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Levels of Hepatocyte Growth Factor/Scatter Factor (HGF/SF) in Seminal Plasma of Patients With Andrological Diseases

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ABSTRACT: Hepatocyte growth factor/scatter factor (HGF/SF) has all the characteristics of a molecule suitable for functioning in regulatory networks of motility, such as the spermatogenic epithelium, where spermatogenic cells must migrate between the cells of Sertoli, and it exerts its effect through binding of its high-affinity receptor (c-*met*). Considering the findings that c-*met* receptor is expressed in the human testis and on spermatozoa, and that HGF/SF in seminal plasma consists of pro-HGF/SF, mature αβ-HGF/SF, and less active forms of HGF/SF, we investigated the concentration and biological activity of HGF/SF in seminal plasma and their correlation with parameters of spermatogenesis to obtain better insight into mechanisms that may be involved in the pathogenesis of male infertility. We also evaluated the potential value of assessment of hepatocyte growth factor concentration and its bioactivity for the diagnosis of certain pathological conditions of male reproduction. We studied the concentration and biological activity of HGF/SF in seminal plasma of normal men and of patients with a range of andrological diseases or conditions by measuring HGF/SF in seminal plasma by enzyme-linked immunosorbent assay and by scatter assay using Madin-Darby canine kidney epithelial cells. We identified three sources of HGF/SF in seminal plasma. In samples from vasectomized men (n = 30; 2.01 ng/ml) and in split ejaculate samples (n = 6; 1° fraction 2.75 ng/ml, 2° fraction 1.62 ng/ml), a prostatic origin can be certified. This HGF/SF has low biological activity (133.3 U/ml). In inflammation of the accessory sex glands (n = 40), a high amount of HGF/SF (3.04 ng/ml) can be generated by white blood cells and has moderate scatter activity (426.7 U/ml). In normozoospermic samples, there is a lower amount of HGF/SF (1.12 ng/ml), with strong scatter activity (1280.0 U/ml). Finally, the clear difference between the low amount of HGF/SF (1.06 ng/ml) with poor scatter activity (106.6 U/ml) in oligozoospermic samples (n = 28) and the high amount of HGF/SF (3.35 ng/ml) with strong scatter activity (853.3 U/ml) in samples from men with azoospermia of primary testicular failure (n = 18) suggests a mainly testicular origin, with different activity in different pathological conditions.

Key words: Testis, spermatozoa, male infertility.
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Growth factors and cytokines play an important role in intercellular communication, and they exert broad pleiomorphic activities. They are distinct from the classical endocrine hormones in that their synthesis is ubiquitous and their action is local to the site of production. These factors are involved in the negative or positive control of cell growth and in induction or modulation of cell differentiation. Hepatocyte growth factor (HGF), also known as scatter factor (SF), induces a pleiotropic response in epithelial cells, including mitogenesis (Rubin et al, 1991; Bussolino et al, 1992; Halaban et al, 1992), stimulation of cell motility and chemotaxis of dissociated cells (Morimoto et al, 1991; Bussolino et al, 1992; Giordano et al, 1993), dissociation of epithelial sheets or cell scattering (Stoker et al, 1987; Gherardi et al, 1989; Weidner et al, 1990, 1991; Naldini et al, 1991b), and promotion of extracellular matrix invasion (Weidner et al, 1990, 1991; Naldini et al, 1991b). HGF/SF is the ligand for the tyrosine kinase receptor encoded by the *MET* proto-oncogene (c-*met* receptor; Bottaro et al, 1991; Naldini et al, 1991a,b). *In vivo,* HGF/SF is thought to act in a paracrine fashion: it is produced by mesenchymal/stromal cells (Stoker et al, 1987), and its receptor is found on epithelial cells nearby (Sonnenberg et al, 1993). HGF/SF is secreted as a single-chain, 92-kDa precursor devoid of biological activity (pro-HGF/SF; Mizuno et al, 1992; Naka et al, 1992; Naldini et al, 1995). Pro-HGF/SF binds the cell surface or the extracellular matrix, presumably via its affinity for heparin-like glycosaminoglycans (Naldini et al, 1991b). Pro-HGF/SF also binds the c-*met* receptor, but with low affinity and without triggering its kinase activity

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(Hartmann et al, 1992; Lokker et al, 1992; Naldini et al, 1995). Limited proteolysis of pro-HGF/SF at the Arg494-Val495 bond yields a disulfide-linked heterodimer of a 60-kDa (α) and a 32–36-kDa (β) chain (αβ-HGF/SF; Nakamura et al, 1987; Godha et al, 1988; Gherardi et al, 1989; Zarnegar and Michalopoulos, 1989; Weidner et al, 1990). Heterodimeric αβ-HGF/SF binds the c-*met* receptor with high affinity and triggers its kinase activity, resulting in biological response of target cells (Bottaro et al, 1991; Naldini et al, 1991a,b).

Maturation of pro-HGF/SF into the bioactive αβ-HGF/SF takes place in the extracellular environment, where urokinase-type plasminogen activator (uPa) activates pro-HGF/SF by a stoichiometric reaction. This occurs both *in vitro* with the pure reagents and on the membrane of target cells through to the formation of a stable complex between pro-HGF/SF and uPa, after the binding of pro-HGF/SF and uPa to their respective receptors (Naldini et al, 1995).

Because of its distinctive ability to act as a mitogen, a motogen, and a morphogen, HGF/SF has all the characteristics of a molecule suitable for functioning in regulatory networks of motility, such as the spermatogenic epithelium, where spermatogenic cells must migrate between the cells of Sertoli, and these effects occur through binding of its high-affinity receptor (c-*met*).

We previously demonstrated expression of c-*met* receptor in the human testis and on spermatozoa (Depuydt et al, 1996b) and showed that immunoreactive HGF/SF, detectable in human seminal plasma by an enzyme-linked immunosorbent assay (ELISA), consists of pro-HGF/SF, heterodimeric mature αβ-HGF/SF, and less biologically active forms of HGF/SF (Depuydt et al, 1997).

Previous studies indicate that HGF/SF is differentially expressed in various parts of the human male genital tract, with slight expression in the seminiferous epithelium (Wolf et al, 1991) and high expression in the epithelium of the epididymis, ductus deferens, prostate and seminal vesicles (Wolf et al, 1991; Humphrey 1995). In the murine male genital tract, no expression of HGF/SF was seen in the testes, caput epididymis, and vas deferens, and the highest expression was observed in the cauda and corpus (distal) epididymidis, followed by expression in the corpus (proximal) epididymidis (Naz et al, 1994).

The purpose of the present study was to investigate the concentration and biological activity of HGF/SF in seminal plasma and their correlation with parameters of spermatogenesis, to obtain better insight into some of the mechanisms that may be involved in the pathogenesis of male infertility. We also wished to evaluate the potential value of assessment of growth factor concentration and its bioactivity for the diagnosis of certain pathological conditions of male reproduction. Therefore, we studied the concentration and biological activity of HGF/SF in seminal plasma of normal men and of patients with a range of andrological diseases or conditions.

Materials and Methods

Semen Samples

Semen samples from 159 men attending the andrology outpatient clinic of the University Hospital of Ghent (Belgium) and split ejaculate samples from six healthy sperm donors were analyzed. Semen samples, produced by masturbation, were allowed to liquefy for 30 minutes. Prior to isolation of spermatozoa, semen samples were analyzed by standard procedures according to the recommendations of the World Health Organization (WHO, 1987). The number of days of sexual abstinence prior to production of the sample, the ejaculate volume and viscosity, and the percentage of spermatozoa with normal morphology were recorded. In addition, sperm concentration and motility characteristics (velocity, linear velocity, and linearity index) were evaluated objectively using a computer-assisted system (AutoSperm, FertiPro, Lotenhulle, Belgium) (Hinting et al, 1988). Spermatozoa with linear velocity >22 μm/second were graded A (grade A mot); spermatozoa with linear velocity <22 μm/second and velocity >5 μm/second were graded B (grade B mot); spermatozoa with nonprogressive motility (velocity <5 μm/second) were graded C (grade C mot); and immotile spermatozoa were graded D (grade D mot) according to the recommendations of the WHO (1987). Usually, four to six fields have to be scanned to classify 100 successive spermatozoa, yielding a percentage for each motility category. The values are expressed as percentages adding up to 100. The modified mixed antiglobulin reaction for immunoglobulin G and A (SpermMar, Fertipro) was performed on fresh semen (Jager et al, 1978; Andreou et al, 1995) for the detection of antisperm antibodies. A value of >10% motile spermatozoa with latex particles attached was considered positive. The classification of round cells as white blood cells (WBCs) was based on their capacity to react in a peroxidase stain (Endtz, 1972; LeucoScreen, FertiPro).

The 159 semen samples were grouped into normozoospermia (n = 19), oligozoospermia (n = 28), asthenozoospermia (fewer than 50% spermatozoa with forward progression, categories A and B, or fewer than 25% spermatozoa with category A movement; n = 12), immunological infertility (n = 12), azoospermia due to primary idiopathic testicular failure (n = 18), and postvasectomy samples (VASEC; n = 30) according to the recommendations of WHO (1993). VASEC samples are used as an example of obstructive azoospermia, to estimate the contribution of the prostate and the seminal vesicles in secretion of HGF/SF. Classification was independent of the causal factor of the semen defect, except for the samples from patients with azoospermia.

For the diagnosis of male accessory gland infection (MAGI; n = 40), the recommendations of the WHO were implemented (1993). Briefly, MAGI was diagnosed if the patient had oligozoospermia, asthenozoospermia, or teratozoospermia (but not azoospermia) and fulfilled the following criteria: 1) history and physical signs, such as history of urinary infection, epididymitis, sexually transmitted disease, thickened or tender epididymis, thickened vas deferens, and/or abnormal rectal examination; 2)

prostatic signs, such as abnormal prostatic expression fluid and/or abnormal urine after prostatic massage; and 3) ejaculate signs, such as more than 1 million peroxidase-positive WBCs per milliliter, culture with significant growth of pathogenic bacteria, and/or abnormal appearance, viscosity, pH, and/or biochemistry of the seminal plasma. Any of the following combinations should be present for diagnosis: 1) a history or physical sign with a prostatic sign, 2) a history or physical sign with an ejaculate sign, 3) a prostatic sign with an ejaculate sign, or 4) at least two ejaculate signs in each ejaculate.

The semen characteristics exhibited by the samples are given in Table 1. Seminal plasma was obtained by centrifugation of the sample at 1,400 × *g* for 15 minutes. Samples were aliquoted and stored at −80°C until analysis.

Determination of Seminal Plasma α-Glucosidase and γ-Glutamyltransferase (γ-GT) Activity

α-Glucosidase activity in seminal plasma was measured to evaluate the function of the epididymides. This was done with a commercial kit (EpiScreen, Fertipro, Lotenhulle, Belgium) as described previously (Depuydt et al, 1996a). γ-GT was measured in seminal plasma using a kinetic enzymatic kit from Boehringer Mannheim (Mannheim, Germany) (Comhaire et al, 1989).

Measurement of Scatter Activity in Seminal Plasma

Scatter activity in seminal plasma was assessed by the scatter factor assay on Madin-Darby canine kidney (MDCK) epithelial cells (Stoker et al, 1987). For this assay, 3000 MDCK cells in 150 μl Earle's minimum essential medium supplemented with 10% fetal calf serum were inoculated into 96-well plates and cultured for 24 hours at 37°C in a carbon dioxide incubator. Semen samples to be tested were diluted serially in serum-free Earle's minimum essential medium in a 96-well titer plate, and a volume of 150 μl was added to each well containing the MDCK cells, so that each well contained a volume of 300 μl (total assay volume, 300 μl). Plates were incubated at 37°C for 20 hours, stained with crystal violet, and examined by light microscopy. The highest dilution of a sample at which significant scattering effect was observed (HGF/SF titer) was divided by 0.3 to obtain the units of scatter activity per milliliter (Gherardi et al, 1989). Significant scattering consisted of spreading of tight cohesive colonies of MDCK cells, elongation of polyclonal cells, and separation into single cells.

Measurement of HGF/SF Concentrations in Seminal Plasma

In all 159 semen samples, HGF/SF was measured by ELISA (Eurogenetics, Tessenderlo, Belgium). This is a sandwich immunoassay, in which a monoclonal antibody against HGF/SF was used as catching antibody (R&D Systems, cat. no. MAB294; Minneapolis, Minnesota) and a polyclonal antibody against HGF/SF (R&D Systems, cat. no. 294-HG-NA) conjugated with peroxidase was used as detecting antibody, as described previously (Depuydt et al, 1997). The semen samples were diluted 1:8 in dilution buffer provided in the kit and incubated for 2 hours at 37°C in microtiter plate coated with the monoclonal antibody against HGF/SF. The microtiter plate was washed five times, and 100 μl biotin–anti-HGF/SF conjugate was added. Af-

Table 1. *Semen profiles of the different groups (median, 10th–90th percentiles)*

Parameter	Normo (n = 19)	Astheno (n = 12)	Immuno (n = 12)	Oligo (n = 28)	MAGI (n = 40)	Azoo (n = 18)	VASEC (n = 30)
Volume (ml)	3.2 (2.2–5.1)	2.6 (1.8–5.9)	3.3 (1.4–5.0)	4.0 (2.7–7.5)	3.6 (2.2–5.3)	2.0 (1.0–3.0)	3.2 (1.0–6.5)
Concentration (10^6/ml)	42.9 (20.9–113.0)	34.1 (20.2–62.9)	10.5 (2.0–101.6)	8.6 (1.2–15.4)	6.1 (0.9–47.5)	0	0
Grade (A + B) motility (%)	56.0 (43.6–70.0)	31.0 (18.1–46.1)	20.0 (6.8–66.4)	33.0 (6.2–62.2)	25.5 (8.4–53.9)	—	—
Linear velocity (μm/s)	22.6 (17.4–31.1)	16.9 (12.0–24.8)	19.4 (14.1–36.6)	17.1 (10.7–24.3)	19.8 (11.2–24.4)	—	—
Normal morphology (%)	20 (10–29)	11 (2–30)	6 (0–29)	4 (0–14)	4 (0–11)	—	—

Normo, normozoospermia; Astheno, asthenozoospermia; Immuno, immunological infertility; Oligo, oligozoospermia; MAGI, male accessory gland infection; Azoo, azoospermia due to primary idiopathic testicular failure; VASEC, postvasectomy samples.

ter incubation for 60 minutes at 37°C, the microtiter plate was washed five times and incubated with 100 µl streptavidin–horseradish peroxidase conjugate for 20 minutes at 37°C. One hundred microliters of phosphate-citrate buffer and 100 µl tetramethylbenzidine solution were added. After 20 minutes incubation at room temperature, 50 µl of 2N H_2SO_4 was added to stop the reaction. Absorbance was measured at 450 nm. The average absorbance values obtained for each standard (recombinant human HGF/SF; R&D Systems, cat. no. 294-HG) were plotted against the corresponding HGF/SF concentration (ng/ml), and a calibration curve with an optimized curve fit was constructed. The sensitivity of the test is <500 pg/ml and the intra-assay and inter-assay percent coefficient of variation is below 6% over the entire dynamic range from 0.25 ng/ml to 5 ng/ml.

Affinity Chromatography

Using its strong affinity for heparin, different forms of HGF/SF can be affinity-purified on a heparin-Sepharose column (Mizuno et al, 1994; Depuydt et al, 1997). Pools of seminal plasma from donors (20 ml) and from patients with MAGI (20 ml) were loaded onto a heparin-Sepharose column and subjected to affinity chromatography as described previously (Depuydt et al, 1997). Bound proteins were eluted with a linear gradient of 0.2–1.5 M NaCl, 2.5-ml fractions were collected, and immunoreactive HGF/SF was measured by ELISA.

Statistical Analysis

Statistical analysis was performed using the MedCalc® program (MedCalc Software, Mariakerke, Belgium) (Schoonjans et al, 1995). The skewed distribution of most variables was transformed into normality by using the square root or logarithmic values. The significance of differences was assessed by the Wilcoxon test. Data are presented as mean and 10th–90th percentiles or as mean and range when the number of observations was small. Correlations were calculated by the Spearman rank test. Receiver operating characteristics curves (Griner et al, 1981; Zweig and Campbell, 1993) were used to determine the discriminative power between groups and to identify criterion values. Specificity and sensitivity for all parameters were calculated for a predefined level of 95% for sensitivity and specificity, respectively (Schoonjans et al, 1996).

Results

Table 2 shows the mean and the 10th–90th percentiles of HGF/SF concentration, scatter activity, and specific activity in seminal plasma of different groups. The concentration of HGF/SF was higher in samples from patients with asthenozoospermia ($P < 0.01$), immunological infertility ($P < 0.05$), MAGI ($P < 0.0001$), azoospermia ($P < 0.0001$), and VASEC ($P < 0.0001$) than with normozoospermia. The scatter activity was significantly lower in samples from patients with oligozoospermia ($P < 0.05$), MAGI ($P < 0.05$), and VASEC ($P < 0.01$) compared to normozoospermia.

In addition, we found the concentration of HGF/SF in

Table 2. *Concentration of HGF/SF, scatter activity, and specific activity in seminal plasma in different groups (median, 10th–90th percentiles)*

Parameter	Normo (n = 19)	Astheno (n = 12)	Immuno (n = 12)	Oligo (n = 28)	MAGI (n = 40)	Azoo (n = 18)	VASEC (n = 30)
HGF/SF (ng/ml)	1.12 (0.52–2.02)	1.73† (1.30–2.44)	1.74* (0.85–4.24)	1.06 (0.59–1.56)	3.04§ (1.95–5.23)	3.35§ (1.74–5.80)	2.01§ (1.36–2.57)
Scatter activity‖ (U/ml)	1,280.0 (277–5,802)	1,706.7 (427–3,413)	853.3 (85–3,072)	106.6* (0–747)	426.7* (0–1,707)	853.3 (213–3,413)	133.3† (0–853)
Specific activity¶ (U/ng)	680.7 (142–2,067)	952.0 (331–2,082)	633.1 (15–1,185)	87.4* (0–614)	165.6‡ (0–612)	164.1* (72–1,206)	50.0‡ (0–449)

Normo, normozoospermia; Astheno, asthenozoospermia; Immuno, immunological infertility; Oligo, oligozoospermia; MAGI, male accessory gland infection; Azoo, azoospermia due to primary idiopathic testicular failure; VASEC, postvasectomy samples.

* $P < 0.05$ compared with the normozoospermic group.

† $P < 0.01$ compared with the normozoospermic group.

‡ $P < 0.001$ compared with the normozoospermic group.

§ $P < 0.0001$ compared with the normozoospermic group.

‖ One unit represents the minimal activity per milliliter that shows clear scattering of Madin-Darby canine kidney epithelial cells.

¶ Scatter activity (U) per nanogram of immunoreactive HGF/SF.

VASEC samples to be significantly lower ($P < 0.0001$) than in samples from patients with azoospermia of testicular origin. Similarly, the scatter activity ($P = 0.003$) was lower in the VASEC than in the azoospermic samples; however, the amount of scatter activity (U) per nanogram immunoreactive HGF/SF (specific activity) was not significantly different between these two groups ($P = 0.08$).

Receiver operating characteristics curve analysis indicates HGF/SF to have a good capacity to discriminate between VASEC (negative group, $n = 30$) and azoospermia of testicular origin (positive group, $n = 18$), with the area under the curve equal to 0.87 (95% confidence interval, 0.72 to 0.96), sensitivity of 86%, and specificity of 95% at the selected criterion value of 2.58 ng/ml. At this criterion value, the positive likelihood ratio of a sample to belong to a patient with primary testicular failure rather than vasectomy is 18.0.

HGF/SF concentration in seminal plasma was negatively correlated with sperm concentration ($r = -0.23$, $P < 0.002$) and percentage normal forms ($r = -0.18$, $P < 0.02$) and was positively correlated with γ-GT activity ($r = 0.24$, $P < 0.003$) and concentration of WBCs ($r = 0.33$, $P < 0.0001$). In cases with spermatozoa present, multiple regression analysis selected the concentration of WBCs, the γ-GT activity, and the percentage normal forms as the only independent variables ($R = 0.16$, $r = 0.42$).

The scatter activity was positively correlated with sperm concentration ($r = 0.36$, $P < 0.0001$), sperm morphology ($r = 0.36$, $P < 0.0003$), α-glucosidase activity ($r = 0.34$, $P < 0.0001$), γ-GT activity ($r = 0.32$, $P < 0.0005$), and concentration of WBCs ($r = 0.21$, $P < 0.02$). Multiple regression analysis showed that in cases with spermatozoa present, γ-GT and concentration of WBCs were the only two independent variables ($R = 0.16$, $r = 0.42$).

The amount of scatter activity (U) per nanogram immunoreactive HGF/SF (specific activity) in seminal plasma was positively correlated with sperm concentration ($r = 0.37$, $P < 0.0001$), sperm morphology ($r = 0.39$, $P < 0.0001$), and α-glucosidase activity ($r = 0.27$, $P < 0.005$) and was negatively correlated with the percentage of immobile spermatozoa ($r = -0.22$, $P < 0.05$). In cases with spermatozoa present, multiple regression analysis selected the percentage normal forms as the only independent variable ($R = 0.08$, $r = 0.28$).

Table 3 shows the concentration of HGF/SF, scatter activity, and specific activity in the two fractions of split ejaculates from six normozoospermic donors. In all six cases, the scatter activity and the HGF/SF concentration were higher in the first fraction than in the second fraction ($P < 0.02$).

Pools of seminal plasma from donors and from patients with MAGI were loaded onto a heparin-Sepharose column and chromatographed by stepwise elution with increasing concentration of NaCl. Figure 1 shows the elution profiles of HGF/SF present in seminal plasma from donors and patients with MAGI, as monitored and quantified by ELISA. Consistent with previous reports (Mizuno et al, 1994; Depuydt et al, 1997), pro-HGF/SF, as well as less biologically active forms of HGF/SF, eluted in a first peak between 0.72 and 0.85 M NaCl, whereas biologically active heterodimeric mature αβ-HGF/SF eluted in a second peak between 0.95 and 1.10 M NaCl. In seminal plasma from donors, only 27.2% of all measured HGF/SF eluted in the first peak (inactive fraction), and 72.8% consisted of heterodimeric mature αβ-HGF/SF. In seminal plasma from patients with MAGI, 41.0% of all measured HGF/SF eluted in the first peak and 59.0% in the second fraction.

Table 3. *Concentration of HGF/SF, scatter activity, and specific activity in seminal plasma in the first and second fractions of split ejaculates (median, range) (n = 6)*

Parameter	First fraction	Second fraction
HGF/SF (ng/ml)	2.75 (2.5–3.1)	1.62 (1.3–1.75)
Scatter activity* (U/ml)	1,706.7 (1,706.7–3,413.3)	853.3 (426.7–853.3)
Specific activity† (U/ng)	641.6 (603.1–1,365.3)	500.8 (328.2–540.1)

* One unit represents the minimal activity per milliliter that shows clear scattering of Madin-Darby canine kidney epithelial cells.
† Scatter activity (U) per nanogram of immunoreactive HGF/SF.

Discussion

HGF/SF and its receptor control essential cellular responses, such as cell proliferation, motility, morphogenesis, and differentiation. Their action is context-dependent in that the activity of each factor depends upon the presence of others. In addition, HGF/SF can be secreted in an inactive form (pro-HGF/SF) or together with binding proteins (uPa) or with soluble receptors that can modulate their (bio)availability to the receptors.

Previous studies indicate that HGF/SF is present along the male genital tract (Wolf et al, 1991; Naz et al, 1994; Humprey et al, 1995), and we reported recently that immunoreactive HGF/SF detectable in human seminal plasma by ELISA consists of pro-HGF/SF, heterodimeric mature αβ-HGF/SF, and less biologically active forms of HGF/SF (Depuydt et al, 1997).

In the present paper, we have been able to identify the sources of HGF/SF present in seminal plasma. Apparently, most of the HGF/SF does not originate from the testis and epididymides, since it is present in VASEC samples. The most probable origin is prostatic, since higher concentrations of HGF/SF were measured in the first fraction

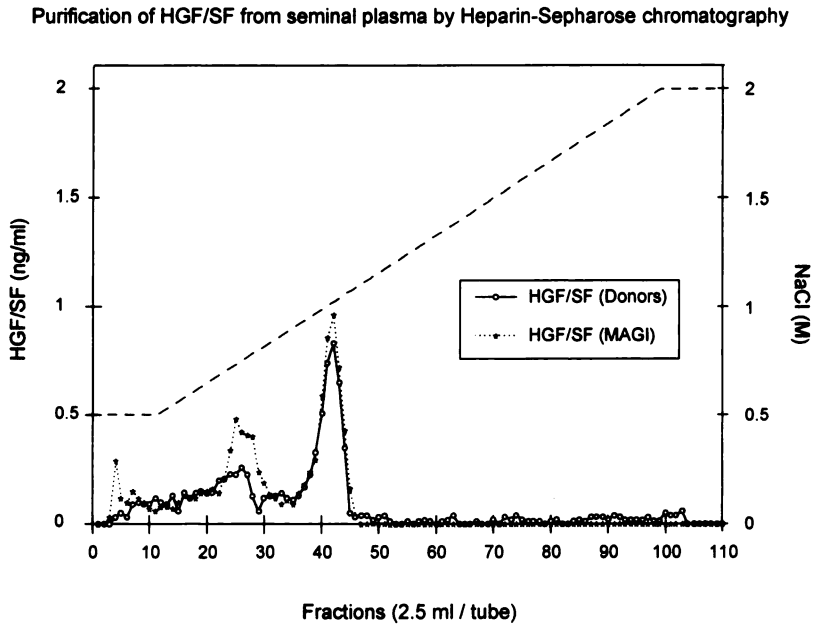

FIG. 1. Purification by heparin-Sepharose chromatography of HGF/SF from human seminal plasma. Seminal plasma pool from donors and patients with MAGI were applied to a heparin-Sepharose column and eluted at 60 ml/hour of a gradient of 0.5–2.0 M NaCl as described. Fractions of 2.5 ml were collected, and the HGF/SF immunoreactivity was measured by ELISA.

of split ejaculates than in the second fraction. However, HGF/SF concentration was higher in samples from patients with azoospermia of primary testicular failure than in samples from patients with normozoospermia, which seems to indicate that at least some HGF/SF is from testicular or epididymal origin in patients with azoospermia. Previous immunohistological studies have revealed HGF/SF to be present in the seminiferous epithelium, the epithelium of the epididymis, ductus deferens, prostate, and seminal vesicles (Wolf et al, 1991). The concentration of HGF/SF is positively correlated with γ-GT, the activity of which in seminal plasma is considered a marker of prostatic function.

Since HGF/SF concentration is higher in samples from patients with MAGI, asthenozoospermia, and immunological infertility than with oligozoospermia and normozoospermia, we suggest that part of HGF/SF in seminal plasma could also have an inflammatory origin. The positive correlation between the concentration of WBCs and HGF/SF concentration corroborates this hypothesis. Others have shown that polymorphonuclear WBCs in diseased livers are, indeed, a cellular source of circulating HGF/SF (Sakaguchi et al, 1994). In inflammation of the accessory sex glands, 41.0% of the HGF/SF present in seminal plasma consists of biologically inactive pro-HGF/SF, as well as less biologically active forms of HGF/SF, explaining the higher concentration of HGF/SF measured by ELISA , but the lower scatter activity as compared to the results obtained in donors.

When the scatter activity of HGF/SF is considered, the highest activity is seen in normozoospermic and asthenozoospermic samples, whereas almost no activity is seen in VASEC samples, suggesting a predominant testicular or epididymal origin. Also, results obtained from split ejaculates, showing that the first fraction has the highest scatter activity, suggest HGF/SF to be of testicular or epididymal origin. The clear difference between the low scatter activity in oligozoospermia and high scatter activity in azoospermia of primary testicular failure suggest that different pathological mechanisms are involved in these conditions. In addition, the significant correlation of the scatter activity with sperm concentration and percentage of normal spermatozoa suggests involvement of HGF/SF during spermatogenesis. However, 13 of 68 semen samples from patients with oligozoospermia and MAGI failed to induce scattering of MDCK cells, although some HGF/SF could be measured by ELISA. This suggests that the HGF/SF present in the seminal plasma of some subfertile patients may not be biologically active and fails to down-regulate c-*met* receptors. This could explain our previous observations that spermatozoa of subfertile patients have significantly more c-*met* receptors than spermatozoa from healthy donors (Depuydt et al, 1996b).

From the preceding, one can deduce that the specific activity of the HGF/SF present in seminal plasma is highest in normozoospermia, asthenozoospermia, and immunological infertility, median in MAGI and azoospermia, and lowest in oligozoospermia and in VASEC. Alternatively, a decreased level of uPa could be present in sem-

inal plasma of particular patients with abnormal liquefaction (Arnaud et al, 1994), since biological activation of pro-HGF/SF by uPa is controlled by a stoichiometric reaction (two molecules of uPa for one pro-HGF/SF molecule; Naldini et al, 1995). Furthermore, we found that the amount of HGF/SF per spermatozoon increases with the number of c-*met* receptors per spermatozoon, which may indicate that, although more HGF/SF is present per c-*met* receptor in samples from oligozoospermic patients, it is not biologically active. This, then, could explain previous observations (Hong et al, 1985), whereby the addition of uPa to spermatozoa increased their motility. The latter suggests that part of the HGF/SF present in seminal plasma is inactive pro-HGF/SF, which can be activated by adding uPa produced by the target cells.

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