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High Seminal Platelet-Activating Factor Acetylhydrolase Activity in Men With Spinal Cord Injury

JIUMING ZHU,*‡ NANCY L. BRACKETT,† TEODORO C. ABALLA,† CHARLES M. LYNNE,† MICHAEL A. WITT,* HILTON I. KORT,* AND WILLIAM E. ROUDEBUSH*

*From the *Reproductive Biology Associates, Atlanta, Georgia; and the †The Miami Project to Cure Paralysis and the Department of Urology, University of Miami School of Medicine, Miami, Florida.*

ABSTRACT: Spinal cord injury (SCI) causes male infertility, with low sperm motility the major long-term cause. It has been suggested in previous studies that some seminal components may be responsible for the pathological asthenozoospermia. It is hypothesized that platelet-activating factor (PAF) acetylhydrolase (PAFah), which originates in the epididymis and other accessory sexual glands, may be a causative factor. This enzyme catalyzes PAF to acetate and biologically inactive lyso-PAF. PAF is well recognized to be an important phospholipid mediator that stimulates sperm motility and enhances sperm capacitation and fertilization. The present study was designed to analyze differences in PAFah activity in semen of men with SCI and age-matched healthy men. PAFah assay reagent kits were used to measure enzymatic activity by monitoring the production rates of 4-nitrophenol on a spectrophotometer during a given interval. The results showed that subjects with SCI had a higher concentration of PAFah than men in the control group ($P < .001$). A statistically significant negative correlation was found between enzymatic activity and sperm motility ($r^2 = 0.8449$; $P < .001$). Further studies will determine whether seminal vesicle dysfunction in men with SCI leads to abnormal PAFah activity, resulting in low sperm motility.

Key words: Sperm, semen, SCI, asthenozoospermia, infertility.
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Spinal cord injury (SCI) causes irreversible infertility in men. The typical symptoms of SCI include erectile dysfunction, ejaculatory dysfunction, and poor semen quality. Dysfunction of erection and ejaculation can be clinically remedied by some drugs, devices, or combination of both, but no treatment is available for poor semen quality (Monga et al, 1999). Studies demonstrate that ejaculate specimens from patients with SCI have a normal sperm concentration but more-immotile sperm than healthy controls (Brackett et al, 1996b). The cause of poor semen quality is unknown, but factors from the seminal plasma have been implicated. For example, the motility of sperm in ejaculate specimens from healthy control subjects decreased significantly after the specimens were incubated with seminal plasma from men with SCI. Conversely, sperm motility for men with SCI increased when ejaculate specimens were incubated with seminal plasma from healthy control subjects (Brackett et al, 1996a). An examination of semen characteristics for 125 subjects with SCI who were injured between 6 weeks and 26 years of age showed that the sperm concentration remained normal during the years after injury, whereas the sperm motility remained low (Brackett et al, 1998). These findings indicate that seminal plasma is a major contributing factor to low sperm motility in men with SCI. This hypothesis was verified in a study showing significantly higher motility among sperm retrieved from the vas deferens, compared with sperm from the ejaculate specimens, for subjects with SCI (Brackett et al, 2000).

These findings are supported by results of a similar study showing a seminal plasma concentration–dependent decrease in sperm motility when sperm from healthy men were incubated with seminal plasma from men with asthenozoospermic SCI (Monga et al, 2001). Taken together, these data suggest that seminal plasma from men with SCI is detrimental to sperm motility.

Platelet-activating factor (PAF) is an important phospholipid mediator. Its involvement in reproduction was first suggested when it was detected in rabbit sperm (Kumar et al, 1988). As an autocrine mediator of sperm motility, PAF functions as a capacitation factor (Wu et al, 2001). It stimulates sperm motility (Ricker et al, 1989) and enhances sperm capacitation and the acro-

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Correspondence to: Dr William E. Roudebush, Clinical Laboratory, Reproductive Biology Associates, 1150 Lake Hearn Dr, Ste 400, Atlanta, GA 30342 (e-mail: roudebush@rba-online.com).

‡Present address: Jarrett Fertility Group, 11725 Illinois St, Ste 520, Carmel, IN 46032.

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some reaction (Sengoku et al, 1993; Fukuda et al, 1994; Huo et al, 2000; Odeh et al, 2003). As a catalytic enzyme of PAF degradation, PAF acetyohydrolase (PAFah) is thought to serve as a decapacitation factor (Muguruma and Johnston, 1997; Zhu et al, 2006). When present in seminal plasma (Letendre et al, 1992; Jarvi et al, 1993a; Hough and Parks, 1997), PAFah catalyzes hydrolysis of esterified PAF at the *sn*-2 position, producing acetate and biologically inactive lyso-PAF. By this action, it prevents sperm from hyperactivation, which is a prerequisite for sperm capacitation (Suarez and Ho, 2003).

A series of studies have shown that seminal plasma PAFah originates in the accessory sexual glands (Jarvi et al, 1993a; Parks and Hough, 1993). Only a very small amount of the enzyme is secreted from the epididymides (Parks and Hough, 1993; Muguruma and Johnston, 1997). The studies indicated that the seminal vesicle and the prostate are 2 major sources of seminal plasma PAFah in humans (Jarvi et al, 1993a). It is, therefore, hypothesized that men with SCI have impaired seminal vesicle function, which may affect synthesis and secretion of PAFah, leading to low sperm motility. Our study was designed to compare seminal PAFah activity between SCI and healthy control subjects.

Materials and Methods

Collection and Preparation of Seminal Plasma

Twenty men with SCI and 20 age-matched healthy controls participated in this study. Subjects were participants in the Male Fertility Research Program of the Miami Project to Cure Paralysis at the University of Miami School of Medicine (Miami, Fla). All subjects were in good general health with no active urinary tract infections. The mean time (±SEM) since SCI was 10.7 ± 1.3 years (range, 1–24 years). The level of injury was C5 to C6 (for 5 patients), T3 to T6 (for 11 patients), and T8 to T11 (for 4 patients). There was no significant difference between the mean age (±SEM) for subjects with SCI (35.8 ± 1.4 years; range, 24–44 years) and the mean age of control subjects (32.6 ± 1.7 years; range, 20–46 years). Control subjects were healthy men with no history of infertility. None of the study participants had taken any medication within 6 months before enrollment that was known to affect semen quality. In subjects with SCI, antegrade semen was collected by penile vibratory stimulation (Brackett, 1999) or by electroejaculation (Brackett, 2002). Control subjects produced a semen specimen by masturbation after 2–5 days of abstinence. Following liquefaction, semen analysis was performed by manual microscopic methods according to the criteria of the World Health Organization (World Health Organization, 1999). Seminal plasma was isolated by centrifugation and frozen at −80°C until shipment on dry ice from the University of Miami to Reproductive Biology Associates in Atlanta for determination of PAFah. Seminal plasma specimens were thawed and centrifuged for 10 minutes at 12 000 *g* to remove remaining cells and debris before enzymatic assay.

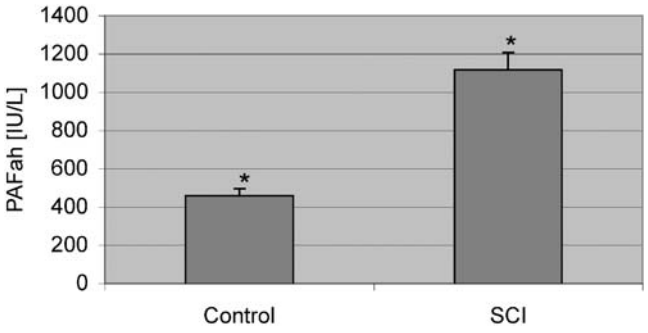

Figure 1. Platelet-activating factor acetylhydrolase (PAFah) activity in semen from men with spinal cord injury (SCI) and from controls (*$P < .001$).

Assay of Enzyme Activity

PAFah activity was analyzed on a Spectronic 21D Spectrophotometer (Milton Roy Co, Rochester, NY) by use of the Azwell Auto PAF-AH Assay Kit (Azwell Inc, Osaka, Japan). After buffers were warmed for 5 minutes, the catalyzing reaction was performed for 3 minutes at 37°C. The yield of 4-nitrophenyl, the end product of the substrate [1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine] digested by the enzyme, was measured by reading differences in absorbance at 505 nm between 1 and 3 minutes after addition of the substrate. The speed of enzymatic digestion, expressed as the net yield of end product within the given period, was converted to PAFah activity in international units per liter of sample (IU/L).

Statistical Analysis of Data

Each sample was measured in duplicate, and a mean value was calculated. Student's *t* test was used to analyze the difference in PAFah activity between the SCI group and the control group. Linear regression analysis was used to compare seminal PAFah concentration with sperm motility. Statistical significance was defined as a *P* value of less than .05.

Results

Group Difference in Enzyme Activity

Of the 20 patients with SCI, the mean concentration (±SEM) of PAFah was 1117.40 ± 99.83 IU/L (range, 601–1881 IU/L), whereas the 20 healthy men had a mean PAFah concentration (±SEM) of 458.60 ± 30.57 IU/L (range, 192–691 IU/L) ($P < .001$) (Figure 1). The mean sperm motility (±SEM) for the control group was 61% ± 2.0% (range, 45%–80%), whereas the value for the SCI group was 25% ± 3.6% (range, 1%–53%) ($P < .001$).

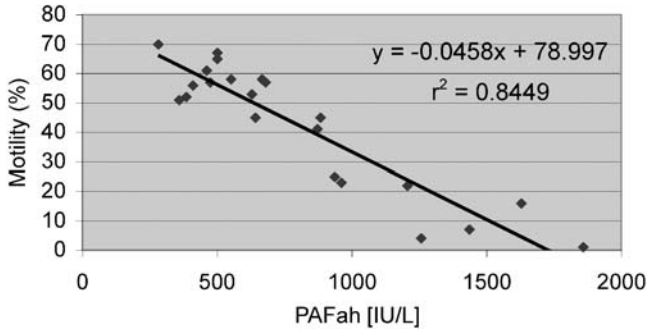

Figure 2. Linear regression analysis revealing a statistically significant negative correlation between human seminal platelet-activating factor acetylhydrolase (PAFah) concentration and sperm motility ($P < .001$).

Correlation Between Enzyme Activity and Sperm Motility

Further analysis by linear regression revealed a statistically significant negative correlation between seminal PAFah concentration and sperm motility ($r^2 = 0.8449$; $P < .001$) (Figure 2). It was indicated from the results presented above that patients with SCI had a significantly higher PAFah activity in their seminal plasma, compared with controls ($P < .001$), whereas patients with SCI had lower sperm motility. Linear regression analysis revealed that these 2 findings also had a statistically significant negative correlation.

Discussion

Our results showed that patients with SCI had markedly higher PAFah activity in their seminal plasma than their healthy counterparts and that the enzymatic activity in seminal plasma was negatively correlated with sperm motility. This result is consistent with our findings from a previous study involving 312 healthy men seeking infertility treatment (Zhu et al, 2006). It was found that semen specimens with a large percentage (at least 50%) of motile sperm had a significantly lower mean PAFah concentration (±SEM) (442.03 ± 14.37 IU/L) than semen specimens from patients who had a lower percentage (ie, less than 59%) of motile sperm (882.16 ± 18.45 IU/L) ($P < .001$) (Zhu et al, 2006). PAFah functionally catalyzes esterified PAF to hydrolyze at the *sn*-2 position, resulting in acetate and biologically inactive lyso-PAF. The increase in PAFah activity leads to a corresponding decrease in PAF activity. PAF is known to functionally stimulate sperm motility and progression (Hellstrom et al, 1991; Krausz et al, 1994; Roudebush et al, 2002; Henkel and Schill, 2003). A high level of seminal PAFah activity thus results in a corresponding decrease in sperm motility.

PAFah also inhibits sperm capacitation and fertilization. Many studies indicate that PAF is a capacitation factor. For example, PAF is biochemically categorized as a phospholipid, a class of molecules that have been positively correlated with sperm capacitation (Davis, 1981). Functionally, PAF meets the criteria for an autocrine mediator of capacitation. Its receptor was detected on the sperm membrane after sperm were washed in a capacitation medium (Roudebush et al, 2000; Wu et al, 2001). The release of PAF by most cells is dependent on extracellular albumin (Ludwig et al, 1985; Clay et al, 1990; Ammit and O'Neill, 1997), which is necessary for sperm capacitation (Huang et al, 2000) and PAF-improved motility (Jarvi et al, 1993b). This characteristic of albumin-dependent release is apparently coupled with the spontaneous and reversible induction of sperm capacitation, because albumin is not generally recognized as a signaling molecule. In vitro capacitation induced by PAF has been further demonstrated in spermatozoa of the mouse model (Sengoku et al, 1992; Shi et al, 1992; Huo et al, 2000; Wu et al, 2001), the bovine model (Aravindakshan and Sharma, 1995), the stallion model (Odeh et al, 2003), and humans (Wu et al, 2001; Henkel and Schill, 2003).

Seminal plasma PAFah originates mainly from the prostate gland and seminal vesicles, and in humans, only a small amount originates from the epididymides (Jarvi et al, 1993a, Parks and Hough, 1993; Muguruma and Johnston, 1997). The specific activity of PAFah in seminal plasma and prostatic fluid was twice the activity of PAFah in serum and 15-fold higher than the activity in fluid from the seminal vesicles and vas deferens. Given that PAFah from the seminal plasma had the same activity as PAFah from the prostate and higher activity than PAFah from any of the other reproductive tract fluids suggests that there are either activators of PAFah in seminal plasma or inhibitors in one or several of the other reproductive tract fluids (Jarvi et al, 1993a). The facts that the seminal vesicles contribute up to 70% of semen volume (Mortimer, 1994) and that the prostate contains 15-fold higher enzymatic activity than the seminal vesicle (Jarvi et al, 1993a) indicate that seminal plasma PAFah is mainly derived from these 2 accessory sexual glands. It is possible that the abnormal PAFah activity observed in semen of men with SCI is related to abnormal function of their seminal vesicles. Studies have indicated that seminal vesicle dysfunction may be, at least in part, responsible for low sperm motility in men with SCI (Brackett et al, 1996a; Ohl et al, 1999; Monga et al, 2001).

The PAF-PAFah roles in fertilization suggest that PAF is a capacitation factor (Wu et al, 2001), whereas PAFah is regarded as a decapacitation factor (Muguruma and Johnston, 1997; Zhu et al, 2006). Thus,

a hypothetical model is proposed for sperm capacitation. In either the epididymis or seminal vesicle, PAFah secretion keeps sperm from hyperactivation by inactivating PAF that is produced by mature sperm. After ejaculation, sperm still remain uncapacitated in the seminal plasma environment, which contains PAFah. During intercourse, however, the low vaginal pH causes a partial reduction of PAFah activity. More importantly, spermatozoa shed seminal plasma as they migrate through the female reproductive tract. In the presence of albumin, which is a major protein in the reproductive tract (Aitken et al, 1977), sperm release PAF, promoting sperm hyperactivation, capacitation, and, subsequently, the acrosome reaction and, eventually, fertilization. Previous clinical studies have demonstrated that, in neurologically intact men with asthenozoospermia, brief incubation of washed sperm in a solution of synthesized PAF improves sperm motility (Wu et al, 2001) and pregnancy rates (Wild and Roudebush, 2001, Roudebush et al, 2004; Grigoriou et al, 2005). Future studies will determine whether it is possible to improve the SCI-related deficiency in sperm motility by washing semen and then incubating sperm with extraneous PAF.

It is possible that other toxins in the seminal plasma, such as inflammatory cytokines, contribute to the pathologic deterioration of sperm motility in men with SCI. The concentration of cytokines was found to be elevated in seminal plasma from men with SCI (Basu et al, 2004), but their adverse effects on sperm motility could be relieved by inactivating the substance (Cohen et al, 2004). Abnormally high concentrations of activated T lymphocytes in the semen of men with SCI (Basu, 2002) may be a source of toxic semen cytokines in these men.

In conclusion, the semen of men with SCI contained an abnormally high concentration of PAFah, compared with healthy control subjects. The concentration of PAFah was negatively correlated with sperm motility. Future studies will determine whether asthenozoospermia in men with SCI may improve after semen washing followed by incubation with extraneous PAF.

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