Seminal Oligouridinosis: Low Uridine Secretion as a Biomarker for Infertility in Spinal Neurotrauma

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BACKGROUND: Compromised sexual health is a major rehabilitative barrier for men with lower–spinal cord injury (SCI). Although studies have revealed decreased sperm motility, the quantitative biochemical changes that underlie the infertility mechanism remain poorly understood.

METHODS: We employed a nontargeted approach combining 800 MHz hydrogen nuclear magnetic resonance (1H NMR) spectroscopy and ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) with pattern recognition methods to analyze seminal fluid metabolite profiles in 10 men with and 8 without SCI above thoracic vertebra 10 (T10).

RESULTS: The metabolic phenotype for SCI could be predicted from the 1H NMR data. The median concentration of uridine in fertile controls was 1.55 mmol/L (range 1.0–5.0 mmol/L), but was undetectable by both NMR and MS in all but 2 individuals from the SCI group, one who later fathered a child without assisted fertility techniques.

CONCLUSIONS: We hypothesize that uridine is likely to be an essential precursor to metabolites required for capacitation and is a potential marker for the prognosis of post-SCI functional fertility recovery. We derived the term “seminal oligouridinosis” to describe this newly identified condition.

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Most of the 200 000 men with spinal cord injury (SCI)4 in the US and about 40 000 in the UK suffer compromised sexual health attributable to a combination of neurophysiologic impairment of ejaculation and poor semen quality. Despite increasing success of semen-harvesting techniques such as vibro- and electroejaculation, understanding is limited regarding biochemical and mechanistic causes of infertility after SCI (1). The definitive observation is that SCI patients have lower percentages of motile sperm (2).

We used high-resolution hydrogen nuclear magnetic resonance (1H NMR) spectroscopy and ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) as unbiased multivariate probes of metabolite profiles to compare seminal plasma from 10 men with SCI above thoracic vertebra 10 (T10) for more than 6 months with seminal plasma from 8 age-matched fertile (fathered a child within 12 months) controls. The use of 1H NMR spectroscopy and MS has gained increasing importance and has been well documented as a top-down systems biology method for understanding pathophysiological states (3) and for analysis of biofluids, including seminal fluid (4).

We obtained ethics approval from the National Health Service Research and Ethics Committee (Ref No. 05/Q0506/17) and informed consent from all study participants. We collected antegrade semen obtained by vibroejaculation from SCI patients and by masturbation from age-matched controls. Samples were snap-frozen in liquid nitrogen immediately after collection and stored at −80 °C. Drug history was checked to rule out any medications known to affect semen parameters, and to our knowledge, apart from the SCI, all the volunteers were free of any comorbidities that would have any direct effects on the semen parameters assessed. WHO-defined sperm characteristics were noted (5). For NMR analysis, semen samples were thawed for 48 h at room temperature to allow all dynamic peptidase reactions to come to equilibrium, then diluted 2:1 in a solution of 50 g/L sodium trimethylsilyl [2,2,3,3-2H4]propionate in D2O. We performed centrifugation for 5 min at 16 000g, then diluted 300 μL of supernatant 1:3 in D2O for NMR analysis. Experiments were performed on a Bruker AvanceII with CryoProbe™ operating at 800.32 MHz. We acquired 128k data points by use of a pulse sequence of the form RD (recycle delay)–90°–3 μs–90°–tm–90°–acquire, during which selective irradiation of

4 Nonstandard abbreviations: SCI, spinal cord injury; 1H NMR, hydrogen nuclear magnetic resonance; UPLC-MS, ultra-performance liquid chromatography–mass spectrometry; T10, thoracic vertebra 10; O-PLS-DA, orthogonal projections to latent structures discriminant analysis.
Fig. 1. (A), Stacked plot of 800 MHz $^1$H NMR spectra from individual human seminal fluid samples, expanded to show only the region containing olefinic and aromatic uridine protons. Spectra are annotated with anonymized patient number and SCI status (cont indicates control). (B), Stacked plot of uridine-extracted ion chromatogram UPLC-MS data from selected samples, along with uridine standard (Std). (C), Plot of O-PLS coefficient loadings as a function of chemical shift for model constructed from 1-dimensional NMR data from human seminal fluid. The color represents the coefficient of determination ($r^2$ on a scale of 0–1) that each variable has with the patient classification group, with positive O-PLS coefficients corresponding to resonances that covaried with control class.
water was applied during the RD (2 s) and mixing time, \( t_\text{m} \) (0.1 s). For processing we applied line broadening of 1 Hz and zero-filling by 128 k data points. For subsequent MS analysis, 100 \( \mu \text{L} \) of sample was added to 200 \( \mu \text{L} \) methanol and then twice mixed, centrifuged, and lyophilized and reconstituted in 100 \( \mu \text{L} \) H\(_2\)O. We used an Acquity UPLC system (Waters) coupled to an LCT Premier (Micromass) operating in the positive electrospray mode, using a scan range of 50–1000 m/z. Samples (5 \( \mu \text{L} \)) were injected onto an HSS T\(_3\) Acquity column (Waters) of 2.1 \( \times \) 100 mm (1.7 \( \mu \text{m} \)) and eluted by use of a 25-min linear gradient of 100% A (water, 0.1% formic acid) to 100% B (acetonitrile, 0.1% formic acid). The capillary voltage was 3.2 kV, sample cone 35 V, desolvation temperature 350 °C, source temperature 120 °C, and desolvation gas flow 900 L/h. Orthogonal projections to latent structures discriminant analysis (O-PLS-DA) models were constructed using in-house software (MetaSpectra, O. Cloarec, Imperial College London). Uridine concentrations were determined by integration of the relatively isolated resonances at 65.90 and 85.92 before and after spiking in a known amount of uridine standard.

A stacked plot of the partial \(^1\text{H}\) NMR spectra from all the samples analyzed (Fig. 1A) showed 2 doublets in the spectra (65.9, \( J_{\text{HH}} = 4.41 \text{ Hz} \) and 85.92, \( J_{\text{HH}} = 8.01 \text{ Hz} \)) that were absent from all but 2 of the spectra from SCI patients. These peaks are known from previous assignment to correspond to uridine (6). This NMR assignment was confirmed by spiking in a known amount of uridine. Selected samples were also analyzed by UPLCMS, confirming the status of uridine in respective samples by comparing retention time and mass spectrum to that of the reference standard (Fig. 1B).

We expanded statistical analyses with O-PLS-DA and found that the \(^1\text{H}\) NMR spectra were modeled with good predictive ability, as indicated by the cross-validation parameter \( Q^2 \) of 0.62 (7). Fig. 1C plots the O-PLS coefficients for the model as a function of chemical shift, colored to highlight resonances that were correlated (Pearson correlation coefficient, \( r^2 \)) with either class, revealing that uridine, N-acetyl glucosamine, tyrosine, and phenylalanine were reduced in SCI patients. We also found that the frequency of spermatozoal motility (23.9% and 87%), forward progression (34% and 79.4%), and normal morphological forms (21.5% and 62%) were substantially different between the groups (SCI and control, respectively).

Our most striking finding was that uridine was undetectable by NMR and MS (i.e., <1 nmol/L) in seminal fluid from all but 2 of the SCI patients participating in this study, yet present (median, 1.55 mmol/L, range 1.0–5.0 mmol/L) in all fertile volunteers. Moreover, 1 SCI patient (spectrum labeled “101” in Fig. 1), whose semen had detectable uridine (0.18 mmol/L), subsequently fathered a child without intervention, suggesting that seminal uridine or its metabolites are necessary for fertilization, and that seminal uridine is a potential prospective biomarker of therapeutic efficacy in male infertility. Determination of the significance of the other metabolites was hindered by peak overlap, and (for the amino acids) complicated by the known changes in the concentrations over time that are attributable to natural peptidase activity in the sample. We are not aware of any other study in which uridine has been shown to be so low in seminal fluid, and hence we have derived the term “seminal oligouridinosis” to describe this (potential) condition.

Uridine, uridine monophosphate, and uridine triphosphate are metabolic precursors of membrane phosphatidylserine, which in turn is metabolized as energy substrate through the pentose phosphate pathway (8). In addition, we established a positive correlation between the presence of uridine in semen and an increased percentage of motile spermatozoa to the zona pellucida of the oocyte. It has been postulated that in human spermatozoa uridine is catabolized to ribose-1-phosphate, which in turn is metabolized as energy substrate during acrosomal exocytosis and the binding of spermatozoa to the zona pellucida of the oocyte. It has been postulated that seminal uridine or its metabolites are suggestive of energy expenditure (8).

The presence of uridine in the seminal plasma of healthy fertile men, the proposed role of uridine in spermatozoal metabolism, and the novel observation of “seminal oligouridinosis” in infertile SCI men suggest that seminal uridine may be an important male fertility biomarker that may also be causally linked to spinal injury-related infertility.

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