Quantification by magnetic resonance spectroscopy of metabolites in seminal plasma able to differentiate different forms of azoospernia

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Human seminal plasma contains a mixture of secretions from the seminiferous tubules, the epididymis and the accessory glands (bulbo-urethral, prostate, seminal vesicles). Information about biochemical markers of the seminal plasma including fructose, zinc, acid phosphatase, free L-carnitine and α-1,4-glucosidase and glycerophosphorylcholine (GPC) has been obtained by classical biochemical analysis with different degrees of sensitivity and intra-assay variation coefficients. However, in cases of azoospermia, this analysis provided limited information. In addition, it is now established that the high follicle stimulating hormone (FSH) concentration present in azoospermia is not always a reliable indicator of testicular failure. In recent years, much in-vivo and in-vitro work using magnetic resonance spectroscopy (MRS) have shown it has a potential use in the study of human testicular function and male infertility evaluation (Arrata et al., 1978; Bahl et al., 1988; Bretan et al., 1989; Chew and Hericak, 1989; Grond et al., 1991). MRS analysis is a simple, reliable method that is able to provide data concerning numerous compounds at the same time in a sample of seminal plasma.

Quantitative and qualitative studies using 1H-MRS spectroscopy in human seminal plasma have provided data suggesting that metabolites including GPC, choline, citrate and lactate can be used to differentiate spermatogenic failure from obstructive azoospernia (Hamamah et al., 1993). More recently, Segalen et al. (1995) showed that the 1H-MRS pattern of human seminal plasma was related to the occurrence of ongoing pregnancies following in-vitro fertilization conditions.

The purpose of this study was to determine whether 1H-MRS of metabolites including GPC, choline, citrate and lactate in human seminal plasma can be used to differentiate between cases of spermatogenic failure and obstructive azoospernia but also to qualify forms of spermatogenic failure. These results demonstrate the potential use of 1H-MRS on human seminal plasma in a new approach in the management of male infertility. Key words: azoospermia/human/magnetic resonance spectroscopy/seminal plasma

Introduction

In spite of important developments in assisted reproductive technology, male infertility remains poorly understood and eludes specific diagnosis. Since the technique of intracytoplasmic sperm injection (ICSI) was introduced by Palermo et al. (1992) for cases of low sperm count, poor sperm morphology, and low sperm motility, it has also been applied to obstructive (Schoysman et al., 1993) and non-obstructive azoospermic patients (Kahraman et al., 1996). However, in non-obstructive azoospermic patients, the criteria for predicting the presence or absence of spermatozoa are lacking.

Human seminal plasma contains a mixture of secretions from the seminiferous tubules, the epididymis and the accessory glands (bulbo-urethral, prostate, seminal vesicles). Information about biochemical markers of the seminal plasma including fructose, zinc, acid phosphatase, free L-carnitine and α-1,4-glucosidase and glycerophosphorylcholine (GPC) has been obtained by classical biochemical analysis with different degrees of sensitivity and intra-assay variation coefficients (Soufir et al., 1981; Guérin et al., 1986; Mieusset et al., 1988; Cooper et al., 1990; Yeung et al., 1990; Bujan, 1995). Many of these biochemical markers relating to seminal vesicles, prostate, epididymis and spermatozoa have been largely investigated, and few have proved to be clinically useful. In cases of non-obstructive azoospermia, such analysis provided limited information. In addition, it is now established that the high follicle stimulating hormone (FSH) concentration present in azoospermia is not always a reliable indicator of testicular failure (Devroey et al., 1995).

In recent years, much in-vivo and in-vitro work using magnetic resonance spectroscopy (MRS) have shown it to have a potential use in the study of human testicular function and male infertility evaluation (Arrata et al., 1978; Bahl et al., 1988; Bretan et al., 1989; Chew and Hericak, 1989; Grond et al., 1991). MRS analysis is a simple, reliable method that is able to provide data concerning numerous compounds at the same time in a sample of seminal plasma.

Quantitative and qualitative studies using 1H-MRS spectroscopy in human seminal plasma have provided data suggesting that metabolites including GPC, choline, citrate and lactate can be used to differentiate spermatogenic failure from obstructive azoospernia (Hamamah et al., 1993). More recently, Segalen et al. (1995) showed that the 1H-MRS pattern of human seminal plasma was related to the occurrence of ongoing pregnancies following in-vitro fertilization conditions.

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Materials and methods

Seminal plasma identification

A total of 75 seminal plasma samples were classified into two groups. Group 1: men with spermatogenic failure (n = 58), in whom surgical exploration did not find any obstruction and whose testicular biopsy...
showed spermatogenic alterations (hypospermatogenesis, spermatogenesis arrest or germinal cell aplasia). FSH was measured in all cases of spermatogenic failure except certain patients who had undergone chemo- or radiotherapy treatment. The testicular biopsy was performed when serum FSH values were normal (4–8 mIU/ml). Group 2: men with obstructive azoospermia (n = 17), in whom surgical exploration showed normal testicles as defined by Bujan (1995).

**Principle of MRS**

The technique of MRS is based on the property of atomic nuclei, composed of an uneven number of nucleons (like hydrogen), to have a magnetic moment, called spin, which makes them behave like small magnets. These spins are arranged at random. However, in a strong external magnetic field, produced by the magnet of the spectrometer, they can be made parallel or antiparallel to the magnetic field, while emitting a specific radiofrequency signal, characteristic of each type of nucleus and of the chemical environment surrounding it. This signal, after reception by appropriate coils, is termed the ‘free induction decay’, and is mathematically analysed by the spectrometer computer to give a spectrum characterized by several peaks each corresponding to a specific chemical species. These peaks allow identification of the corresponding molecules in the sample studied.

**Seminal plasma preparation for MRS analysis**

After liquefaction the semen was centrifuged at 750 g for 15 min to remove cells and spermatozoa. Azoospermia was confirmed by the absence of a sperm sediment. Seminal plasma was stored at −20°C and analysed immediately after thawing.

For MRS analysis, a Bruker AM 200 WB spectrometer (4.7 T) operating at 200.13 MHz and equipped with a 5 mm 1H/13C probe thermostatted at 25°C with the sample spinning was used. Seminal plasma samples (500 µl) were transferred into 5 mm (outer diameter) MRS sample tubes as previously reported by Hamamah et al. (1993). Proton chemical shifts were determined with respect to the external MRS standard sodium 3-trimethylsilylpropionate. Two-dimensional MRS experiments were performed using the homonuclear shift-correlated (COSY) technique. The peak areas were determined using the integration software of the spectrometer (DISNMR, Bruker). Despite the precautions taken to obtain quantitative measure of area peaks, the use of a selective pulse for water suppression could disturb these analyses. To prevent this problem, we used the ratio of area peaks to compare results from different seminal plasma.

The results for choline/citrate, citrate/lactate, choline/lactate and GPC/choline peak ratios were expressed as mean ± SEM. Statistical analysis to compare the two groups of patients was performed using the Mann–Whitney U-test.

**Results**

GPC, choline, citrate and lactate were identified in a typical 1H-MRS obtained from seminal plasma from a patient with obstructive azoospermia and compared with a group with spermatogenic failure. The peak area ratios for choline/citrate and choline/lactate were significantly different between these two groups (Table I).

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Obstructive azoospermia (n = 17)</th>
<th>Spermatogenic failure (n = 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline/citrate</td>
<td>1.6 ± 0.1*</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Citrate/lactate</td>
<td>4.8 ± 0.8</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>Choline/lactate</td>
<td>6.9 ± 1.0*</td>
<td>8.4 ± 0.6</td>
</tr>
<tr>
<td>GPC/choline</td>
<td>0.08 ± 0.01*</td>
<td>0.14 ± 0.01</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.01. GPC = glycerophosphorylcholine.

When the serum FSH values were normal, there was a significant difference (P < 0.01) for choline/lactate ratio between excretory and secretory azoospermia (Table II). Significant differences in GPC/choline ratio were observed between the patients with spermatogenic failure and normal FSH and those with obstructive azoospermia (0.12 ± 0.01 versus 0.08 ± 0.02 respectively). When the FSH values were low (<3 mIU/ml), the GPC/choline ratio was the only factor able to differentiate cases of spermatogenic failure from obstructive azoospermia (0.20 ± 0.05 versus 0.09 ± 0.02 respectively).

In the secretory azoospermic group, a slight decrease in testosterone concentration in comparison with the obstructive azoospermic group was observed (mean ± SD: 491 ± 143 versus 520 ± 135 ng/0.11 respectively). According to the mean of their serum FSH values, spermatogenic failure patients were subdivided into three groups: (i) low FSH = 3.6 (2.6–3.9) mIU/ml, n = 7, (ii) normal FSH = 7.3 mIU/ml (4.1–8.8), n = 9, and (iii) high FSH = 18.6 (9.5–75.0) mIU/ml, n = 23. The GPC/choline ratio was significantly higher in the group with low FSH compared with the other two groups (Figure 1). Also, the comparison between groups with high FSH and patients treated by chemo- or radiotherapy with high FSH showed a significant difference only for GPC/choline ratio (Table III; P < 0.01).

**Discussion**

In male infertility evaluation, there are several biochemical markers in human seminal plasma which may be used for the
In order to understand the molecular profile of the perturbation to seminal plasma which results in infertility, new techniques are required to measure quantitative and qualitative changes in the constituents of seminal plasma. MRS is one such tool which has many advantages compared to conventional techniques (Grond et al., 1991; Hamamah et al., 1993, 1995; Burt and Ribolow, 1994; Lynch et al., 1994; Segalen et al., 1995; Kamp and Lauterwein, 1995). The aim of the present study was to determine whether $^1$H-MRS of metabolites including GPC, choline, citrate and lactate can be used to differentiate patients with different forms of azoospermia.

In this study, regardless of the serum FSH concentrations of men with spermatogenic failure, $^1$H-MRS spectroscopic data showed that the choline/citrate, choline/lactate and GPC/choline ratios were increased compared to those observed in the obstructive azoospermia group. A significant difference in the GPC/choline peak area ratio was observed between cases with spermatogenic failure and those with obstructive azoospermia when serum FSH values were normal, whereas no significant differences were found for choline/citrate or citrate/lactate peak area ratios. When FSH concentration increased (indicative of a severe alteration in spermatogenesis or of spermatogenesis arrest prior to the appearance of spermatids), the comparison between patients after chemo- or radiotherapy treatment with elevated FSH and others with non-obstructive azoospermia also having high FSH showed that the GPC/choline ratio may be an important parameter able to differentiate between these two groups. The ability of the GPC/choline ratio to differentiate different forms of spermatogenic failure was established by calculating the sensitivity and specificity of the discrimination using several cut-off points. Obviously, the sensitivity and specificity of such a parameter varies with the cut-off value.

If $^1$H-MRS is employed as an alternative method of studying human semen in male infertility management, the use of GPC/choline changes may be important in the study of non-obstructive azoospermia: (i) to study the kinetics of recovery of spermatogenesis, as well as accessory gland activities, following arrest of chemo- or radiotherapy treatment, since the value of this ratio is already known in fertile men (Hamamah et al., 1993), (ii) to differentiate between men with spermatogenic failure and normal FSH levels and those with obstructive azoospermia. In these cases, this non-invasive technique will avoid the need for testicular biopsies. These results demonstrate the potential use of $^1$H-MRS as a means of differentiating between several subgroups of non-obstructive azoospermia according to serum FSH concentration and also between cases of obstructive azoospermia and spermatogenic failure. In addition, the MRS technique may be used to monitor the human seminal plasma of patients with severe oligoasthenozoospermia before referral to an ICSI procedure.

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References


Table III. $^1$H-Magnetic resonance spectroscopy values of non-obstructive azoospermia: patients undergoing chemo- or radiotherapy treatment compared with spermatogenic failure patients with a high serum follicle stimulating hormone concentration. Table 3.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Patients undergoing chemo- or radiotherapy treatment ($n = 6$)</th>
<th>Spermatogenic failure ($n = 23$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline/citrate</td>
<td>2.3 ± 0.2</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td>Citrate/lactate</td>
<td>4.5 ± 0.8</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Choline/lactate</td>
<td>8.4 ± 1.0</td>
<td>9.5 ± 1.2</td>
</tr>
<tr>
<td>GPC/choline</td>
<td>0.10 ± 0.02*</td>
<td>0.16 ± 0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *$P < 0.01$.

GPC = glycerophosphorylcholine.

Figure 1. Glycerophosphorylcholine (GPC)/choline peak area ratio of spermatogenic failure azoospermic patients according to serum follicle stimulating hormone (FSH) levels. Values are mean ± SEM.


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MRS profile of seminal plasma in azoospermia