occurs in the female reproductive tract.

Keywords Semen · Catalase · Superoxide dismutase · Vasectomy · Spermatozoa

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Introduction

Seminal plasma is an important source of antioxidants and, as such, is believed to play a key role in supporting sperm function [1]. Most importantly, seminal plasma can protect spermatozoa from the potentially harmful effects of reactive oxygen species (ROS) [14]. The susceptibility of mammalian spermatozoa to oxidative stress stems from the abundance of unsaturated fatty acids in the sperm plasma membrane [2, 5]. ROS can also cause DNA damage [3, 18].

Human seminal plasma possesses both superoxide dismutase (SOD)-like and catalase-like activities [12, 23]. However, little is known about the putative source of these key antioxidant activities in human semen. We measured catalase-like and SOD-like activities (two major antioxidant activities) in the semen of fertile and vasectomized men to gain insight into the potential source(s) and function(s) of these antioxidants in semen.

Patients and methods

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and were at least reagent grade.

Sperm preparation, treatments and motility analysis

Semen samples were obtained from consecutive men presenting for vasectomy (pre-vasectomy, $n=11$) and post-vasectomy ($n=16$) at our institution. All men had previously fathered children. This study was undertaken with ongoing internal review board approval. Samples were produced by masturbation after 3–5 days of sexual abstinence and allowed to liquefy at room temperature. After liquefaction of semen, standard semen parameters (volume, density, motility, morphology) were obtained according to World Health Organization (WHO) guidelines [19]. Semen samples were centrifuged at $10{,}000 \times g$ for 10 min, and the supernatants were collected and stored at –20°C for later assessment of antioxidant activity. All of the pre-vasectomy semen samples had motile sperm, and none had significant leukocytospermia as per WHO guidelines [19].

SOD-like and catalase-like activities

SOD-like activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to the superoxide anion generated by the combination xanthine + xanthine oxidase [23]. The reaction mixture was buffered with phosphate-buffered saline (PBS, pH 7.4) and contained xanthine (0.6 mM), diethylene triamine penta-acetic acid (DETAPAC, 2 mM), catalase (1.5 U/mL), NBT (0.2 mM) and various volumes of seminal plasma. The reaction was started by adding xanthine oxidase (0.05 U/ml) and monitored (at 25ºC) by the increase in absorbance at 545 nm. Under these conditions the reduction of NBT by the superoxide anion is linear for the first 30 min, and measurements were taken every 3–5 min [23]. One unit of SOD-like activity was defined as the quantity of seminal plasma capable of decreasing the reduction of NBT by 50%.

Catalase-like activity was measured by the decrease in concentration of H_2O_2 after incubation with the test samples. The presence of H_2O_2 was assessed using the horseradish peroxidase-dependent oxidation of phenolsulphonphthalein (phenol red) to a blue derivative (absorbance 630 nm) [23]. The reaction mixture was buffered with PBS (pH 7.4) and contained H_2O_2 (0.05 mM) and various volumes of seminal plasma. After a 1-h incubation at room temperature (25ºC), horseradish peroxidase (20 U/ml) and phenol red (0.56 mM) were added to react with the remaining H_2O_2. The reaction mixtures were alkalinized (50 mM NaOH), and the absorbance read 10 min later at 630 nm. One unit of catalase-like activity was defined as the quantity of seminal plasma capable of decreasing the amount of H_2O_2 present in solution by 50%.

Statistical analysis

Values are expressed as mean ± 1 standard deviation (SD). Differences between the pre-vasectomy and post-vasectomy parameters were estimated by parametric and non-parametric tests as appropriate. Pearson's correlation coefficient was used to evaluate the association between individual parameters (SAS Institute, Cary, NC, USA). Values $p < 0.05$ were considered statistically significant.

Results

The mean sperm density and percent sperm motility ($\pm$1SD) of the pre-vasectomy semen samples were 34 ± 20 sperm/ml and $41\% \pm 20\%$, respectively. All post-vasectomy semen samples were azoospermic. The mean age and mean semen volume of the pre-vasectomy and post-vasectomy groups were not significantly different (Table 1). Mean seminal catalase-like activity ($\pm$1SD) in the fertile group was not significantly different from that of the post-vasectomy group (389 ± 163 and 325 ± 119 U/ml, respectively; Table 1). Similarly, mean seminal SOD-like activity ($\pm$1SD) in the fertile group was not significantly different from that of the post-vasectomy group (37 ± 10 and 36 ± 10 U/ml, respectively; Table 1).

There were no significant correlations between seminal catalase-like or SOD-like activities and semen volume or patient age. However, we did observe a significant correlation between the catalase-like and SOD-like activity in semen ($r = 0.43$, $p = 0.02$).

Table 1. SOD-like and catalase-like activities in human seminal plasma (values are expressed as means ± 1 SD)

	Pre-vasectomy	Post-vasectomy	*p*-value
Age (years)	43 ± 6	41 ± 6	0.43[a]
Semen volume (ml)	2.6 ± 1.4	2.8 ± 1.3	0.69[b]
SOD-like activity (U/ml)	37 ± 10	36 ± 10	0.80[a]
Catalase-like activity (U/ml)	389 ± 163	325 ± 119	0.22[b]

[a] *t*-test
[b] Mann-Whitney rank sum test

Discussion

Antioxidant enzymes are ubiquitous in aerobic biological systems. Therefore, not surprisingly, a number of studies have confirmed the localization of antioxidant enzyme mRNA and protein throughout the male reproductive tract [10, 13, 16, 17, 20, 21, 22]. SOD, catalase and glutathione peroxidase mRNA have been detected in most reproductive tract tissues (testis, epididymis, vas, prostate, seminal vesicles) and with the exception of catalase, most studies have reported the highest levels of these mRNAs in the testis and epididymis. However, as many of these enzymes are not secreted, their relative contribution to the antioxidant activity of reproductive tract fluids may be negligible. Furthermore, much of the antioxidant activity in semen is non-enzymatic and is likely derived from small molecules such as albumin, taurine and hypotaurine [11, 23]. Therefore, the reported antioxidant enzyme mRNA and protein localization throughout the reproductive tract may not reflect the antioxidant activity of the reproductive tract fluids.

The results of this study suggest that under normal conditions, semen SOD-like and catalase-like activities are primarily derived from post-epididymal (i.e. seminal vesicle, prostate) secretions with at most a negligible contribution from the testis and/or epididymis. These data are in apparent conflict with the observed antioxidant (in particular, SOD and glutathione peroxidase) mRNA and protein expression in the testis and epididymis [10, 13, 21, 22]. A likely explanation for our findings is that testicular and epididymal antioxidant enzymes are largely non-secretory forms and, thus, represent only a negligible component of the total SOD-like or catalase-like activity in seminal fluid. Alternatively or additionally, it is likely that much of the seminal antioxidant activity is non-enzymatic and, therefore, does not correlate with the reported mRNA and protein localization studies. It has been shown that much of the SOD-like and catalase-like activities in semen are derived from small, non-enzymatic molecules [11, 23]. Jeulin et al. [12] have previously suggested that catalase-like activity is primarily of prostatic origin, and this is in keeping with molecular studies on catalase expression in the male rat reproductive tract [20]. We suspect that under pathological conditions (e.g. epididymitis, prostatitis), the relative production of antioxidants in the different reproductive organs would potentially differ substantially from the normal, disease-free state.

The characteristic features of the testis and epididymis favour conditions of low oxidative stress. The scrotal position of the testis, the pampiniform venous plexus and the characteristics of the scrotal wall result in a lower testicular (and epididymal) temperature compared with the core body temperature [8, 15]. It is reported that the scrotal position of the epididymis helps maintains epididymal temperature as much as 6–8ºC below body temperature [6, 7]. In addition, the tail of the epididymis receives little blood supply, and consequently, pO_2 is reportedly low [9]. The low epididymal temperature and low pO_2 together effectively reduce oxidative stress to the spermatozoa and probably contribute to enhanced sperm survival [4]. In contrast, the female reproductive tract (vagina, uterus, fallopian tubes) is characterized by higher levels of oxidative stress. Alvarez and Storey [4] have shown that mammalian sperm show significant loss of motility and viability under oxidative incubating conditions that mimic oviductal fluid characteristics (high pO_2, 37ºC). Taken together with these observations, our finding that most of the antioxidant activity in semen is post-epididymal supports the notion that semen antioxidants are primarily designed to protect spermatozoa once they have been deposited in the female reproductive tract.

The experimental design employed in the present study does not allow us to conclusively demonstrate the origin of seminal SOD-like and catalase-like activities. For example, the data derived from the present study do not allow us to exclude the possibility that vasectomy may induce antioxidant production in the post-epididymal tissues (i.e. prostate and seminal vesicles). Although technically difficult, measuring antioxidant activity from fluids taken directly from tissues or ducts in the reproductive tract (e.g. vasal, epididymal and seminal vesicle fluid) would provide the most conclusive evidence with respect to the origin of seminal antioxidants. Using experimental models (animal models) could also be helpful in this respect.

In summary, our data suggest that the testis and epididymis are not an important source of catalase-like and SOD-like activities in ejaculated semen. These findings support the idea that semen antioxidants are primarily designed to protect spermatozoa once they have been deposited in the female reproductive tract.

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