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PHYSIOLOGY

Matrix metalloproteinase (MMP)-2 and MMP-9 in seminal plasma

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Abstract
Purpose To evaluate the latent and active forms of MMP-2 and MMP-9 in human semen samples and to investigate their association with semen parameters.
Methods Basic semen analysis was performed in 82 semen samples. Seminal plasma was analyzed with gelatin zymography.
Results Both latent and active forms of MMP-2 and MMP-9 were detected in human seminal plasma. The latent forms were the predominant ones. MMP-2 and MMP-9, either in latent or active forms, were not correlated with semen parameters. ProMMP-9 levels were higher in semen samples with abnormally low concentration ($\leq 19\times10^6$/ml) compared with semen samples with concentration $\geq 50\times10^6$/ml.
Conclusion MMP-2 and MMP-9 are both present in human semen. The latent forms of both MMPs are the predominant ones. ProMMP-9 is elevated in samples of low sperm concentration.

Both latent and active forms of MMP-2 and MMP-9 are present in human seminal plasma; ProMMP-9 is associated with low concentration and poor sperm morphology.

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Introduction

Matrix metalloproteinases (MMPs) belong to a large family of enzymes (the metzincins) that are zinc-dependent proteinases. They are known as the main enzymes digesting the extracellular matrix (ECM) components [1]. This is the main function for MMPs, though recently it has been reported that they are also involved in the release and activation of growth factors and cytokines [2]. MMPs are classified into four groups: gelatinases, mainly targeting type IV collagen fibers; stromelysins, targeting non-collagen molecules; collagenases, targeting fibrillar forms of collagen; MT-MMPs, a group of transmembrane enzymes not only cleaving ECM components, but also activating other MMPs [1]. MMPs are secreted as latent forms which are activated through cleavage of the inhibitory pro-peptide. The active MMPs have a reltive molecular mass of about 10 kDa less than the latent forms.

There is a complex mechanism controlling MMP activation. This mechanism includes regulation at the level of gene expression, cleavage of the latent forms as well as inhibition of active MMPs by endogenous inhibitors, primarily tissue inhibitors of metalloproteinases (TIMPs) [1].

Seminal plasma contains many proteinases originating either from testicular cells or from prostate and other accessory sex glands [3, 4]. There are a limited number of studies focused on the presence of MMPs in semen [5–8]. According to previous reports, there are two types of MMPs in human seminal plasma: MMP-2 and MMP-9 [7, 8].

In this study, we evaluated the levels of two gelatinases: MMP-2 and MMP-9 in seminal plasma and investigated the

association between the patterns of these enzymes and six semen parameters: sperm concentration, total sperm count, sperm motility, total count of motile sperm, percentage of morphologically normal sperm and total count of normal sperm.

Materials and methods

Semen samples

Semen samples were obtained by masturbation after 3–4 days of abstinence from 82 men (mean age: 32; range: 27–40) attending the "Embryokosmogenesis" infertility clinic (Alexandroupolis, Greece) for in vitro fertilization cycles. Only one semen sample from each patient was included in the study. There were no exclusion criteria such as smoking or alcohol consumption. The patients gave verbal consent and did not receive any monetary compensation for participating in the study.

All semen samples were evaluated according to basic semen analysis and at the same time samples of seminal plasma were prepared and stored in −20°C for further analysis. Basic semen analysis revealed that 22 semen samples were normal (concentration $\geq 20\times10^6$/ml, motility ≥50%, morphology ≥14%) whilst 62 were abnormal (mainly due to decreased motility and poor morphology).

Gelatin zymography

Gelatin zymography was performed in seminal plasma as described by Brown et al. [9]. This technique can distinguish between the 72 and 92 kDa type IV collagenases. Additionally, the method can detect the inactive proforms of collagenases because SDS causes activation of the enzymes without proteolytic cleavage of the inhibitory N-terminal sequence [10].

Aliquots of samples (15 μl) homogenized in loading buffer, were applied directly without heating or reduction to a 5% stacking polyacrylamide gel overlayed on top of a 8% resolving gel, containing 1 mg/ml gelatin and 0.1% (*w*/*v*) SDS. Gels were run at room temperature at 150 V till completion. After incubation of gels in 2.5% Triton X-100 for 30 min to remove SDS, the gels were incubated for 16 h at 37°C in 50 mM Tris–HCl, pH 7.6, containing 0.2 M NaCl, 5 mM CaCl2 and 0.02% Brij-35. Gels were stained for 3 h in 15% methanol/7.5% glacial acetic acid containing 0.5% Coomassie Brilliant Blue G 250 and distained in the same solution in the absence of dye. Bands denoting the MMP enzymes came out white against a blue background.

The distained gels were finally scanned, and relative intensities of the MMP bands were quantified using the Gelpro3 image analysis software (Media Cybernetics).

Satistical analysis

The levels of latent and active forms of MMP-2 and MMP-9 were correlated to sperm concentration, total sperm count, sperm motility, total count of motile sperm, percentage of morphologically normal sperm and total count of normal sperm.

Furthermore, in order to investigate the possible association of MMPs with sperm count, semen samples were divided into four groups with increasing concentration within the following ranges: group A ($\leq 19\times10^6$/ml; $n=8$), group B ($20–49\times10^6$/ml; $n=26$), group C ($50–100\times10^6$/ml; $n=26$), group D ($>100\times10^6$/ml; $n=22$).

The statistical analysis included descriptive statistics for the above parameters, comparisons between groups of semen samples by means of Mann–Whitney U-test, Kolmogorov–

Table 1 Spearman rank order correlations between MMPs and semen parameters

Parameters	Total sperm count	Motility (%)	Total count of motile sperm	Morphology (%)	Total count of normal sperm	proMMP-2	MMP-2	proMMP-9	MMP-9
Sperm concentration	0.84*	0.35*	0.83*	0.44*	0.68*	−0.03	−0.004	−0.16	−0.04
Total sperm count		0.2	0.91*	0.29*	0.67*	−0.06	0.07	−0.18	−0.12
Motility (%)	0.2		0.53*	0.47*	0.37*	−0.02	0.05	−0.06	−0.07
Total count of motile sperm	0.91*	0.53*		0.41*	0.73*	−0.07	0.08	−0.17	−0.14
Morphology (%)	0.29*	0.47*	0.41*		0.85*	0.07	0.09	−0.16	−0.03
Total count of normal sperm	0.67*	0.37*	0.73*	0.85*		0.03	0.1	−0.18	−0.06

*Statistically significant at $p<0.05$.

Table 2 Descriptive statistics of basic semen parameters and MMPs in four groups of semen samples with increasing sperm concentration

Parameters	Group A (n=8) ≤19×10^6/ml	Group B (n=26) 20–49×10^6/ml	Group C (n=26) 50–100×10^6/ml	Group D (n=22)>100×10^6/ml
Semen volume (ml)	4.88±0.67	4.62±0.53	4.4±0.35	3.44±0.37
Total sperm count	49.34±14.15*	141.48±18.73*	359.04±39.12*	566.43±52.55*
Motility (%)	18.13±6.12*	44.42±3.42*	48.85±3.7*	52.27±3.08*
Total count of motile sperm	11.1±6.41*	58.94±8.66*	170.79±22.24*	295.38±35.72*
Sperm morphology (%)	2.13±1.86*	9.52±2.3*	11.56±2.41*	13.5±1.81*
Total count of normal sperm	2.1±1.95*	14.09±4,43*	39.34±9.27*	77.3±14.02*
proMMP-9	28.95±2.32*	20.9±2.82	18.3±2.32*	15.42±2.43*
MMP-9	9.07±1.94	6.07±1.04	5.44±0.78	5.99±1
proMMP-2	10.27±2.84	17.71±2.27	17.32±2.99	16.39±2.42
MMP-2	6.41±2.12	10.39±1.73	10.61±1.56	9.95±2.17

Values are presented as mean±standard error.
*Values indicated are significantly different (p<0.05).

Smirnov test, Median test and Kruskal–Wallis test. Correlations between the studied parameters were evaluated with the Spearman Rank test. The two-tailed significant level was set at p<0.05. The software we used for statistical analysis was STATISTICA 6.0 (StatSoft Inc., Tulsa, OK, USA). Values are expressed as mean±standard error.

Results

Both latent and active forms of MMP-2 and MMP-9 were detected in all samples, with the latent being the predominant form for both. In general, the levels of active MMP-2 were higher than those of the active MMP-9.

There was no significant correlation between the levels of MMPs and the studied semen parameters. There were strong correlations between the latent and the active forms of both MMPs (proMMP-2 to MMP-2: R=0.83, p<0.05; proMMP-9 to MMP-9: R=0.41, p<0.05) (Table 1).

In a second step, semen samples were classified into four groups according to sperm counts (Table 2). The four groups differed significantly regarding total sperm count (Kruskal–Wallis test; H=51.24, p=0.00), morphology (Krukal–Wallis test; H=1.63, p=0.00), total count of normal sperm (Kruskal–Wallis test; H=32.84, p=0.00), motility (Kruskal–Wallis test; H=15.44, p=0.00) and total count of motile sperm (Kruskal–Wallis test; H=53.57, p=0.00). Group A had significantly higher levels of proMMP-9 than group C and group D. Even when the levels of both forms of MMP-9 were taken together, group A (38.03±2.84) had significantly higher levels than groups C (23.73±2.82; p=0.035) and D (21.42±2.81; p=0.004). The levels of proMMP-2 and active MMP-2 were lower in group A than in the other three groups but without reaching statistical significance.

Discussion

During the last 10 years, many investigators have addressed the role of MMPs in reproductive functions [11–17]. Most of the studies have been focused on the potential involvement especially of MMP-2 and MMP-9 in the female reproductive organs, namely the ovary, where there is a periodic and extensive remodeling activity throughout adulthood. Follicular development, ovulation, implantation and corpus luteum degradation require lysis and remodeling of ECM, processes where MMPs actively participate.

On the other hand, very little is known about the expression and the role of MMPs in the male reproductive organs where there is a continuous and massive production of semen. MMP-2 and MMP-9 have been detected in human seminal fluids [7, 8], although their origin and consequently their function are still uncertain.

MMP-2 has been detected in rat Sertoli cell cultures, therefore Sertolli cells are potential sites of MMP-2 secretion [6]. Split ejaculate analysis has shown that accessory sex glands such as seminal vesicles and prostate are also sites of MMPs secretion [5]. Moreover, MMP-2 activity has been demonstrated in prostatic secretions of benign hyperplasic tissue [4, 5]. Baumgart et al [7] also detected latent and active forms of MMP-2, but not MMP-9, in sperm lysates.

It has been postulated that MMPs participate in seminal liquefaction after ejaculation, together with other proteinases as prostatic specific antigen [8], although this may be only a part of their entire role in semen functions.

The findings of the present study have confirmed previous reports that MMP-2 and MMP-9 are present in seminal plasma [7, 8]. Both latent and active forms of MMP-2 and MMP-9 were detected, with the latent forms being the predominant ones.

One of the main questions put forward by clinicians is whether the levels of MMPs are correlated with basic semen parameters. In the present study, we found significantly higher levels of proMMP-9, but not of active MMP-9, in semen samples with sperm concentration $\leq 19 \times 10^6$ compared with those having sperm concentration $\geq 50 \times 10^6$. This possibly implies impairment at the level of the activation of this enzyme. On the other hand, the total levels of MMP-9 were higher in group A compared with groups C and D, a fact indicating a higher production of this enzyme in patients with low sperm concentration.

Conclusions

This study confirmed the presence of latent and active forms of MMP-2 and MMP-9 in seminal plasma. The latent forms are the predominant ones. ProMMP-9 seems to be elevated in semen samples of low sperm concentration.

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