Secretory leukocyte protease inhibitor (SLPI) concentrations in seminal plasma: SLPI restores sperm motility reduced by elastase

Akihiro Moriyama, Koichiro Shimoya, Akiko Kawamoto, Kazumasa Hashimoto, Isao Ogata, Ichiro Kunishige, Kazutomo Ohashi, Chihiro Azuma, Fumitaka Saji and Yuji Murata

Department of Obstetrics and Gynecology, Faculty of Medicine, Osaka University, 2–2 Yamada-oka, Suita city, Osaka 565–0871, Japan

1To whom correspondence should be addressed

In this study, we quantified secretory leukocyte protease inhibitor (SLPI) and elastase in ejaculates from normal donors and infertile patients with or without leukospermia and investigated the effect of SLPI on sperm motility reduced by elastase. Western blot analysis revealed that SLPI protein was detected in the seminal plasma. The SLPI titre in the seminal plasma with leukospermia was lower than that in the seminal plasma without leukospermia and in the seminal plasma of fertile donors. The elastase concentration in the seminal plasma with leukospermia was significantly higher than that in the seminal plasma without leukospermia. A significant correlation between SLPI and elastase concentrations in the seminal plasma \( r = 0.36, P < 0.01 \) was observed. There was a positive correlation between SLPI titre in the seminal plasma and sperm motility \( r = 0.51, P < 0.001 \). SLPI recovered the sperm motility reduced by elastase in a dose-dependent manner. Our results suggest that SLPI is a potential substance to treat infertile patients with leukospermia.

Key words: elastase/leukospermia/secretory leukocyte protease inhibitor/seminal plasma/sperm motility

Introduction

Human semen contains spermatozoa and leukocytes, as well as various proteins such as enzymes, hormones and those with unknown functions. In some semen samples associated with male genital tract infection and infertility, large numbers of leukocytes have been detected (Caldamone et al., 1980; Berger et al., 1982). It has been reported that an elevated concentration of leukocytes impairs the fertilizing ability of spermatozoa (Maruyama et al., 1985). We have reported that seminal plasma contains large amounts of cytokines, such as interleukin (IL)-6, IL-8, and monocyte chemotactic and activating factor (MCAF) (Shimoya et al., 1993, 1995). These cytokines are produced, possibly by monocytes, endothelial cells and fibroblasts, in the male genital tract in an infection-free state. However, cytokine concentrations in the seminal plasma are markedly elevated in genital infections, such as leukospermia (Shimoya et al., 1993, 1995). These cytokines may accumulate and activate leukocytes in the male genital tract. Activated leukocytes produce large amounts of elastase in the genital tract. The titration of polymorphonuclear (PMN) elastase in seminal plasma rapidly and accurately diagnoses silent male genital tract inflammation (Jochum et al., 1986; Wolff and Anderson, 1988; Micic et al., 1989). It was also demonstrated that PMN elastase is an inhibitor of sperm motility (Satoh et al., 1990; Wolff et al., 1990). We have also demonstrated that nitric oxide concentrations in the seminal plasma of infertile males are higher than those in the seminal plasma of fertile males and that nitric oxide is an inhibitor of sperm motility (Nobunaga et al., 1996).

Secretory leukocyte protease inhibitor (SLPI) is a protein found in various fluids, including parotid secretions (Thompson and Ohlsson, 1986), bronchial and nasal mucus (Frykemark et al., 1989), cervical mucus (Helming et al., 1995), and seminal plasma (Ohlsson et al., 1995). SLPI is a protein with a molecular mass of 11.7 kDa that was originally isolated from human parotid gland secretions (Thompson and Ohlsson, 1986). SLPI has been found to be a potent inhibitor of human leukocyte elastase, human cathepsin G, and human trypsin (Thompson and Ohlsson, 1986). SLPI has also been shown to inhibit mast cell chymase (Fink et al., 1986), a protease released during mast cell degranulation, and to inhibit histamine release from mast cells \( \text{in vitro} \) (Dietze et al., 1990). Several studies have now established that the gene for SLPI is expressed in a tissue-specific manner by cells at a variety of mucosal surfaces such as those of the lung, cervix, parotid duct, and seminal vesicles (Abe et al., 1991). The concentrations of SLPI in biological samples have been monitored to correlate these concentrations with pathological conditions (Kidä et al., 1992; Kouchi et al., 1993; Sluis et al., 1994). Increased concentrations of SLPI in nasal secretions and in bronchoalveolar lavage fluids may be indicative of inflammatory lung conditions or allergic reactions (Frykemark et al., 1989; Vogelmeier et al., 1991; Lee et al., 1993). Ohlsson et al. (1995) showed that seminal plasma contains SLPI and a strong immunostaining for SLPI was demonstrated in the epithelial cells within the granular lumina of the prostate gland, seminal vesicles and epididymis. Ohlsson et al. (1995) also suggested that SLPI has a local protective function against proteolytic degradation of the male reproductive tract tissues.

In this study, we quantified SLPI and elastase in ejaculates
from normal donors and infertile patients with or without leukospermia and examined the effects of SLPI and elastase on sperm motility.

Materials and methods

Reagents
Goat anti-SLPI polyclonal antibodies and recombinant (r-) SLPI were purchased from R&D systems (Minneapolis, MN, USA). Recombinant elastase was purchased from Sigma Chemical Co. (St Louis, MO, USA).

Semen collection
Semen was obtained by masturbation after 5 days of abstinence. Samples were collected in a sterile container and examined within 1 h after ejaculation. Semen samples were obtained from 44 infertile and eight proven fertile men. The fertile men had fathered at least one child and had no recent history of venereal infection. Informed consent to use seminal plasma was obtained from all patients in this study.

Semen analysis
Semen analysis, including the determination of motility, morphological features, the number of sperm cells, and viscosity of the ejaculate, was performed by the morphological techniques and the method of peroxidase staining as previously described. Leukospermia was diagnosed as >10⁶ white blood cells/ml semen.

Preparation of seminal plasma
Thirty minutes after collection, liquefied semen samples were first centrifuged at 1000 g for 10 min, and the supernatants were recentrifuged at 10 000 g for 15 min to remove cellular elements and debris. After centrifugation, the clear seminal plasma was collected and stored at −80°C until titration of SLPI and elastase.

Western blot analysis
To determine SLPI protein in the seminal plasma, we performed Western blotting analysis using an anti-human SLPI polyclonal antibody. Seminal plasma (5 µl) was electrophoresed on a 15% sodium dodecyl sulphate–polyacrylamide gel and transferred onto nitrocellulose membranes (0.45 µm; Schleicher and Schuell, Dassel, Germany). The membrane was incubated with 5% dried milk protein followed by anti-human SLPI polyclonal antibody. The primary antibody was used at a final concentration of 1.0 µg/ml. The SLPI immunoreactivity was visualized using an ECL Western blotting analysis system (Amersham, Aylesbury, UK).

Determination of SLPI in the seminal plasma by enzyme-linked immunosassay (ELISA)
To determine concentrations of SLPI in the seminal plasma, ELISA kits specific for SLPI (R&D Systems) were used. No excessive effects of the diluted seminal plasma on the measurement of SLPI using this ELISA kit were observed. Seminal plasma concentrations of SLPI detected covered amounts 62.5 pg/ml. No cross-reactivity with growth factors, such as epidermal growth factor (EGF), and proteases, such as leukocyte elastase, trypsin, and chymotrypsin could be found in this kit. Intra-assay variability of the SLPI kit was 4.2–8.0% and its interassay variability was 4.9–9.5%.

Determination of elastase titre in the seminal plasma by ELISA
To measure titres of elastase in seminal plasma, enzyme-linked immunoassay kits specific for elastase (E.Merck, Darmstadt, Germany) were used. Seminal plasma titres of elastase which the kit detected covered the ranges of >1.0 µg/l. Intra-assay variability of the elastase kit was 2.7–5.2% and its interassay variability was 4.9–9.5%.

Sperm preparation
Semen samples, classified as normal according to World Health Organization criteria, were obtained from five healthy fertile volunteers after 3 days of abstinence. The semen was liquefied for 30 min at room temperature, and motile spermatozoa, collected by the swim-up method, were suspended in modified Biggers–Whitten–Whittingham (mBWW) medium containing 0.5% human serum albumin (HSA; Fuso Pharmaceutical Industries, Osaka, Japan). The prepared spermatozoa were adjusted to 1×10⁶/ml and immediately pre-incubated with 100 or 500 µM of r-elastase, or control medium with or without r-SLPI. After 0.5, 1, 2, 4 and 6 h of incubation in an atmosphere of 5% CO₂ and 95% air at 37°C, sperm motility was determined by a computer-assisted semen analyser (Hamilton–Thorn Research, MA, USA).

Statistical analysis
Statistical analysis of SLPI and elastase concentrations in seminal plasma was conducted using Welch’s t-test, and P < 0.05 was considered significant. The correlation was analysed by simple linear regression.

Results
To quantify SLPI protein in the seminal plasma, we performed a Western blot analysis. As shown in Figure 1, SLPI protein was detected as a 12 kDa band in the seminal plasma. These results show the presence of SLPI in the seminal plasma. Table I shows the SLPI titres and elastase titres in the seminal plasma of infertile men with (n = 19) and without leukospermia (n = 27). The mean SLPI titre in the seminal plasma with leukospermia (3990 ± 400 ng/ml) was significantly lower than both that in the seminal plasma without leukospermia (4310 ± 500 ng/ml, P < 0.05) and in the seminal plasma of the fertile donors (4650 ± 640 ng/ml, n = 8, P < 0.01). There was no significant difference between the SLPI titre of infertile patients without leukospermia and that of fertile donors. The elastase concentration in the seminal plasma with leukospermia (296 ± 153 ng/ml) was significantly higher than that in the seminal plasma without leukospermia (74 ± 43 ng/ml, P < 0.0001). Figure 2 shows a statistically significant negative correlation between SLPI and elastase concentrations in the seminal plasma.
Table I. Secretory leukocyte protease inhibitor (SLPI) concentrations, elastase concentrations, and SLPI/elastase ratio in the seminal plasma of infertile patients with or without leukospermia and of the controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>SLPI (ng/ml)</th>
<th>Elastase (ng/ml)</th>
<th>SLPI/elastase ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukospermia (+)</td>
<td>3990 ± 400(^b)</td>
<td>296 ± 153(^c)</td>
<td>22 ± 5(^c)</td>
</tr>
<tr>
<td>(n = 19)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukospermia (-)</td>
<td>4310 ± 500</td>
<td>74 ± 43(^d)</td>
<td>85 ± 11</td>
</tr>
<tr>
<td>(n = 27)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>4650 ± 640</td>
<td>37 ± 6</td>
<td>128 ± 9</td>
</tr>
</tbody>
</table>

\(^a\) The SLPI/elastase ratio was calculated for each individual, and from these values the mean and SD of the SLPI/elastase ratio in each group was derived.

\(^b\) Significantly different from leukospermia (-): \(P \leq 0.05\); and significantly different from control: \(P < 0.01\).

\(^c\) Significantly different from leukospermia (-) and control: \(P < 0.001\).

\(^d\) Significantly different from control: \(P < 0.05\).

Discussion
The present study demonstrated that a certain amount of SLPI is contained in human seminal plasma as reported previously (Ohlsson et al., 1995) and that the SLPI titre in the seminal plasma of patients with leukospermia was significantly lower...
than that in patients without leukospermia and in fertile donors. SLPI was revealed in the epithelial cells of the prostate gland and epididymis by an immunohistochemical analysis (Ohlsson et al., 1995). Several inflammatory mediators enhance SLPI mRNA concentrations and the production of SLPI protein in the HS-24 human bronchial epithelial cell line (Maruyama et al., 1994). Neutrophil elastase increased SLPI transcript concentrations in primary human airway epithelial cells whereas tumour necrosis factor-α and IL-8 produced little or no effect on SLPI transcript concentrations (Abbinante et al., 1993). Up-regulation of SLPI plays a defensive role in the epithelial surface of inflammatory lung diseases (Abbinante et al., 1993). However, in contrast to the respiratory system, we did not detect elevated SLPI concentrations in the seminal plasma of infertile patients with leukospermia. In this study we found a negative correlation between elastase and SLPI (r = −0.36). These results suggest that male genital tract infections, such as leukospermia, affect the production of SLPI in the epithelial cells of the prostate gland and epididymis or that SLPI in the seminal plasma is consumed when infection occurs in the male genital tract. In this study, SLPI in the seminal plasma was shown to be a possible marker to diagnose leukospermia. In particular, the elastase/SLPI ratio is a useful marker to diagnose leukospermia.

Large numbers of leukocytes in the semen are associated with male genital tract infection and infertility (Caldamone et al., 1980; Berger et al., 1982). Recently Thomas et al. (1997) reported that PMN granulocytes in the seminal plasma are associated with increased numbers of spermatozoa. Elevated elastase concentrations in the seminal plasma reduce semen quality (Micic et al., 1989). In this study, we demonstrated that r-elastase inhibited sperm motility in vitro and that the inhibition was restored with the treatment of r-SLPI. The binding of SLPI to elastase is accelerated by polyanions (Ying et al., 1994). The mechanism by which r-SLPI improves sperm motility damaged by elastase is unknown. Therefore further studies are necessary to investigate how SLPI affects sperm motility.

Various kinds of drugs are clinically used to improve sperm quality. A clinical trial of clomiphene citrate in oligozoospermic men revealed that clomiphene citrate improved sperm concentration and sperm motility (Micic and Dotlic, 1985). It has been reported that other drugs such as low dose androgen and zinc sulphate, kallikrein, and pentoxifylline, improved the sperm motility (Schill, 1979; Takahara et al., 1983; Marrama et al., 1985). Recently gluthione therapy was performed to improve sperm quality (Lenzi et al., 1993); however, such clinical treatments were not effective. SLPI is a potential therapeutic tool in inflammatory lung diseases such as cystic fibrosis and pulmonary emphysema (McElvaney et al., 1993; Stolk et al., 1994). A short-term study in patients with cystic fibrosis was performed with aerosalized r-SLPI (McElvaney et al., 1993). Further investigations would be necessary to examine whether SLPI is a potential drug to treat infertile men, especially those with leukospermia.

Acknowledgement

This study was supported in part by Grants-in-Aid for Scientific Research (Nos. 8671888, 861389, 9470359, 9671679 and 9716777) from the Ministry and Education, Science and Culture of Japan.

References


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*Received on March 20, 1998; accepted on July 9, 1998*