Hepatocyte growth factor in human semen and its association with semen parameters

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Hepatocyte growth factor (HGF) is a structurally unique growth factor with potent motogenic (motility inducing) effects. Studies in the murine male genital tract have suggested important associations between HGF and the acquisition of sperm motility during epididymal maturation. The aim of this study was, therefore, to determine the concentration of HGF in human semen and assess its correlation, if any, with sperm motility and other semen parameters. Semen samples were collected by masturbation and analysed using standard procedures. HGF concentrations were measured in duplicate using an enzyme-linked immunoassay technique. Total protein estimations were also made in a subset of samples. The 95 subjects were divided into three groups for analysis: normozoospermic, subnormal semen and azoospermic. HGF was detected in all samples (median 0.456, 25th centile 0.388, 75th centile 0.556 ng/ml). No significant correlations were found between semen HGF concentrations and sperm concentration, motility, total sperm count or total motile count. There were no significant differences in mean HGF concentrations between the three subgroups. In conclusion HGF is present in human semen in significant quantities. The data do not suggest HGF concentrations are correlated with parameters of sperm motility.

Key words: hepatocyte growth factor/human/infertility/motility/spermatozoa

Introduction

Hepatocyte growth factor (HGF), also known as scatter factor (SF), is a structurally unique growth factor secreted by cells of mesenchymal origin. HGF acts on epithelial cells, through its receptor, c-met, a membrane spanning tyrosine kinase and the product of the c-met proto-oncogene (Lyon and Gallagher, 1992; Michalopoulos and Zarnegar, 1992; Rubin et al., 1993). It is a potent mitogen, morphogen and motogen. Its effect on cell motility varies between different cell lines and according to the HGF concentration. Cultured epithelial and endothelial cells ‘scatter’ on addition of HGF (a combination of cell dissociation and migration). HGF has been implicated in tumour metastasis in man, as well as angiogenesis and tissue repair. Cell dissociation and migration are pivotal to these processes.

The role of HGF in sperm motility has not been extensively investigated. Studies in the murine male genital tract suggest that HGF is differentially expressed, with the highest concentrations detected in the distal region of the corpus and cauda epididymides (Naz et al., 1994). This pattern coincides with the acquisition of both the potential for motility and fertilizing capacity by mouse spermatozoa and suggests HGF may be involved in the process of acquisition of the potential for sperm motility during epididymal maturation. Incubation of immotile mouse spermatozoa from the caput epididymis with HGF induced motility in 5–15% of these spermatozoa. c-met is also present in the acrosomal region of human spermatozoa and is phosphorylated when incubated with HGF (Herness and Naz, 1999). Depuydt et al. (1996) showed that c-met, the receptor for HGF, is expressed on human seminiferous epithelium and spermatozoa. The same group have also shown that HGF is present in seminal plasma (Depuydt et al., 1997) and recently reported associations between HGF concentration in human seminal plasma and various andrological diseases (Depuydt et al., 1998).

Human male infertility may be due to decreased numbers of spermatozoa, increased numbers of abnormal spermatozoa, poorly motile spermatozoa or a combination of these factors. Therefore the aim of this study was to determine the relationship between the concentrations of HGF in seminal plasma and parameters of sperm function in fertile and sub-fertile males and to determine whether HGF concentrations increase in post-vasectomy semen.

Materials and methods

Ethical approval was obtained from the Ethics of Human Research Committee of The Queen Elizabeth Hospital and from the South Australian Council on Reproductive Technology.

Subjects

Participants in the study were recruited by the Andrology Laboratory at The Queen Elizabeth Hospital. To ensure a range of values, especially for motility, semen samples were obtained from: (i) male partners of couples seeking infertility treatment in the Reproductive Medicine Units at The Queen Elizabeth Hospital and Wakefield Clinic (n = 80), (ii) men having post-vasectomy semen checks (n = 7), (iii) fertile, anonymous semen donors (n = 16). Recruitment was
undertaken sequentially, without any sample or subject pre-selection. Informed written consent was obtained from all participants, who were free to withdraw at any time. No patient had clinical evidence of infection. Anti-sperm antibodies were not routinely measured.

Collection and analysis of semen samples
One sample from each subject was collected by masturbation and allowed to liquefy at room temperature before analysis using standard techniques (World Health Organization, 1992; Mortimer, 1994). Ejaculatory abstinence for 2–5 days was requested prior to sample collection. Semen volume was measured using a graduated pipette, sperm concentration was determined using a haemocytometer, progressive sperm motility was assessed visually at ×400 using phase contrast optics and sperm morphology was assessed after modified Papanicolaou staining. A subjective motility grade (0–4) was used to describe the vigour of motility. Following semen analysis, an aliquot (0.2–0.5 ml) of each sample was stored at −65°C for HGF analysis. All samples were coded to ensure unbiased HGF analyses.

HGF analysis
The HGF analysis was performed in duplicate at the Department of Paediatrics, The University of Adelaide, using a commercial immunoassay (Immuns HGF enzyme immunoassay; Institute of Immunology Co. Ltd, Tokyo, Japan). This was a sandwich immunoassay, in which monoclonal antibody against HGF was used as a capture antibody and monoclonal antibody conjugated with peroxidase was used as detecting antibody. No cross-reactivity was detected using this assay with other substances displaying kringle motif repeats [such as lipoprotein(a), insulin-like growth factor (IGF-I, IGF-II, fibroblast growth factor, transforming growth factor β (TGFβ)] and plasminogen (Khan et al., 1996). Past intra-assay and interassay coefficients of variation (CV) using serum were 1.9% and 10.9% respectively (Khan et al., 1996). The samples were centrifuged at 4000 rpm for 2 min to pellet the spermatozoa, after which the supernatant was removed by micropipette and analysed. Total protein was measured colorimetrically at 570 nm using a Dynatech MR7000 spectrophotometer (Guernsey, Channel Islands) in 17 samples using the Pierce bicinchoninic calorimetric assay (BCA) (Pierce, Rockford, IL, USA) (Smith et al., 1985).

Statistical analysis
Data were analysed by regression analysis and the Mann–Whitney U test using Statworks statistical software on a Macintosh LC 545 computer. Results were considered statistically significant at P value < 0.05. Samples were analysed in the three groups (infertility patients, semen donors and post-vasectomy). Samples were also categorized into three groups based on World Health Organization (WHO, 1992) and published (Duncan et al., 1993) reference ranges: normozoospermic, subnormal and azoospermic. The threshold values used to classify samples as normozoospermic were: concentration = 20×10⁹/ml, motility = 50% progressive, morphology = 20% normal. Samples were classified as subnormal if one or more parameters were below these values.

Results

Samples
Samples were obtained from 103 males (median age 35 years, range 22–53). Eight samples (seven subnormal, one azoospermic, all infertility patients) were excluded from analysis because of a high CV (>15%) on repeat testing, which was related to increased semen viscosity. Of the 95 samples analysed, there were 27 normozoospermic samples, 63 subnormal samples and five azoospermic samples. Two post-vasectomy samples had some spermatozoa and were classified in the sub-normal group. One infertility patient had azoospermia. The semen profiles and HGF values for the groups are summarized in Tables I and II.

HGF concentration
HGF was detected in every semen sample (median 0.465, 25th–75th centiles 0.388–0.556 ng/ml). The concentration was higher than those previously reported by our laboratory for normal adult serum (median 0.23, 25th–75th centiles 0.14–0.31 ng/ml; Khan et al., 1996), but lower than those reported by Depuydt et al. (1998) for human semen (mean 1.12 ng/ml for normozoospermic samples, mean 3.35 ng/ml for azoospermic samples).

There was a statistically significant, negative correlation between the HGF concentration and the subjective motility grade (r = 0.265, P = 0.009). The HGF concentration was correlated with progressive motility (r = 0.11), but this did not reach statistical significance. The HGF concentration was not correlated with ejaculate volume (r = 0.155, not significant), sperm concentration (r = 0.155, not significant), total sperm count (r = 0.091, not significant), or total motile count (r = 0.084, not significant). An association between HGF and period of ejaculatory abstinence was not assessed.

The median (range) HGF concentrations in the six subgroups were: (i) normozoospermic 0.469 ng/ml (0.250–0.738 ng/ml); (ii) sub-normal 0.426 ng/ml (0.248–1.213 ng/ml); (iii) azoospermic 0.507 ng/ml (0.344–1.419 ng/ml); (iv) infertility 0.445 (0.248–1.213); (v) donor 0.473 (0.313–1.141); (vi) post-vasectomy 0.5 (0.285–1.419). See Tables I and II and Figures 1 and 2.

There were no significant differences between the groups using the Mann–Whitney U test for non-parametric data (normal versus subnormal; normal versus azoospermic; subnormal versus azoospermic; infertility versus donor; infertility versus post-vasectomy; and donor versus post-vasectomy).

There was no correlation between the HGF concentration and total protein in a subgroup of 17 samples (r = 0.2). These samples had a median (range) HGF concentration of 0.51 ng/ml (0.250–1.141 ng/ml) and a median (range) protein concentration of 23.838 mg/ml (11.478–60.079 mg/ml). There was no difference in protein concentration between the groups, although only two azoospermic (one infertility, one post-vasectomy) samples were analysed.

Discussion
HGF is a mesenchymally elaborated growth factor which has mitogenic, motogenic and morphogenic effects on epithelial and endothelial cells. A transgenic mouse knock-out model, produced using targeted HGF gene disruption is lethal in utero (Uehara et al., 1995). In vitro, HGF has a number of effects on cell motility, which vary with cell type and include cell dispersion and cell migration. Naz et al. (1994) examined the relationship between HGF and development of sperm motility and fertilizing capacity in the murine genital tract. Using four
Table I. Semen profiles and hepatocyte growth factor (HGF) concentrations, grouped by semen parameters. Values are expressed as median (with range in parentheses)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Volume (ml)</th>
<th>Concentration (&lt;10^6/ml)</th>
<th>Motility (Grade 0–4)</th>
<th>Morphology (% normal)</th>
<th>HGF (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normozoospermic</td>
<td>27</td>
<td>3.8 (1.4–10.1)</td>
<td>69.0 (26–328)</td>
<td>55 (50–66)</td>
<td>28 (20–45)</td>
<td>0.469 (0–250)–0.738</td>
</tr>
<tr>
<td>Subnormal</td>
<td>63</td>
<td>3.3 (1.5–8.5)</td>
<td>31.0 (0.1–275)</td>
<td>44 (0–66)</td>
<td>12 (0–44)</td>
<td>0.428 (0.248–1.213)</td>
</tr>
<tr>
<td>Azoospermic</td>
<td>5</td>
<td>3.2 (0.5–7.5)</td>
<td>0.0</td>
<td>–</td>
<td>–</td>
<td>0.507 (0.344–1.419)</td>
</tr>
</tbody>
</table>

Table II. Semen profiles and hepatocyte growth factor (HGF) concentrations, original patient groups. Values are expressed as median (range)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Volume (ml)</th>
<th>Concentration (&lt;10^6/ml)</th>
<th>Motility (Grade 0–4)</th>
<th>Morphology (% normal)</th>
<th>HGF (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>72</td>
<td>3.6 (1.4–10.1)</td>
<td>49 (0–328)</td>
<td>49 (0–66)</td>
<td>16.5 (0–45)</td>
<td>0.445 (0.248–1.213)</td>
</tr>
<tr>
<td>Donors</td>
<td>16</td>
<td>3.4 (1.6–7.3)</td>
<td>58.5 (3–214)</td>
<td>51 (18–64)</td>
<td>19.5 (7–39)</td>
<td>0.473 (0.313–1.141)</td>
</tr>
<tr>
<td>Post-vasectomy</td>
<td>7</td>
<td>3 (0.5–7.7)</td>
<td>0 (0–2.5)</td>
<td>0</td>
<td>0</td>
<td>0.5 (0.285–1.149)</td>
</tr>
</tbody>
</table>

Depuydt et al. reported the presence of c-met, the receptor for HGF, on human spermatozoa (Depuydt et al., 1996), the presence of HGF in human semen (Depuydt et al., 1997) and an association between HGF concentration in seminal plasma and andrological diseases (Depuydt et al., 1998). Their studies suggest that most seminal HGF in man probably has its origin in the prostate gland, and that concentrations may be higher in inflammatory or immunological disorders involving the male genital tract, possibly because of production by leucocytes. It is also possible that inflammation leads to the up-regulation of HGF expression in tissues of the male reproductive tract.

Immunohistochemical studies in rats and humans have shown moderate expression of HGF in testicular Leydig cells and strong expression in the epithelium of the epididymis, ductus deferens, prostate and seminal vesicle (Wolf et al., 1990). This suggests HGF may have a testicular role, although the prostate and seminal vesicles appear responsible for the majority of HGF production. The results of the current study, with high HGF concentrations in post-vasectomy samples are consistent with this. Depuydt et al. also found the scatter activity of HGF to be highest in normozoospermic and asthenozoospermic samples, with no activity in post-vasectomy samples, and suggested a testicular origin for this function.

It was also sought to determine whether any association existed between seminal HGF concentrations and human sperm motility. It was shown that HGF was present in human semen in quantities approximately double those found in human adult.
serum, but lower than those reported (Depuydt et al., 1998). A possible explanation for this difference would be cross-reactivity with other compounds with kringle repeats such as lipoprotein(a), IGF-I, IGF-II, TGFβ, fibroblast growth factor (FGF) and macrophage stimulatory protein (MSP, hepatocyte growth factor like protein) in the method used for HGF measurement (Depuydt et al., 1998). The results suggest that HGF is either concentrated in or secreted into semen. However, it was not possible to demonstrate a relationship between HGF concentrations and sperm motility, other than motility grade, using semen samples with a wide range of sperm motility.

The presence of HGF in human semen in these quantities, together with the previous findings, suggests that it may have some functional role in semen. There are several possible explanations for the difference in the current results compared with findings in mice and those of Depuydt et al. (1998). First, HGF may have a functional role in the initiation of sperm motility which is independent of total semen concentration (and perhaps reflects tissue concentrations at specific sites within the genital tract). Second, the numbers in the azoospermic group in the current study were small and may not have been sufficient to show a difference between these and normal or sub-normal semen. Third, presence of immunoreactive HGF using an ELISA does not necessarily mean a correlation with function. Fourth, there may be a difference between mice and man in the importance of HGF in the acquisition of sperm motility. Of interest is the difference noted (Depuydt et al., 1998) between HGF concentration and scatter activity in semen. They suggest this relates to less biologically active forms of HGF. Another explanation would be that their method measures scatter activity, but not scatter activity due to HGF. Other compounds, such as MSP, which, together with its tyrosine kinase receptor STK/RON is expressed in the rat male genital tract (Ohshiro et al., 1996) and has scatter activity (Wang et al., 1994, 1996), may be responsible for these results. Scatter activity should be assessed following pre-incubation with an HGF blocking antibody to establish whether this is the case.

In summary, HGF was found to be present in human semen in significant quantities. The data do not support the hypothesis that HGF concentrations in seminal plasma serve as an indicator of sub-optimal human sperm motility. However, the study has shown a weak association between HGF and motility grade and does not rule out the possibility that HGF may have a functional role in the motility of human spermatozoa. Further studies investigating its role in the ontology of human sperm motility are warranted.

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References


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